Wissenschaftlicher Bericht
Scientific Report 2012–2013

ZENTRUM FÜR
INFEKTIONSFORSCHUNG
RESEARCH CENTER FOR
INFECTIOUS DISEASES

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

ZINF
IMIB

SCIENTIFIC REPORT 2012-2013
CONTENT

01

GENERAL REMARKS

SPEAKER'S REPORT 2012–2013

Infectious diseases have remained a global challenge to human health and thus a prominent topic of public discussion during 2012-2013 at both the academic and political levels. Release of reports from major health organisations has sounded the alarm for the sweeping development of antibiotic resistance throughout the world. Heightened rhetoric from UK Chief Medical officer Sally Davies and Thomas Frieden, Director of the Centers for Disease Control and Prevention (CDC), raised the perception that increasing levels of antimicrobial resistance is currently one of the most serious worldwide health threats. This was compounded more recently by the World Health Organisation (WHO) warning that the world is poised to enter a post-antibiotic era. This emphasizes the urgency to develop new antibiotics or even new strategies that would be refractory to the development of resistance.

During this time a more positive aspect of host-microbe interactions has fully emerged, the densely populated resident microbiota. It is becoming increasingly clear that the microbiota plays an important role in the induction, training and function of the host immune system, and may impact humans in previously unrecognized ways. There is also hope that, as we understand the composition of the microbiota it can be designed to control and treat diseases.

The collaborative Research Center for Infectious Diseases (ZINF) at the University of Würzburg has been addressing the molecular principles of host-pathogen interactions since 1993, by bringing together experts in microbiology, parasitology, virology and immunology as well as chemists and clinicians. Founded by Volker ter Meulen, Werner Goebel and colleagues with initial financial contributions from the Federal Ministry of Education and Research (BMBF), ZINF developed into a major scientific research programme at the University of Würzburg under the long-term leadership of Jörg Hacker. In recognition of its extraordinary success, the Bavarian Government and the University of Würzburg have continued to fund this programme. I have had the honour to serve as the Spokesperson of ZINF since 2011.

The present report covering the years 2012 and 2013 highlights the scientific projects and achievements of the Young Investigator groups that lie at the heart of ZINF, and aims to provide an overview of the research activities of ZINF members with relevance to infectious diseases. These were exceptionally busy and exciting years, with respect to changes in personnel, as well as networking and scientific achievements. Importantly 2013 ushered in an important milestone with the ZINF and IMIB celebrating the 20th anniversary of their founding. A symposium titled "The New Infection Biology" provided platform for people currently and previously associated with the ZINF to reflect on the impact made by the center on infectious diseases research both at the University and also world-wide. Speakers included the President of the University, Alfred Forchel, the Dean of the Medical Faculty and ZINF member Matthias Frosch, former ZINF Spokesperson Jörg Hacker and a former Young Investigator Ute Hentschel Humeida. From further afield the impact of research at ZINF was outlined by Christoph Dehio, the Chairperson of the Scientific Advisory Board.

Subsequently, John Mekalanos from Harvard Medical School kicked off the scientific presentations with his work on the type VI secretion system, at the current cutting edge of research. This was followed by a glimpse of the future of infection biology research with presentations from the Young Investigators at the ZINF, Daniel Lopez, Cynthia Sharma, Nicolai Siegel and Ana Eulalio. The symposium was brought to an end by two speeches from Pascale Cossart and Michael Gilmore who represented a European and American view, respectively, on the future of infectious diseases research. The day culminated in a festive dinner overlooking the city of Würzburg, where both past and present members were able to reflect on two decades of ZINF activities.

Since its beginning the core element of ZINF has been a Young Investigator programme whose four junior research groups have been considered as a paradigm for the promotion of early scientific independence within German universities. In addition, the success of the program is evident in our ability to continuously attract highly promising young investigators through other highly competitive funding programs. In 2012, Ana Eulalio (ICGEB, Trieste) was successful in obtaining one of five Junior Research Groups in the Bavarian Research Network for Molecular Biosystems, which is funded through the Bavarian State Ministry of Education, Science and the Arts. Her work will focus on using high-throughput screen approaches to understand the role of RNA

molecules in bacterial host-pathogen interactions. During 2013, we organised a symposium to recruit a new young investigator, with the funds coming from the Interdisciplinary Center for Clinical Research (IZKF), which organises the internal research funding of the Medical Faculty at the University of Würzburg. This resulted in Christian Perez joining us from the University of California at San Francisco to establish a group working on the regulatory networks underlying the interactions of the fungus *Candida albicans* with the host microbiota. Finally, Sebastian Geibel (University College London) has been awarded a junior group position form the Bayern Elite Network starting in 2014; he will work on the structural biology of Mycobacteria secretion systems.

As ever, our international Scientific Advisory Board (SAB) was instrumental in selecting new group leaders and providing us with critical feedback on current and planned research activities. Their continual support is highly appreciated. 2012 saw the departure of Eliora Ron (Tel Aviv) as she completed her second term on the Board. We are indebted to her very active participation and support during the transition of leadership at the ZINF. Concomitantly, the SAB was expanded with Agneta Richter-Dahlfors (Stockholm), David Holden (London), Michael Gilmore (Boston), Gisela Storz (Bethesda) and Tone Tønjum (Oslo) all joining us in 2012. We look forward to working closely with the SAB in the coming years.

The recent establishment of several research networks (see below) has further strengthened infectious disease research in Würzburg. This has also enabled us to further expand the ZINF membership. We welcome as new ZINF members Andreas Beilhack (Dept. Internal Medicine II), Niklas Beyersdorf (Institute for Virology and Immunobiology), Martin Fraunholz (Dept. of Microbiology), Ulrike Holzgrabe (Institute for Pharmacy and Food Chemistry), Vera Kozjak-Pavlovic (Dept. of Microbiology), Jürgen Löffler (Dept. of Internal Medicine II), Knut Ohlsen (Institute for Molecular Infection Biology), Tobias Ölschläger (Institute for Molecular Infection Biology), Jürgen Schneider-Schaulies (Institute for Virology and Immunobiology), Christoph Schoen (Institute for Hygiene and Microbiology) and Andrew Ullmann (Dept. of Internal Medicine II).

The success of the infectious disease researchers at the University of Würzburg is evident by a number of prestigious awards and appointments. Of the Young Investigators, in 2013 Daniel Lopez was awarded a European Research Council (ERC) Starting Grant over five years to work on the architecture of bacterial lipid rafts and their potential as targets of anti-infective treatment. He plans to use drugs whose action will differ from those of current antibiotics thereby placing less of a selective pressure for the development of antibiotic resistance. Cynthia Sharma received the fellowship of the Bavarian Academy of Sciences and received the 2013 Young Investigator award of the foundation of the German Association for Hygiene and Microbiology (DGHM). Ana Eulalio received the 2013 Federation of European Biochemical Societies (FEBS) Distinguished Young Investigator Award.

ZINF members have held important positions in several societies. Heidrun Moll completed her term as the President of the German Society of Parasitology and August Stich was elected Vice President of the German Society of Tropical Medicine and International Health and Vice President of the German Leprosy and Tuberculosis Relief Association. In 2013 Jörg Vogel was elected to the German National Academy of Sciences (Leopoldina) as well as to the American Academy of Microbiology.

We have continued our efforts to improve the scientific environment for infectious diseases research at the University of Würzburg through third-party funding. Milestones in 2012-2013 were successful funding of the collaborative research centre DFG TransRegio 124 *Pathogenic fungi and their human host: Networks of interaction* (speaker Axel Brakhage, vice-speaker Hermann Einsele) involving several ZINF groups together with research groups in Jena. Also in 2013 was the successful application of DFG Research Unit 2123 *Sphingolipid dynamics in infection control* (speaker Sibylle Schneider-Schaulies), which involves groups in both Würzburg and Essen. ZINF members secured research grants in several national and European networks aimed at a better understanding of infectious diseases, including ERA-NET, DFG TRR34 *Pathophysiology of Staphylococci,* DFG SPP1316 *Host-Adapted Metabolism of Bacterial Pathogens,* DFG Priority Program SPP1617 *Phenotypic Heterogeneity and Sociobiology of Bacterial Populations,* BMBF MedVet-Staph - *Interdisciplinary Research Network on the Zoonotic Impact of Staphylococcus aureus/MRSA,* the BMBF-funded network *RNomics of Infections* and the Bavarian Research Network for Molecular Biosystems (BiosysNet). The Interdisciplinary Center for Clinical Research (IZKF) recently elected ZINF board member Thomas Hünig as their spokesperson, reflecting the fact that IZKF also promotes infectious disease research with clinical relevance.

The training of graduate students and postdocs remains a key mission of ZINF. The Graduate School of Life Sciences, University of Würzburg of which ZINF member Caroline Kisker serves as the dean, has been awarded further funding from 2012 to 2017 during the second phase of the German Excellence Initiative. The Infection

SPRECHERBERICHT FÜR DEN ZEITRAUM 2012–2013

Infektionskrankheiten blieben auch in den Jahren 2012 und 2013, die dieser Bericht umfasst, eine Herausforderung für das globale Gesundheitssystem. Die Problematik wird dabei nicht länger nur in akademischen Kreisen diskutiert, sondern rückt zunehmend auch in das öffentliche und politische Bewußtsein. Alarmierende Studien zur weltweiten Zunahme von Antibiotikaresistenzen bewogen führende Vertreter von Gesundheitsbehörden wie Sally Davies, Chefberaterin der Britischen Regierung in Gesundheitsfragen, und Thomas Frieden, Direktor der Centers for Disease Control and Prevention (CDC) in den USA dazu, Antibiotikaresistenzen als eine der derzeit größten Gefahren für die menschliche Gesundheit zu benennen. Diese Einschätzung wird von der Weltgesundheitsorganisation WHO geteilt, welche kürzlich eindringlich vor einem drohenden Rückfall der Menschheit in die präantibiotische Ära warnte. Daraus wird wieder die Dringlichkeit deutlich, neue Antibiotika zu entwickeln und die Resistenzausbreitung einzudämmen, aber auch innovative Strategien zur Therapie und Prävention von Infektionen in den Blick zu nehmen.

Zu diesen innovativen Ansätzen gehört sicherlich auch der derzeit zu beobachtende Paradigmenwechsel in der mikrobiologischen Forschung. Hier wird nicht mehr ausschließlich die isolierte Interaktion einzelner Erreger mit dem Wirt untersucht. Vielmehr konzentriert man sich nun zusätzlich auf die komplexen Gemeinschaften von Mikroorganismen, die zum Beispiel unsere Haut oder den Verdauungstrakt besiedeln. Diese sogenannten Mikrobiome spielen eine enorme Rolle bei der Reifung und Arbeit des Immunsystems. Man darf gespannt sein, welche weiteren bisher unbekannten Funktionen der Mikrobiome demnächst aufgedeckt werden. In diesem Zusammenhang besteht auch die Hoffnung, dass ein besseres Verständnis dieser Gemeinschaften und ihrer Zusammensetzung interessante Ansätze liefern wird, um Krankheiten künftig besser zu behandeln.

Das Zentrum für Infektionsforschung (ZINF) der Universität Würzburg widmet sich seit 1993 der Erforschung von molekularen Prinzipien von Pathogen-Wirt-Interaktionen. Dazu bringt es Experten aus Mikrobiologie, Parasitologie, Virologie und Immunologie sowie Chemiker und klinisch tätige Ärzte zusammen. Gegründet von Volker ter Meulen, Werner Goebel und zahlreichen weiteren Kollegen entwickelte sich das ZINF unter der langjährigen Leitung von Jörg Hacker zu einer herausragenden Forschungsinstitution an unserer Universität. In Anerkennung dieses Erfolgs wurde die anfängliche Finanzierung durch das Bundesministerium für Bildung und Forschung (BMBF) später von der Bayerische Staatsregierung und der Universität Würzburg übernommen. Seit 2011 habe ich die Ehre, als Sprecher des Zentrums zu fungieren.

Der vorliegende Bericht zu den vergangenen zwei Jahren stellt die wissenschaftlichen Projekte und Leistungen der Nachwuchsgruppen dar, die nach wie vor das Herzstück des ZINF bilden. Ebenso wird ein Überblick über die Aktivitäten der ZINF-Mitglieder auf dem Gebiet der Infektionsforschung gegeben. Hinsichtlich personellen Veränderungen, Forschungsvernetzung und wissenschaftlichen Ergebnissen waren dies zwei aufregende und arbeitsreiche Jahre. Ein Höhepunkt war der 20. Jahrestag der Gründung des ZINF und des IMIB, der im Sommer 2013 mit einem internationalen Symposium zum Thema “Die neue Infektionsbiologie” gefeiert wurde. Die Tagung bot jetzigen und früheren ZINF-Mitgliedern Gelegenheit, über den Einfluss des Zentrums auf die Infektionsforschung sowohl hier in Würzburg als auch weltweit zu diskutieren. Sprecher waren der Präsident der Universität Würzburg Alfred Forchel, der Dekan der Medizinischen Fakultät und ZINF-Mitglied Matthias Frosch, der frühere Sprecher Jörg Hacker und die ehemalige Nachwuchsgruppenleiterin Ute Hentschel-Humeida. Eine Außenperspektive auf die Bedeutung des ZINF lieferte Christoph Dehio, der Sprecher des wissenschaftlichen Beirats.

Die wissenschaftlichen Präsentationen wurden von John Mekalanos von der Harvard Medical School eingeleitet, der seine bahnbrechenden Arbeiten zu Typ-IV-Sekretionssystemen vorstellte. Einen Eindruck von der Zukunft der Infektionsbiologie vermittelten dann die Vorträge der Nachwuchsgruppenleiter des ZINF, Daniel Lopez, Cynthia Sharma, Nicolai Siegel und Ana Eulalio. Das Symposium endete mit Beiträgen von Pascale Cossart und Michael Gilmore, die jeweils die europäische und die amerikanische Sichtweise auf die Infektionsforschung und ihre Bedeutung darlegten. Das überaus stimulierende Symposium fand seinen festlichen Abschluss bei einem gemeinsamen Abendessen Am Stein, bei dem die Teilnehmer mit Blick über Würzburg noch einmal über 20 Jahre gemeinsamer Arbeit im ZINF reflektieren konnten.

& Immunity section of the GSLS is headed by ZINF members Thomas Hünig and Joachim Morschhäuser and contains three training programs (infection, immunomodulation, and antiinfectives). Furthermore, Jörg Vogel, Cynthia Sharma, Ana Eulalio and Stan Gorski successfully applied for funding from the European Molecular Biology Organisation (EMBO) to run an international EMBO Practical Course *Non-coding RNA in Infection* in 2014. More on the international level, 2013 saw the completion of the Research Training Group 1522, which is funded by DFG and its South African sister organisation NRF and involves Axel Rethwilm as the German speaker.

Our students and postdocs also benefitted from several high-profile meetings in Würzburg with relevance to infectious diseases research. In addition to the symposium associated with the 20th Anniversary of the ZINF, Jörg Vogel and Daniel Lopez together with several of the ZINF Young Investigators partnered with the editors of the journal Molecular Microbiology to organise the Second Molecular Microbiology Meeting held in Würzburg. The Institute for Molecular Infection Biology also hosted the 14th Drug Design & Development Seminar of the German Society for Parasitology, the organisers including Heidrun Moll and Klaus Brehm. Würzburg was also the scene for the 18th International Pathogenic Neisseria Conference organised by Matthias Frosch, Thomas Rudel and Ulrich Vogel. Jörg Vogel was also one of the main organizers of the DFG supported *Regulating with RNA in Bacteria* international conference, which in addition to leading experts from around the world also contained a plenary lecture from Chemistry Nobel Prize winner Sidney Altman.

Students in Würzburg also benefit from several high-quality seminar series that cover different aspects of infection biology. These include those hosted by the Institute for Virology and Immunobiology and the Tuesday night Microbiology Colloquium, which is jointly organised by the Institute for Hygiene and Microbiology, the Dept. of Microbiology and the Institute for Molecular Infection Biology. These seminars are extremely well attended, by students and faculty alike, and have included many fantastic speakers, for example, Craig Roy (New Haven), Kim Lewis (Boston), Gunther Hartmann (Bonn), Aaron Mitchell (Pittsburgh), Søren Molin (Lyngby), and Maria Mota (Lisbon).

Future plans are being put into place to nurture interactions between groups in the ZINF including those with the clinics. This is also the case with other strongholds of infection biology such as the Welcome Trust Sanger Center in Hinxton, UK. Taken together, ZINF has been a vital instrument to bring together scientists with a keen interest in understanding the molecular cause of infectious diseases, and to encourage interdisciplinary research locally and internationally.

Let me finish by gratefully acknowledging the generous support given by the Bavarian State Ministry of Education, Science and the Arts and the presidium of the University of Würzburg for their continued support of the centre. ZINF is still going strong and new initiatives have been started to help us meet the challenges of infection disease research in the twenty-first century. We are looking forward to the next years of exciting research activities in ZINF.

Jörg Vogel
Spokesperson ZINF
Würzburg, July 2014

ropäischen Programmen ein. Zu nennen sind hier das ERA-NET-Programm, der DFG-geförderte Transregio-SFB Pathophysiologie von Staphylokokken in der Post-Genom-Ära, das DFG-Schwerpunktprogramm SPP1316 Wirtsadaptierter Metabolismus bakterieller Infektionserreger, das DFG-Schwerpunktprogramm SPP1617 Phänotypische Heterogenität und Soziobiologie bakterieller Populationen, das BMBF-MedVetStaph-Netzwerk *Staphylococcus aureus* als Zoonose-Erreger, das BMBF-geförderte Netzwerk RNomics in Infektionen sowie das Bayerische Forschungsnetzwerk für Molekulare Biosysteme (BioSysNet). Das Interdisziplinäre Zentrum für Klinische Forschung (IZKF) wählte kürzlich Thomas Hünig zu seinem Sprecher, was noch einmal die enge Verbindung zwischen Infektionsforschung und klinischer Medizin in Würzburg unterstreicht.

Die Ausbildung von Doktoranden und Postdocs ist und bleibt eine Schlüsselaufgabe des ZINF. Die Graduate School of Life Sciences (GSLS), mit dem ZINF-Mitglied Caroline Kisker als deren Dekanin, wird im Rahmen der zweiten Runde der Exzellenzinitiative für weitere fünf Jahre (2012-2017) gefördert. Die GSLS-Klasse Infektion und Immunität wird von Thomas Hünig und Joachim Morschhäuser als Sprecher geleitet, und bietet derzeit drei Trainingsprogramme zu den Themen Infektion, Immunmodulation und Antiinfektiva an. Zusätzlich waren Jörg Vogel, Cynthia Sharma, Ana Eulalio und Stan Gorski erfolgreich bei der Einwerbung von Mitteln der European Molecular Biology Organisation (EMBO), um einen internationalen Laborkurs zum Thema Non-coding RNA in Infection im Jahr 2014 abzuhalten. Auf internationaler Ebene fand das DFG-geförderte Deutsch-Südafrikanische Graduiertenkolleg 1522 seinen Abschluss, als dessen Sprecher Axel Rethwilm fungierte.

Unsere Studenten und Postdocs profitierten darüber hinaus von zahlreichen hochkarätigen Tagungen in Würzburg. Zusätzlich zum erwähnten Symposium zum 20. ZINF-Jubiläum, schlossen sich Jörg Vogel und Daniel Lopez sowie einige andere Nachwuchsgruppenleiter mit den Editoren der Fachzeitschrift Molecular Microbiology zusammen, um das *2nd Molecular Microbiology Meeting* hier in Würzburg zu veranstalten. Ebenso war das Institut für Molekulare Infektionsbiologie Gastgeber für das *14th Drug Design & Development* Seminar 2013 der Deutschen Gesellschaft für Parasitologie, das von Heidrun Moll und Klaus Brehm organisiert wurde. Würzburg war auch Tagungsort der *18th International Pathogenic Neisseria Conference (IPNC)* mit Matthias Frosch, Thomas Rudel und Ulrich Vogel als Organisatoren. Schließlich war Jörg Vogel einer der Hauptverantwortlichen für die Gestaltung der DFG-unterstützten internationalen Konferenz *Regulating with RNA in Bacteria,* die neben hochkarätigen Beiträgen anerkannter Kollegen aus aller Welt auch einen stimulierenden Plenarvortrag des Nobelpreisträgers für Chemie, Sidney Altman, bot.

Die regelmäßigen Seminarserien zu verschiedenen Aspekten der Infektionsbiologie waren eine weitere vielgenutzte Plattform zum Austausch innerhalb des ZINF. Hier sind die Seminare des Instituts für Virologie und Immunbiologie und das "Dienstagsseminar" zu erwähnen, das gemeinsam vom Institut für Hygiene und Mikrobiologie, dem Lehrstuhl für Mikrobiologie und dem Institut für Molekulare Infektionsbiologie veranstaltet wird. Die Seminare waren überaus gut besucht, sowohl durch die Studenten als auch durch die ZINF-Mitglieder, und boten hochinteressante Beiträge durch exzellente Sprecher wie zum Beispiel Craig Roy (New Haven), Kim Lewis (Boston), Gunther Hartmann (Bonn), Aaron Mitchell (Pittsburgh), Soren Molin (Lyngby) oder Maria Mota (Lisbon).

In der nahen Zukunft wollen wir die Zusammenarbeit zwischen dem ZINF und der klinischen Forschung verstärken und ausbauen. Das gilt ebenso für die Kontakte zu anderen "Leuchttürmen" der Infektionsbiologie wie zum Beispiel dem Welcome Trust Sanger Centre in Hinxton (Großbritannien). Insgesamt hat sich das ZINF als äußerst lebendige Institution etabliert, um Wissenschaftler mit einem starken Interesse an molekularen Ursachen von Infektionskrankheiten miteinander zu vernetzen und interdisziplinäres Arbeiten auf lokaler und internationaler Ebene zu ermöglichen.

Lassen Sie mich am Ende noch einmal dem Bayerischen Staatsministerium für Wissenschaft, Forschung und Kunst für die großzügige Förderung, und dem Präsidium der Universität Würzburg für die kontinuierliche Unterstützung und Ermutigung danken. Das ZINF ist weiterhin erfolgreich und neue Initiativen wurden bereits gestartet, um die Herausforderungen auf dem Gebiet der Infektionskrankheiten im 21. Jahrhundert zu meistern. Wir freuen wir uns auf die nächsten Jahre mit spannender Wissenschaft und vielen neuen Forschungsaktivitäten im ZINF.

Prof. Dr. Jörg Vogel
Sprecher des ZINF
Würzburg, Juli 2014

Von Anfang an bildete ein Nachwuchsgruppenprogramm den Kern des ZINF, welches beispielgebend für die frühe Förderung unabhängiger junger Wissenschaftler in Deutschland ist. Die Attraktivität des Programmes zeigt sich nicht zuletzt dadurch, dass es uns weiterhin gelingt, vielversprechende junge Wissenschaftler, auch durch andere hochkompetitive Ausschreibungen, für eine Arbeit am ZINF zu gewinnen. So bekam 2012 Ana Eulalio, zuvor am ICGEB in Trieste, die Förderung für eine von fünf BioSysNet-Juniorgruppen in ganz Bayern zugesprochen. Dieses Forschungsnetzwerk für Molekulare Biosysteme wird durch das Bayerische Staatsministerium für Bildung, Wissenschaft und Kunst gefördert. Ihre Arbeit konzentriert sich auf die Anwendung von Hochdurchsatztechnologien zur Untersuchung von RNA-Molekülen und deren Funktion bei Pathogen-Wirt-Interaktionen. 2013 organisierten wir außerdem ein Symposium zur Etablierung einer Nachwuchsgruppe, die durch das Interdisziplinäre Zentrum für Klinische Forschung (IZKF) der Würzburger Medizinischen Fakultät finanziert wird. Hier konnten wir Christian Perez von der University of California, San Francisco gewinnen, der eine Arbeitsgruppe zu regulatorischen Netzwerken bei der Interaktion von *Candida albicans* mit Wirtsmikrobiomen aufbauen wird. Ebenso erhielt Sebastian Geibel (University College London) eine Nachwuchsgruppenleiterposition über das Elitenetzwerk Bayern. Er wird ab 2014 am ZINF zur Strukturbiologie von Sekretionssystemen in Mykobakterien arbeiten.

Wie auch in den vergangenen Jahren war die Unterstützung durch den wissenschaftlichen Beirat (SAB) für uns von unschätzbarem Wert. Wir möchten uns an dieser Stelle für die Arbeit des SAB sowohl bei der Auswahl neuer Gruppen als auch bei der kritischen Begleitung laufender und künftiger Forschungsvorhaben herzlich bedanken. 2012 schied Eliora Ron (Tel Aviv) nach zwei Amtsperioden aus dem Beirat aus. Wir sind ihr für die überaus aktive und konstruktive Unterstützung, insbesondere während der Zeit des Wechsels in der ZINF-Leitung, zu besonderem Dank verpflichtet. Gleichzeitig wurde der Beirat mit Agneta Richter-Dahlfors (Stockholm), David Holden (London), Michael Gilmore (Boston), Gisela Storz (Bethesda) und Tone Tønjum (Oslo) als neue Mitglieder erweitert, die uns alle seit 2012 in unserer Arbeit beraten. Wir freuen uns auf die enge Zusammenarbeit mit dem SAB in den kommenden Jahren!

Die aktuelle Etablierung verschiedener neuer Forschungsprogramme (siehe unten) hat die Infektionsforschung in Würzburg weiter gestärkt. Das hat uns in die glückliche Lage versetzt, das ZINF um eine Reihe neuer Mitglieder zu erweitern. Wir freuen uns, Andreas Beilhack (Innere Medizin II), Niklas Beyersdorf (Institut für Virologie und Immunbiologie), Martin Fraunholz (Lehrstuhl für Mikrobiologie), Ulrike Holzgrabe (Institut für Pharmazie und Lebensmittelchemie), Vera Kozjak-Pavlovic (Lehrstuhl für Mikrobiologie), Jürgen Löffler (Innere Medizin II), Knut Ohlsen (Institut für Molekulare Infektionsbiologie), Tobias Ölschläger (Institut für Molekulare Infektionsbiologie), Jürgen Schneider-Schaulies (Institut für Virologie und Immunbiologie), Christoph Schoen (Institut für Hygiene und Mikrobiologie) und Andrew Ullmann (Innere Medizin II) in unseren Reihen begrüßen zu dürfen.

Der Erfolg der Infektionsforscher an der Universität Würzburg zeigt sich nicht zuletzt in einer Reihe von angesehenen Preisen und Auszeichnungen. Von den Nachwuchsgruppenleitern wurde Daniel Lopez 2013 durch den European Research Council (ERC) eine fünfjährige Förderung für ein Projekt zur Architektur von bakteriellen Lipidinseln zuerkannt. Diese Strukturen könnten Angriffsorte für neue Antibiotika darstellen. Daniel Lopez plant, Substanzen zu untersuchen, die anders als klassische Antibiotika wirken und damit einen geringeren Selektionsdruck für die Entwicklung von Resistenzen ausüben. Cynthia Sharma wurde 2013 in die Bayerische Akademie der Wissenschaften aufgenommen und erhielt den Förderpreis der Deutschen Gesellschaft für Hygiene und Mikrobiologie. Ana Eulalio wurde, ebenfalls 2013, der Nachwuchspreis der Federation of European Biochemical Societies (FEBS) verliehen.

ZINF-Mitglieder sind in zahlreichen wissenschaftlichen Gesellschaften aktiv. Heidrun Moll beendete ihre Amtszeit als Präsidentin der Deutschen Gesellschaft für Parasitologie, und August Stich wurde zum Vizepräsidenten der Deutschen Gesellschaft für Tropenmedizin und Internationale Gesundheit sowie der Deutschen Lepra- und Tuberkulosehilfe gewählt. 2013 wurde Jörg Vogel in die Nationale Akademie der Wissenschaften (Leopoldina) und die Amerikanische Akademie für Mikrobiologie aufgenommen.

Auch im vergangenen Berichtszeitraum haben wir unsere Bemühungen fortgesetzt, das Umfeld für die Infektionsforschung an der Universität Würzburg durch die Einwerbung von Drittmittel noch attraktiver zu machen. Meilensteine waren dabei in den Jahren 2012/2013 die Förderung des Transregio-Sonderforschungsbereiches 124 Pathogene Pilze und ihr Wirt: Netzwerke und Interaktionen (Sprecher: Axel Brakhage, Vizesprecher: Hermann Einsele) durch die DFG. In diesem TR-SFB arbeiten ZINF-Mitglieder mit Kollegen aus Jena zusammen. Ebenfalls 2013 wurde eine DFG-Forschergruppe zum Thema Sphingolipid-Dynamik und Infektionskontrolle (Sprecherin: Sibylle Scheider-Schaulies) erfolgreich etabliert, die Arbeitsgruppen aus Würzburg und Essen miteinander vernetzt. Darüber hinaus warben ZINF-Mitglieder Drittmittel in zahlreichen nationalen und eu-

EXECUTIVE COMMITTEE

PROF. DR. JÖRG VOGEL
(Spokesperson of the ZINF)
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15 97080Würzburg
T +49.931.318 2576
M joerg.vogel@uni-wuerzburg.de

PROF. DR. HERMANN EINSELE
Medizinische Klinik und Poliklinik II
Gebäude ZIM, Ebene: A3.-1
Oberdürrbacher Straße 6 97080 Würzburg
T +49.931.201 400 00
M einsele_h@klinik.uni-wuerzburg.de

PROF. DR. MATTHIAS FROSCH
Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/E 1
97080 Würzburg
T +49.931.314 6160
M mfrosch@hygiene.uni-wuerzburg.de

PROF. DR. THOMAS HÜNIG
Institut für Virologie und Immunbiologie
Versbacher Str. 7
97078 Würzburg
T +49.931.318 1541
M huenig@vim.uni-wuerzburg.de

PROF. DR. AXEL RETHWILM
Institut für Virologie und Immunbiologie
Versbacher Str. 7
97078 Würzburg
T +49.931.318 1672
M axel.rethwilm@mail.uni-wuerzburg.de

PROF. DR. THOMAS RUDEL
Lehrstuhl für Mikrobiologie
Theodor-Boveri-Institut für Biowissenschaften
Biozentrum
Am Hubland
97074 Würzburg
T +49.931.318 4401
M thomas.rudel@biozentrum.uni-wuerzburg.de

SCIENTIFIC COORDINATOR

DR. STAN GORSKI
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 9079
M stan.gorski@uni-wuerzburg.de

YOUNG INVESTIGATORS

DR. CYNTHIA SHARMA
Young Investigator Group Leader
ZINF (since 2010)
Zentrum für Infektionsforschung
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 2560
M cynthia.sharma@uni-wuerzburg.de

DR. DANIEL LÓPEZ
ZINF (since 2010)
Zentrum für Infektionsforschung
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 3831
M daniel.lopez@uni-wuerzburg.de

DR. T. NICOLAI SIEGEL
ZINF (since 2012)
Zentrum für Infektionsforschung
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 8499
M nicolai.siegel@uni-wuerzburg.de

DR. ANA EULALIO
Young Investigator Group Leader
BioSysNet (since 2012)
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 9078
M ana.eulalio@uni-wuerzburg.de

DR. J. CHRISTIAN PÉREZ
ZINF/IZKF (since 2014)
Zentrum für Infektionsforschung
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 3815
M christian.perez@uni-wuerzburg.de

DR. SEBASTIAN GEIBEL
Elite Netzwerk Bayern (since 2014)
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 4590
M sebastian.geibel@uni-wuerzburg.de

SCIENTIFIC MEMBERS OF THE RESEARCH CENTER FOR INFECTIOUS DISEASES

DR. ANDREAS BEILHACK
Experimentelle Stammzelltransplantation
der Medizinische Klinik und Poliklinik II
Klinikstraße 6-8
97070 Würzburg
T +49.931.201 27637
M beilhack_a@klinik.uni-wuerzburg.de

PD DR. NIKLAS BEYERSDORF
Institut für Virologie und Immunbiologie
Versbacher Str. 7
97078 Würzburg
T +49.931.318 1527
M niklas.beyersdorf@vim.uni-wuerzburg.de

PROF. DR. KLAUS BREHM
Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/E 1
97080 Würzburg
T +49.931.314 6168
M kbrehm(at)hygiene.uni-wuerzburg.de

PROF. DR. GERHARD BRINGMANN
Lehrstuhl für Organische Chemie I
Biozentrum
Am Hubland
97074 Würzburg
T +49.931.318 5323
M bringman@chemie.uni-wuerzburg.de

PROF. DR. THOMAS DANDEKAR
Bioinformatik
Theodor-Boveri-Institut für Biowissenschaften
Am Hubland
97074 Würzburg
T +49.931.318 4551
M dandekar@biozentrum.uni-wuerzburg.de

PROF. DR. MARKUS ENGSTLER
Lehrstuhl für Zell- und Entwicklungsbiologie
Theodor-Boveri-Institut für Biowissenschaften
Biozentrum
Am Hubland
97074 Würzburg
T +49.931.318 0060
M markus.engstler@biozentrum.uni-wuerzburg.de

DR. MARTIN FRAUNHOLZ
Lehrstuhl für Mikrobiologie
Theodor-Boveri-Institut für Biowissenschaften
Biozentrum
Am Hubland
97074 Würzburg
T +49.931.318 3242
M martin.fraunholz@uni-wuerzburg.de

PROF. DR. ROY GROSS
Lehrstuhl für Mikrobiologie
Theodor-Boveri-Institut für Biowissenschaften
Biozentrum
Am Hubland
97074 Würzburg
T +49.931.318 4403
M roy@biozentrum.uni-wuerzburg.de

PROF. DR. UTE HENTSCHEL-HUMEIDA
Julius-von-Sachs-Institut für Biowissenschaften
Julius-von-Sachs-Platz 3
D - 97082 Würzburg
T +49.931.318 2581
M ute.hentschel@uni-wuerzburg.de

PROF. DR. THOMAS HERRMANN
Institut für Virologie und Immunbiologie
Versbacher Str. 7
97078 Würzburg
T +49.931.318 1538
M herrmann-t@vim.uni-wuerzburg.de

PROF. DR. ULRIKE HOLZGRABE
Institut für Pharmazie und Lebensmittelchemie
Am Hubland
97074 Würzburg
T +49.931.318 5460
M u.holzgrabe@pharmazie.uni-wuerzburg.de

PROF. DR. CAROLINE KISKER
Rudolf-Virchow-Zentrum
Josef-Schneider-Str.2/ D15
97080 Würzburg
T +49.931.318 0381
M caroline.kisker@virchow.uni-wuerzburg.de

PROF. DR. HARTWIG KLINKER
Medizinische Klinik und Poliklinik II
Zertifiziertes Zentrum für Infektiologie (DGI)
Oberdürrbacher Straße 6
97080 Würzburg
T +49.931.201 40043
M Klinker_H@medizin.uni-wuerzburg.de

PD DR. VERA KOZJAK-PAVLOVIC
Lehrstuhl für Mikrobiologie
Theodor-Boveri-Institut für Biowissenschaften
Biozentrum
Am Hubland
97074 Würzburg
T +49.931.318 8061
M vera.kozjak@uni-wuerzburg.de

PROF. DR. JÜRGEN LÖFFLER
Medizinische Klinik und Poliklinik II
Oberdürrbacher Straße 6
97080 Würzburg
T +49.931.201 36412
M Loeffler_J@ukw.de

PROF. DR. MANFRED LUTZ
Institut für Virologie und Immunbiologie
Versbacher Str. 7
97078 Würzburg
T +49.931.318 1553
M m.lutz@vim.uni-wuerzburg.de

PROF. DR. HEIDRUN MOLL
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 2627
heidrun.moll@uni-wuerzburg.de

PROF. DR. JOACHIM MORSCHHÄUSER
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 2152
M joachim.morschhaeuser@uni-wuerzburg.de

PD DR. KNUT OHLSEN
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 2155
M knut.ohlsen@uni-wuerzburg.de

DR. TOBIAS ÖLSCHLÄGER
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 2150
M t.oelschlaeger@uni-wuerzburg.de

PD DR. CHRISTOPH SCHOEN
Institut für Hygiene und Mikrobiologie
Josef-Schneider-Straße 2 / E1
97080 Würzburg
T +49.931.314 6162
M cschoen@hygiene.uni-wuerzburg.de

PROF. DR. JÜRGEN SCHNEIDER-SCHAULIES
Institut für Virologie und Immunbiologie
Versbacher Str. 7
97078 Würzburg
T +49.931.318 1564
M jss@vim.uni-wuerzburg.de

PROF. DR. SIBYLLE SCHNEIDER-SCHAULIES
Institut für Virologie und Immunbiologie
Versbacher Str. 7
97078 Würzburg
T +49.931.318 1566
M s-s-s@vim.uni-wuerzburg.de

PROF DR. ALEXANDRA SCHUBERT-UNKMEIR
Institut für Hygiene und Mikrobiologie
Josef-Schneider-Straße 2 / E1
97080 Würzburg
T +49.931.314 6721
M aunkmeir@hygiene.uni-wuerzburg.de

SCIENTIFIC ADVISORY BOARD (SAB)

PROF. DR. AUGUST STICH
Missionsärztliche Klinik
Abt. Tropenmedizin
Salvatorstr. 7
97067 Würzburg
T +49.931.791 2821
M stich@missioklinik.de

PROF. DR. MAX TOPP
Med. Klinik und Poliklinik II
Versbacherstraße 5
Gebäude E4
97078 Würzburg
T +49.931.201 40015
M Topp_M@medizin.uni-wuerzburg.de

PROF. DR. HEIKE WALLES
Lehrstuhl Tissue Engineering und Regenerative Medizin
Röntgenring 11
97072 Würzburg
T + 49.931.318 0183
M heike.walles@uni-wuerzburg.de

PROF. DR. ANDREW ULLMANN
Schwerpunkt Infektiologie
Medizinische Klinik und Poliklinik II
Oberdürrbacher Straße 6
97080 Würzburg
T +49.931.201 40080
M Ullmann_A@ukw.de

PROF. DR. ULRICH VOGEL
Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/ Bau E1
97080 Würzburg
T +49.931.314 6802
M uvogel@hygiene.uni-wuerzburg.de

PD DR. WILMA ZIEBUHR
Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/ D15
97080 Würzburg
T +49.931.318 2154
M w.ziebuhr@mail.uni-wuerzburg.de

PROF. DR. AXEL BRAKHAGE (SINCE 2009)
Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie
Hans-Knöll-Institut
Beutenberg 11 a
07745 Jena
T +49.3641.532 1001
M axel.brakhage@hki-jena.de

PROF. DR. CHRISTOPH DEHIO (SINCE 2009)
Biozentrum der Universität Basel
Abteilung Infektionsbiologie
Klingelbergstr. 50/70
4056 Basel / SCHWEIZ
T +41.61.267 2140
M christoph.dehio@unibas.ch

PROF. DR. RICHARD LUCIUS (SINCE 2009)
Humboldt-Universität zu Berlin
Lehrstuhl für Molekulare Parasitologie
Institut für Biologie
Philippstr. 3
10115 Berlin
T +49.30.209 36053
M richard.lucius@rz.hu-berlin.de

PROF. JEAN LANGHORNE (SINCE 2009)
The National Institute for Medical Research
Division of Parasitology
The Ridgeway, Mill Hill
London, NW7 1AA / UK
T +44.208.816 2558
M jlangho@nimr.mrc.ac.uk

PROF. DR. SEBASTIAN SUERBAUM (SINCE 2009)
Medizinische Hochschule Hannover
Institut für Medizinische Mikrobiologie
und Krankenhaushygiene
Carl-Neuberg-Str. 1
30625 Hannover
T +49.511.532 6769
M suerbaum.sebastian@mh-hannover.de

DR. MARIAGRAZIA PIZZA (SINCE 2009)
Novartis Vaccines and Diagnostics
Via Fiorentina 1
5300 Siena / ITALY
T +39.577.243 390
M mariagrazia.pizza@novartis.com

PROF. MICHAEL GILMORE (SINCE 2012)
Harvard Medical School
Department of Ophthalmology
(Microbiology and Immunobiology)
Mass Eye and Ear Infirmary
243 Charles St.
Boston, MA 02114 -USA
T +1.617.573 3845
M michael_gilmore@meei.harvard.edu

PROF. DAVID HOLDEN (SINCE 2012)
Imperial College London
Centre for Molecular Microbiology
and Infection
2.21 Flowers Building
South Kensiington Campus
London. SW7 2AZ – UK
T +44.20.7594 3073
M d.holden@imperial.ac.uk

PROF. DR. AGNETA RICHTER-DAHLFORS (SINCE 2012)
Karolinska Institutet
Department of Neuroscience
Retzius väg 8
S-17177 Stockholm – SWEDEN
T +46.8524.874 25
M Agneta.Richter.Dahlfors@ki.se

DR. GISELA STORZ (SINCE 2012)
National Institutes of Health
Cell Biology and Metabolism Program
NICHD, Building 18T, Room 101
18 Library Dr MSC 5430
Bethesda, Maryland 20892-5430 – USA
T +1.301.402 0968
M storz@helix.nih.gov

PROF. TONE TØNJUM (SINCE 2012)
University of Oslo
CMBN and Institute of Microbiology
Rikshospitalet
NO-0027 Oslo – NORWAY
T +47.2307.4065
M tone.tonjum@medisin.uio.no

1. GENERAL REMARKS

1.3. STRUCTURE OF THE ZINF

The Research Center for Infectious Diseases (ZINF) at the University of Würzburg was founded in 1993. It brings together bacteriologists, mycologists, parasitologists, virologists, immunologists, chemists, bioinformaticians, structural biologists and clinicians from the Medical, Biology and Chemistry Faculties.

ZINF

INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

YOUNG INVESTIGATORS

INSTITUTE FOR HYGIENE AND MICROBIOLOGY

INSTITUTE FOR VIROLOGY AND IMMUNOBIOLOGY

DEPARTMENT OF MICROBIOLOGY

DEPARTMENT OF INTERNAL MEDICINE II

ZINF MEMBERS ASSOCIATED WITH OTHER INSTITUTES

STAN GORSKI
Scientific Coordinator

EXECUTIVE COMMITTEE

JÖRG VOGEL
Institute for Molecular Infection Biology, Spokesperson

HERMANN EINSELE
Deptartment of Internal Medicine II

MATTHIAS FROSCH
Institute for Hygiene and Microbiology

THOMAS HÜNIG
Institute for Virology and Immunobiology

AXEL RETHWILM
Institute for Virology and Immunobiology

THOMAS RUDEL
Chair of Microbiology

SCIENTIFIC ADVISORY BOARD

AXEL BRAKHAGE
Jena

RICHARD LUCIUS
Berlin

SEBASTIAN SUERBAUM
Hannover

CHRISTOPH DEHIO
Basel

MICHAEL GILMORE
Boston

DAVID HOLDEN
London

JEAN LANGHORNE
London

MARIAGRAZIA PIZZA
Siena

AGNETA RICHTER-DAHLFORS
Stockholm

GISELA STORZ
Bethesda

TONE TONJUM
Oslo

MMMW 2012

2ND MOLMICROMEETING WÜRZBURG

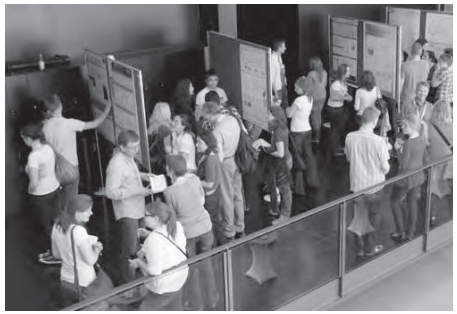
The second Molecular Microbiology Meeting was held in Würzburg from 25–27 April 2012.

PICTURES
top: key note lecture by Jeff Errington, at the Institute for Molecular Infection Biology in the central foyer
middle: Audience
bottom: get together

RNA2013

REGULATING WITH RNA IN BACTERIA

The Regulating with RNA in Bacteria meeting took place at IMIB between 4-8 June 2013, bringing together international experts in the structural and functional aspects of regulatory RNAs in prokaryotes.



PICTURES

left: boat trip on the main river
middle left: Cari Vanderpool, Éric Massé, Cynthia Sharma, Joel Belasco and Susan Gottesman
bottom left: Jörg Vogel in discussion with participants
right page: poster sessions, Petra Dersch, Emanuelle Charpentier, Tyrell Conway and Poul Valentin-Hansen

20 YEARS OF ZINF

SYMPOSIUM 2013

A symposium to celebrate the 20th anniversary of the ZINF in July 2013 brought together people both past and present that have contributed to the success of the centre.

PICTURES

top: Fritz Melchers, Hermann Bujard, Jörg Hacker, Jörg Vogel, Reinhard Burger
middle: Jörg Hacker with former coworkers of the ZINF-IMIB
bottom: Werner Goebel and Michael Gilmore

PICTURES

top: Pascale Cossart , Hermann Bujard and Stan Gorski
bottom, left: Eliora Ron, former ZINF SAB member;
Former and current ZINF Young investigator group leaders (from left to right): N. Siegel, H. Moll, C. Hauck, S. Hammerschmidt, C. Sharma, J. Morschhäuser, S. Krappmann, A. Eulalio, D. Lopez

CYNTHIA SHARMA (ZINF)

DANIEL LOPEZ (ZINF)

NICOLAI SIEGEL (ZINF)

ANA EULALIO (BIOSYSNET)

CHRISTIAN PEREZ (ZINF/IZKF)

SEBASTIAN GEIBEL (ELITE NETWORK BAVARIA)

02

YOUNG INVESTIGATOR GROUPS OF THE ZINF

regulation (activation or repression) by affecting the translation of *tlpB* mRNA. This example represents an unexpected twist in sRNA-mediated regulation and a new connection between variable simple sequence repeats and fine-tuning of post-transcriptional regulation.

Since Epsilonproteobacteria lack an apparent Hfq homolog, we are searching for alternate RNA-protein (RNP)-complexes and RNA binding proteins as well as RNases that are important for post-transcriptional regulation in these bacteria. We are especially interested in whether sRNAs in these bacteria require a different RNA binding protein or act independently by novel mechanisms. Using co-immunoprecipitation combined with global RNA-seq we have investigated direct RNA binding partners of the translational regulator CsrA in *Campylobacter* and identified the 5'UTR of a flagellin mRNA as a novel potential mRNA antagonist of this protein.

Infection studies of human pathogens are often impeded by the use of artificial *in vitro* cell culture or animal models which are limited in their ability to comprehensively mimic the infection situation and disease development in the human host. In a collaborative project with Prof. Heike Walles we are developing new three-dimensional (3D) infection models using tissue engineering to mimic the microenvironment of human tissue and we have begun to establish infection conditions with *Campylobacter* using a small-intestine model.

FUTURE DIRECTIONS

In addition to studying sRNA functions and mechanisms and identifying novel associated protein factors, we aim to further develop deep sequencing-based approaches and bioinformatics-based analyses to study bacterial infections. To identify RNA regulators of virulence genes we will employ RNA-seq to monitor the transcriptomes of host and pathogen in parallel using cell culture as well as 3D tissue models. In this direction we are developing a new 3D stomach tissue model to further investigate *Helicobacter* infections. These models and approaches will also be useful to study other pathogens and the development of new therapeutic and diagnostic tools.

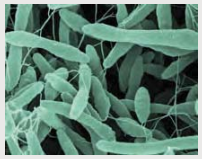


Fig. 1: Colorized scanning electron microscopy of the food-borne pathogen *Campylobacter jejuni* at 15,000x magnification.

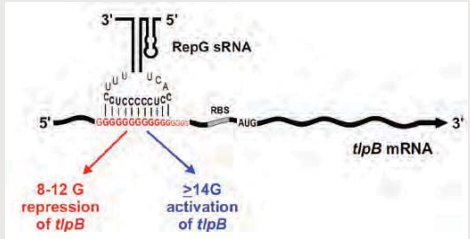


Fig. 2: RepG sRNA from *Heliocbacter pylori* directly interacts with its C/U-rich loop with a length-variable homopolymeric G-repeat in the mRNA leader of the chemotaxis receptor TlpB. The length of the G-repeat determines the outcome of RepG-mediated regulation and leads to the repression of tlpB for 8-12Gs and to activation of *tlpB* for longer repeats of more than 13G.

SELECTED PUBLICATIONS

Pernitzsch SR, Tirier S, Beier D, **Sharma CM** (2013) *A variable homopolymeric G-repeat defines small RNA-mediated post-transcriptional regulation of a chemotaxis receptor in Helicobacter pylori.* **PNAS** in press

Dugar G, Herbig A, Förstner KU, Heidrich N, Reinhardt R, Nieselt K, **Sharma CM** (2013) *High-resolution transcriptome maps reveal strainspecific regulatory features of multiple Campylobacter jejuni isolates.* **PLoS Genetics** *9:e1003495*

Pernitzsch SR, **Sharma CM** (2012) *Transcriptome complexity and riboregulation in the human pathogen Helicobacter pylori.* **Frontiers in Cellular and Infection Microbiology** *2:14*

Rieder R, Reinhardt R, **Sharma CM,** Vogel J (2012) *Experimental tools to identify RNA-protein interactions in Helicobacter pylori.* ***RNA Biology*** *9:520-31*

Sharma CM, Heidrich N (2012) *Small RNAs and virulence in bacterial pathogens.* **RNA Biology** *9:361-3*

PRIZES AND AWARDS

2013 DGHM-Förderpreis: Young Investigator award of the foundation of the German Association for Hygiene and Microbiology

2012 Young Academy fellowship of the Bavarian Academy of Sciences

2012 Young Investigator fellowship of the Daimler-and-Benz-Foundation

2. YOUNG INVESTIGATORS

2.1. DEEP SEQUENCING APPROACHES TO PATHOGENESIS

Our research focuses on the identification and characterization of small regulatory RNAs (sRNAs) and associated RNA-binding proteins in the bacterial pathogens *Helicobacter pylori* and *Campylobacter jejuni*. We are especially interested in the roles and underlying molecular mechanisms of sRNAs during the stress response and virulence control of these widespread human pathogens.

DR. CYNTHIA M. SHARMA
RESEARCH CENTER FOR INFECTIOUS DISEASES

Zentrum für Infektionsforschung (ZINF)
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3182560
M cynthia.sharma@uni-wuerzburg.de

INTRODUCTION

Post-transcriptional regulation represents a central level of gene expression control in the cell. Small RNAs and RNA-binding proteins have been described as key players in this regulatory process. The 50-200 nt long sRNAs from bacteria are a heterogeneous class of molecules that regulate bacterial gene expression in response to adverse growth and environmental stress conditions. Although a large number of sRNAs have been identified in a wide range of bacteria during the last two decades, most of the functional insight on sRNAs comes from work on enterobacteria. In contrast, little is known about sRNA-mediated regulation (riboregulation) in Epsilonproteobacteria. These include *Helicobacter pylori*, the causative agent of gastric cancer, and *Campylobacter jejuni*, the most common cause of bacterial food-borne disease to date. Epsilonproteobacteria, like 50% of all bacteria, lack a homolog of the RNA chaperone Hfq, a key player in sRNA-mediated regulation in enterobacteria, and thus were previously assumed to lack riboregulation.

The development of next generation sequencing technology and its application to cDNA has enabled the analysis of most RNA molecules in a cell in a single sequencing experiment at unprecedented resolution. This has greatly facilitated transcriptome annotation including the determination of transcript boundaries and identification of novel transcripts. For example, our differential RNA-sequencing approach (dRNA-seq) revealed a very high transcriptome complexity of the relatively small *H. pylori* genome and allowed us to define a genome-wide map of transcriptional start sites (TSS). Moreover, besides extensive antisense transcription we also identified an unexpected number of sRNAs in *H. pylori*.

RESEARCH HIGHLIGHTS

Helicobacter and *Campylobacter* display very high genotypic and phenotypic diversity among strains. To understand how transcriptome differences contribute to phenotypic differences among closely related strains, we have applied a comparative dRNA-seq approach to multiple *Campylobacter* strains and developed a novel, generic method for the automated annotation of TSS to provide genome-wide promoter maps. Our comparative approach revealed that the majority of TSS are conserved among strains, but also strain-specific promoter usage and sRNA repertoires were observed, which could contribute to strain-specific gene regulation.

Based on our transcriptome datasets, we are now using *Helicobacter* and *Campylobacter* as new model organisms for riboregulation in virulent bacteria and bacteria that lack Hfq. Using biochemical, molecular biology and genetics approaches we are elucidating the functions and physiological roles of sRNAs along with their underlying molecular mechanisms in stress response and virulence. For example, the abundant RepG sRNA from *Helicobacter*, directly targets a homopolymeric G-repeat in the 5'UTR of a chemotaxis receptor mRNA and thereby represses the expression of the pH-sensing chemotaxis receptor TlpB. While the sRNA sequence is conserved, the G-repeat varies in length in different *Helicobacter* strains. Using a variety of *in vivo* and *in vitro* experiments we have shown that the length of the G-repeat determines the outcome of small RNA-mediated

genetically manipulate the pathogen and generate a number of transcriptional and translational reporter strains. We have successfully applied these tools to study the role of the cytoskeleton in defining cell shape in stapylococci.

We have also been studying the function of lipid rafts in *B. subtilis* and *S. aureus*. In *B. subtilis* we have begun to try to understand the principles involved in the organization and assembly of lipid rafts in bacteria. This has involved determining the composition of lipid rafts and their organization using a variety of methods including proteomics, transcriptomics and super-resolution microscopy. We have also investigated the role of a major protein component of lipid rafts, flotillin. The overproduction of flotillin alters specific signal transduction pathways that are associated with the membrane microdomains of bacteria. This resulted in significant physiological defects in cell division and cell differentiation caused by an unusual stabilization of the raft-associated protease FtsH.

In addition, we are interested in the role of co-evolution of different bacterial strains within a clonal community and how the emerging mutants spatially segregate specific traits within the community. At the molecular level, we are focused on unraveling the signaling pathways that drive these evolutionary processes.

FUTURE DIRECTIONS

We are expanding the studies of bacterial lipid rafts to other clinically-relevant species and gram-negative bacteria. Moreover, we will further characterize the architecture of bacterial lipid rafts and their protein and lipid composition, to understand their assembly and identify and characterize the signalling pathways that depend on the presence of microdomains. We are also using a collection of small molecules to alter the integrity of bacterial lipid rafts and inhibit several infection related physiological processes. We are interested in implementing these anti-raft compounds as antimicrobial agents to ultimately develop new antimicrobial strategies to fight infectious diseases.

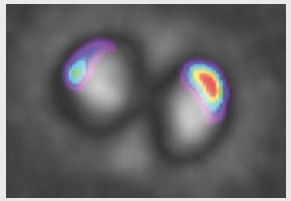


Fig. 1: S. aureus cells expressing a fluorescently labeled copy of the protein flotillin. Flotillin accumulates in bacterial lipid rafts and it localizes in discrete regions of the membrane.

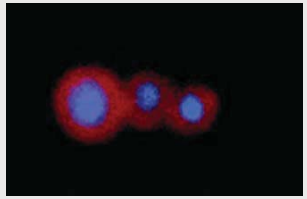


Fig. 2: Our molecular toolbox allows us a fine genetic manipulation of *S. aureus* cells to precisely modify the physiology of cells. Here we show a microscopy picture of *S. aureus* cells in which the activity of the cell wall biosynthesis machinery has been altered by genetic manipulation. As a consequence, cells exhibit different growth sizes. Nile Red dye has been used for membrane staining. DNA staining (blue).

SELECTED PUBLICATIONS

Mielich-Suss B, Schneider J, **Lopez D** (2013) *Overproduction of flotillin influences cell differentiation and shape in Bacillus subtilis.* **MBio** 4: e00719-00713

Yepes A, Schneider J, Mielich B, Koch G, Garcia-Betancur JC, Ramamurthi KS, Vlamakis H, **Lopez D** (2012) *The biofilm formation defect of a Bacillus subtilis flotillin-defective mutant involves the protease FtsH.* **Molecular Microbiology** 86:457-471

PRIZES AND AWARDS

2013 European Research Council (ERC) Starting Grant

2. YOUNG INVESTIGATORS

2.2. CELL-CELL COMMUNICATION AND SIGNAL TRANSDUCTION

Bacteria often reside within complex communities called biofilms. My group investigates the signaling networks within biofilm-dwelling bacteria that are involved in cell-cell communication and the molecular principles that bacteria use to translate these signals into the relevant physiological response.

DR. DANIEL LOPEZ

RESEARCH CENTER FOR INFECTIOUS DISEASES

Zentrum für Infektionsforschung (ZINF)
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-318381
M daniel.lopez@uni-wuerzburg.de

INTRODUCTION

The survival, proliferation, and differentiation of bacteria depends on their ability to interpret the signals they receive from the surrounding environment and to translate them into the correct physiological responses. If the signaling process does not function correctly then the organism is at risk. The organisation of signalling molecules into networks enables cells to robustly sense the presence of extracellular small-molecule signals that activate specific signaling transduction pathways, which in turn, induce the expression of specific genes required to adapt the cell's physiology.

In multicelluar organisms, cell-to-cell signalling occurs by diverse and sophisticated molecular mechanisms from direct contact (juxtacrine signaling) to signalling over short (paracrine signaling) or large distances (endocrine signaling). However, cell-cell signalling is also crucial for the survival of unicellular organisms, such as bacteria. Cell communication in bacteria is mediated by the production, release, and community-wide detection of small molecules called autoinducers. Autoinducers provide a mechanism for the bacteria to monitor the population density and to modulate gene expression accordingly. Although bacterial signal transduction has traditionally viewed as simpler than that of eukaryotes due to the reduced number of signalling components, they are able to respond to complex environmental changes and undergo extremely complex developmental processes. Therefore, it is possible that additional and yet unappreciated organization levels of signaling networks provide an important level of regulation in processing and integrating signalling information in bacteria.

We recently discovered a new level of spatial organization of bacterial signaling networks. Bacteria organize their signal transduction components into functional membrane microdomains constituted by specific lipids that are structurally similar to lipid rafts described in eukaryotic cells. The assembly of bacterial lipid rafts involves the biosynthesis and aggregation of polyisoprenoid lipids in the membrane and their co-localization with flotillin proteins. Flotillin proteins are membrane-bound chaperones that localize exclusively to lipid rafts, where they potentially recruit the protein cargo to lipid rafts and facilitate their oligomerization. The perturbation of bacterial lipid rafts inevitably leads to a potent and simultaneous impairment of all raft-harbored signal transduction pathways. Consequently, the disassembly of lipid rafts in pathogens such as *Staphylococcus aureus* simultaneously inhibit numerous infection-related processes. Therefore we are interested in understanding the molecular principles of the role of lipid rafts in controlling important signalling pathways in physiology and pathophysiology.

RESEARCH HIGHLIGHTS

The identification of membrane platforms in bacteria that are functionally and structurally equivalent to eukaryotic lipid rafts revealed an unexpected level of sophistication in signaling transduction and membrane organization in bacteria. We have been using *Bacillus subtilis* and the pathogen *S. aureus* as model organisms to perform a comprehensive molecular and functional characterization of bacterial lipid rafts. As an initial starting point we have developed a molecular toolbox for *S. aureus* which enables us to efficiently

2. YOUNG INVESTIGATORS

2.3. TRYPANOSOMA GENE REGULATION

Using the protozoan parasite *Trypanosoma brucei,* the causative agent of African sleeping sickness, the group studies the epigenetic mechanisms leading to the formation of chromatin structures that promote and repress transcription. One key question is how changes in chromatin structure can help the parasite to evade the host immune response via antigenic variation.

DR. T. NICOLAI SIEGEL

RESEARCH CENTER FOR INFECTIOUS DISEASES

Zentrum für Infektionsforschung (ZINF)
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3188499
M nicolai.siegel@uni-wuerzburg.de

INTRODUCTION

To elucidate the fundamental mechanisms involved in the regulation of gene expression, we are using *Trypanosoma brucei,* an extracellular protozoan parasite that causes sleeping sickness in humans and nagana in cattle. Every year in Sub-Saharan-Africa these diseases lead to the deaths of thousands of people and loss of livestock worth billions of dollar. To escape elimination by the host immune response, the parasite periodically switches its coat of variant surface glycoproteins (VSG), a process referred to as antigenic variation. The molecular mechanism of antigenic variation is not well understood, but several findings indicate that distinct chromatin structures may ensure that only one of several hundred VSG genes is expressed at any given time.

For transcription of a specific gene to occur, RNA polymerase II (pol II) must locate the correct transcription start site (TSS) against a large background of genomic DNA sequences. In higher eukaryotes this involves the assembly of an elaborate complex of transcription factors to target the polymerase to the correct genomic locus. However, correct targeting also depends on the accessibility of these factors to the TSS since most DNA is packaged into chromatin, which is composed of DNA and proteins including arrays of nucleosomes. While the organization of DNA into dense chromatin structures presents an obstacle to the transcriptional machinery, it also provides an opportunity to regulate gene expression because chromatin structures can be locally and globally modified, making them highly dynamic. Structural changes in chromatin can be induced by the post-translational modification of histones or by the replacement of canonical histones with histone variants. While there is extensive knowledge of the enzymes that add or remove specific histone modifications or replace canonical histones with histone variants, it is not understood in any organism how these enzymes are targeted to specific genomic loci. Furthermore, it is not well established how transcriptionally active or repressed chromatin structures are established.

Thus, using trypanosomes to study the formation of chromatin structures, we hope to elucidate some of the very fundamental and evolutionarily conserved mechanisms of chromatin formation, while at the same understanding the role of epigenetics in antigenic variation.

RESEARCH HIGHLIGHTS

Unusually for a eukaryote, *T. brucei* lacks RNA pol II promoter motifs and its genes are arranged in long polycistronic transcription units, a feature that greatly reduces the number of TSSs. Previously, using ChIP-seq, we revealed that TSSs and transcription termination sites are marked by distinct chromatin domains that are strongly enriched in specific histone modification and contain different histone variants. The small number of TSSs in combination with the distinct chromatin signature marking the boundaries of polycistronic transcription units makes this parasite an ideal organism to study the mechanism of targeted histone variant deposition. Therefore, we are currently investigating how, in the absence of DNA sequence motifs in *T. brucei*, the different histone modifications and

histone variants are targeted to specific loci along the genome and what role they play in forming transcriptionally active or repressed chromatin regions across the nucleus.

Beyond chromatin dynamics, our group is interested in post-transcriptional mechanisms of gene regulation in trypanosomes. The organization of genes in long polycistronic transcription units allows for very little regulation at the level of transcription initiation suggesting an important role for post-transcriptional gene regulation, i.e. RNA maturation, stability and translation. However, the relative contribution of these processes to the final steady state levels of proteins is not well understood for any organism. To obtain genome-wide information on protein synthesis, we adapted a ribosome profiling approach to *T. brucei*. This technique is based on high-throughput sequencing of ribosome-protected RNA fragments and enabled us to perform the first genome-wide analysis of RNA translation and translational efficiency in a eukaryotic pathogen. We found translational efficiency to vary greatly between life cycle stages of the parasite and ~100-fold between genes. Currently, we are searching for proteins and sequence motifs that promote or inhibit efficient translation. Importantly, the ability to map ribosome positions at sub-codon resolution also allowed us to differentiate coding from noncoding RNA and enabled us to identify numerous long ncRNA and hundreds of putative small peptides, many of which are regulated in a life-cycle specific manner.

FUTURE DIRECTIONS

We are aiming to combine system-wide approaches such as ChIP-seq, RNAseq and ribosome-profiling with 'classical' biochemical and molecular techniques to establish a comprehensive understanding of chromatin dynamics in trypanosomes, especially at regions involved in antigenic variation. A better knowledge of how gene expression is regulated and different chromatin structures are formed in *T. brucei* should help us understand how the parasite undergoes antigenic variation and may eventually facilitate medical intervention.

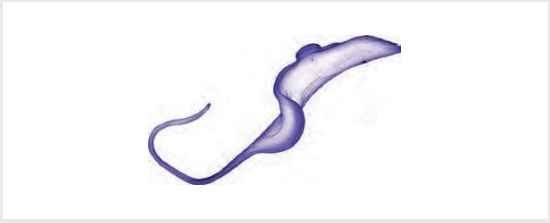


Fig 1: Scanning electron micrograph of a long slender bloodstream form *T. brucei* parasite. (Courtesy of Thierry Blisnick, Philippe Bastin laboratory, Institut Pasteur, Paris.)

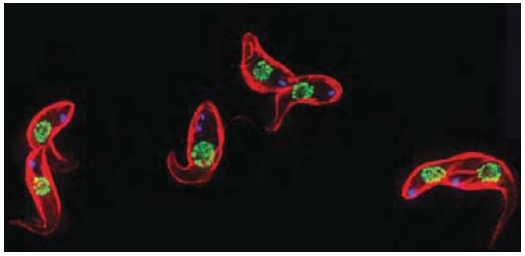


Fig 2: Immunofluorescence analysis of *T. brucei.* Acetylated histone H4K10 (green), tubulin (red) and DNA (blue)changes.

SELECTED PUBLICATIONS

Vasquez JJ, Hon CC, Vanselow JT, Schlosser A, **Siegel TN** (2013) *Comparative ribosome profiling reveals extensive translational complexity in different Trypanosoma brucei life cycle stages.* **Nucleic Acids Research** 42: 3623-3

Nguyen TN, Muller LS, Park SH, **Siegel TN**, Gunzl A (2013) *Promoter occupancy of the basal class I transcription factor A differs strongly between active and silent VSG expression sites in Trypanosoma brucei.* **Nucleic Acids Research** 42: 3164-76

Lopez-Rubio JJ, **Siegel TN,** Scherf A (2013) *Genome-wide chromatin immunoprecipitationsequencing in Plasmodium.* **Methods in Molecular Biology** 923: 321–333

Chene A, Vembar SS, Riviere L, Lopez-Rubio JJ, Claes A, **Siegel TN,** Sakamoto H, Scheidig-Benatar C, Hernandez-Rivas R, Scherf A (2012) *PfAlbas constitute a new eukaryotic DNA/RNAbinding protein family in malaria parasites.* **Nucleic Acids Research** 40: 3066–3077

(e.g. invasion, maturation of the *Salmonella* containing vacuole, replication). In parallel, by performing RNA-seq analysis of the small RNA population of *Salmonella* infected cells, we identified miRNAs that are regulated upon infection. Interestingly, this analysis revealed that some of the miRNAs that potently inhibit infection in our functional screening are downregulated during infection. As an example, we have found that the members of the miR-15 miRNA family inhibit *Salmonella* infection very efficiently and that the expression of miR-15 family members is decreased upon infection. The identification of the targets of these infection-relevant miRNAs has led to the characterization of several host cell cycle proteins as critical regulators of *Salmonella* infection. These findings have uncovered a novel mechanism whereby *Salmonella* renders host cells more susceptible to infection through the active modulation of cellular miRNAs.

Beyond the miRNA pathway, we are also interested in the interplay between bacterial pathogens and RNA granules (P-bodies and stress-granules). By comparing the impact of *Salmonella* Typhimurium and the closely related pathogen *Shigella flexneri* on the integrity of these RNA granules, we have discovered that *Shigella* inhibits the formation of stress-granules in host cells, while both bacteria are able to induce P-body disassembly. These findings reveal a clear interdependence between bacterial infection and host cell RNA metabolism.

FUTURE DIRECTIONS

In the next years we will characterize in detail the role played by selected miRNAs, identified by the combination of high-throughput screening and RNA-seq approaches, in the infection by *Salmonella*. The identification of the targets of these miRNAs is expected to lead to the characterization of other previously unappreciated pathways relevant for the *Salmonella* infection. Furthermore, we plan to apply these approaches to other bacterial pathogens. Overall, we aim to obtain a better understanding of the interplay between bacterial infection and host cell RNA metabolism.

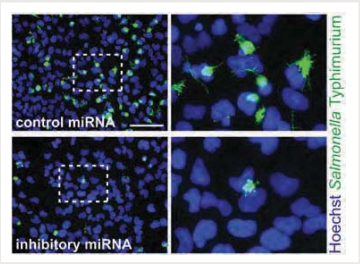


Fig. 1: Overexpression of selected microRNAs inhibits *Salmonella* infection. HeLa cells were transfected with selected microRNAs, followed by infection with *Salmonella* Typhimurium expressing GFP. The regions highlighted by the white squares are enlarged on the rightmost panels.

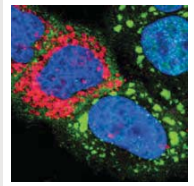


Fig. 2: Shigella infection inhibits stress-granule formation. HeLa cells were infected with *Shigella flexneri* expressing DsRed, and stress-granules were detected by staining the cells with anti-TIAR antibody.

SELECTED PUBLICATIONS

Huntzinger E, Kuzuoglu-Öztürk D, Braun JE, **Eulalio A**, Wohlbold L, Izaurralde E (2013) The interactions of *GW182 proteins with PABP and deadenylases are required for both translational repression and degradation of miRNA targets.* **Nucleic Acids Research** 41:978-994

Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M (2012) *Functional screening identifies microRNAs inducing cardiac regeneration.* **Nature** 492:376-381

Eulalio A, Schulte L, Vogel J (2012) *The mammalian microRNA response to bacterial infections.* **RNA Biology** 9:742-750

PRIZES AND AWARDS

2013 Federation of European Biochemical Societies (FEBS) Distinguished Young Investigator Award

2. YOUNG INVESTIGATORS

2.4. HOST RNA METABOLISM

The main focus of the group is to understand the impact of bacterial infections on host cell RNA metabolism, with a special interest in the microRNA pathway, as well as the reciprocal role of host RNA metabolism on the bacterial life cycle.

DR. ANA EULALIO
BioSysNet

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3189078
M ana.eulalio@uni-wuerzburg.de

INTRODUCTION

During the course of an infection, bacterial pathogens manipulate a vast range of host cellular functions to ensure their survival and replication. Among others, bacterial pathogens are known to induce the reorganization of the host cell cytoskeleton, modulate signal transduction pathways, membrane trafficking and pro-inflammatory responses. However, a largely unexplored question in host-pathogen interactions is the interplay between bacterial infection and RNA metabolism in the host cell. A properly functioning RNA metabolism is essential to a number of crucial host cell processes and therefore it is not surprising that pathogens have evolved sophisticated mechanisms to subvert these pathways for their own benefit.

MicroRNAs (miRNAs) are a class of genome-encoded small RNAs that play a major role in the post-transcriptional control of eukaryotic gene expression, by repressing target transcripts containing complementary binding sites. In addition to their well-established functions in physiological and pathological host processes, it is becoming clear that miRNAs also play crucial roles during infection by different bacterial pathogens. Recent efforts have focused on the identification, in mammalian cells, of miRNAs that are regulated in response to different bacterial pathogens. This analysis revealed a common set of miRNAs as key players in the host innate immune response against bacteria, specifically miR-146, miR-155, miR-21, miR-125 and let-7. These miRNAs were shown to be regulated upon infection with various bacterial pathogens, including *Helicobacter pylori*, *Listeria monocytogenes*, *Francisella tularensis*, *Salmonella enterica* and *Mycobacteria* species. While the expression of several host miRNAs is altered as part of the immune response to bacterial infections, considerably less is known regarding miRNAs that regulate bacterial infections and whether bacterial pathogens are also able to exploit host miRNAs to promote their own intracellular survival and proliferation.

The relationship between bacterial infections and RNA granules, in particular P-bodies (also known as mRNA processing bodies) and stress-granules, is another aspect of the bacterial-host interaction for which very little information is available. Considering that the formation and stability of RNA granules is strictly dependent on the cellular RNA metabolism, any perturbation induced by bacterial pathogens on RNA granules is most likely a consequence of their effect on host RNA metabolism.

RESEARCH HIGHLIGHTS

We have been using systems biology approaches, such as high-throughput screening and RNA-sequencing (RNA-seq), to identify and characterize miRNAs critical for infection with the model bacterial pathogen *Salmonella* Typhimurium, one of the most important causative agents of lethal food-borne diseases.

By performing a functional, high-throughput screening using a genome-wide library of miRNA mimics, we have recently identified 17 miRNAs that decrease *Salmonella* infection by at least 2-fold, and 11 miRNAs that increase *Salmonella* infection by at least 2-fold. These miRNAs affect *Salmonella* infection at different stages of the infection cycle

2. YOUNG INVESTIGATORS

2.5. REGULATORY NETWORKS IN PATHOGENESIS

The group, which was established in 2014, studies the regulatory circuitry that enables the human commensal and opportunistic fungal pathogen, *Candida albicans,* to colonize different niches of the human body. *C. albicans* serves as a model system to gain insights into the general strategies employed by members of our microbiota to proliferate as harmless commensals and how some of these microbes become life-threatening pathogens.

DR. J. CHRISTIAN PEREZ

IZKF–INTERDISCIPLINARY CENTER FOR CLINICAL RESEARCH

Zentrum für Infektionsforschung (ZINF)
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3183815
M christian.perez@uni-wuerzburg.de

INTRODUCTION

Human mucosal surfaces are laden with trillions of microorganisms from all three domains of life. While these microbes are, for the most part, harmless (or beneficial) to us, on occasion some of them can cross the mucosal barriers and cause systemic, potentially fatal infections. In fact, many life-threatening infections in humans are caused by the very same bacterial or fungal species that constitute our own microbiota. Despite their obvious medical importance, little is known about the mechanisms whereby microbes that typically reside within us as commensals can turn into deadly pathogens and cause disease. A fuller understanding of the nature of the interactions with members of our microbiota—opportunistic pathogens in particular—has the potential to open new avenues of intervention for populations at risk of developing infections caused by microbes that spread from our mucosal surfaces into the bloodstream.

Candida albicans is the most prominent fungal species residing in humans and also the major cause of serious fungal infections. *C. albicans* thrives in multiple parts of the human body, including skin, gastrointestinal and genitourinary tracts. The intestine is, however, the most common habitat of this fungus. In fact, the majority, if not all, of healthy adults carry *C. albicans* as part of their regular gut microbiota. On occasion, the fungus spreads from the human gut into the bloodstream and produces deep-seated, systemic infections, particularly in individuals with debilitated immune systems. *Candida* species, for example, are the third most common cause of pediatric health care-associated bloodstream infection in Europe and the United States.

RESEARCH AND FUTURE DIRECTIONS

C. albicans has the ability to proliferate in multiple sites of the human body: skin, mouth, gastrointestinal and genitourinary tracts. This diversity of sites raises the question of whether *C. albicans* uses overlapping or largely independent genetic programs to colonize each niche. We have examined the gene repertoire involved in intestinal colonization and bloodstream infection and plan to incorporate additional mouse models (*e.g.* of oropharyngeal and vulvovaginal candidiasis) to capture the full breadth of niches where this organism proliferates. Combining genetic screens in these animal models with genome-wide molecular biology approaches (RNA-seq, ChIP-seq) will reveal not only genes and cellular functions, but also the strategies that the fungus uses to grow and cause disease in each tissue.

The microorganisms that live in our gastrointestinal tract interact not only with the human host but also with thousands of other microbial species. Global studies of the composition and dynamics of our microbiota indicate that there are strong interdependencies among specific groups of microbes; therefore, it is likely that, just as the status of the immune system of the host influences the severity of *Candida* infections, the composition of the microbiota also plays a role in *C. albicans* proliferation. We plan to use germ-free mice to explore this hypothesis and understand to what extent the regulatory circuitry that *C. albicans* employs to colonize the gastrointestinal tract is designed to cope with, or to rely on, other members of the microbiota.

C. albicans as well as other microbes residing in the human host must be able to continuously gauge their surroundings and adjust their behavior accordingly to prosper. We are interested in understanding which signals, either chemical or physical, denote given environments in the host and, thus, are sensed by the fungus. A first step in this direction is to experimentally determine which sensors and signal transduction pathways feed into *C. albicans* "pathogenesis" genes through the use of reporter genes. The extensive body of knowledge on the signal transduction pathways operating in the model yeasts *S. cerevisiae* and *S. pombe* guide this effort.

Overall we expect that these approaches will provide new insight into the regulatory circuits underlying *C. albicans* proliferation in disparate host niches, the role of the host microbiota in *C. albicans* gut colonization and also the signals sensed by microbes in the host.

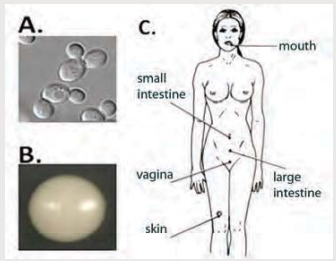


Fig. 1: The yeast *C. albicans* can proliferate in multiple sites of the human body. (A) Morphology of *C. albicans* cells observed under the microscope. (B) Morphology of a *C. albicans* colony grown on standard agar plates. (C) Cartoon illustrating the different sites of the human body where *C. albicans* typically proliferates as a commensal organism. The same yeast can also behave as an aggressive pathogen, colonizing virtually every internal organ and leading to death in as many as 50% of bloodstream infections.

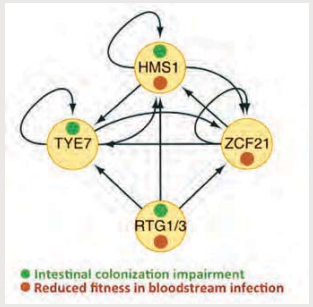


Fig. 2: Core transcriptional circuit governing the ability of *C. albicans* to proliferate in mammalian hosts. Orange circles are transcription regulators; arrows depict protein-DNA interactions as mapped by ChIP. The phenotypes ascribed to each regulator are indicated in green/red. The highly interwoven topology of the circuit suggests that commensalism and pathogenicity are intertwined traits. Modified from Pérez et al., 2013.

SELECTED PUBLICATIONS

Pérez JC, Johnson AD (2013) *Regulatory circuits that enable proliferation of the fungus Candida albicans in a mammalian host.* **PLoS Pathogens** 9:e1003780

Pérez JC, Kumamoto CA, Johnson AD (2013) *Candida albicans commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit.* **PLoS Biology** 11:e1001510

Fraser HB, Levy S, Chavan A, Shah HB, **Perez JC**, Zhou Y, Siegal ML, Sinha H (2012) *Polygenic cis-regulatory adaptation in the evolution of yeast pathogenicity.* **Genome Research** 22:1930-9

Zwir I, Latifi T, **Perez JC**, Huang H, Groisman EA (2012) *The promoter architectural landscape of the Salmonella PhoP regulon.* **Molecular Microbiology** 84:463-85

2. YOUNG INVESTIGATORS

2.6. STRUCTURAL BIOLOGY OF MYCOBACTERIA

Pathogenic mycobacteria have evolved sophisticated mechanisms to subvert the human immune defence. Our group, starting in 2014, aims to understand one of their most important mechanisms - the secretion of proteins by the type VII secretion system.

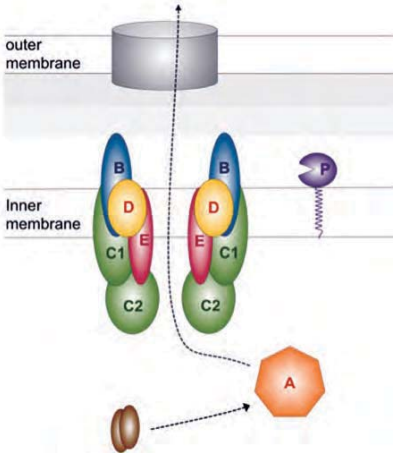


Fig. 1: Working model of the type VII secretion system.

DR. SEBASTIAN GEIBEL
ELITE NETWORK BAVARIA

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3184590
M sebastian.geibel@uni-wuerzburg.de

INTRODUCTION AND FUTURE DIRECTIONS

Tuberculosis is an infectious disease caused by various strains of mycobacteria, which according to recent figures from the World Health Organisation (WHO) accounted for 1.3 million deaths in 2012. Currently about one third of the world's population carries a latent tuberculosis infection, which makes tuberculosis a major health threat.

Mycobacteria are commonly spread through aerosols such as those generated by coughing. Once inhaled, these pathogens are engulfed in the lungs by alveolar macrophages and targeted for destruction. However, mycobacteria can escape this fate through secretion of specific proteins by a major protein secretion pathway, the ESX/type VII secretion system, which comprises of five distinct secretion machines, each forming multi-component nano-machines of immense size that are situated in the cell envelope. Each secretion machine has a differential substrate specificity and is believed to be employed at different stages of the infection. For example, ESX-1 secreted proteins enable mycobacteria to escape the phagolysosome in the macrophage, the compartment in which the bacteria are lysed, while ESX-5 secreted proteins trigger granuloma formation. A granuloma is a cluster of immune cells which mycobacteria exploit as replication niches, or for long term persistence until reactivation causes the outbreak of tuberculosis.

Little is known about the structure and the secretion mechanism of the type VII machinery (see Figure). It has been shown that a set of six conserved proteins EccA-E and MycP are essential for secretion of the type VII substrate. There are four core components EccBCDE which are embedded in the cytoplasmic membrane where they assemble into a stable ~1.5 MDa complex, which is presumably a secretion pore. The protein transport across the type VII secretion system is powered by one or more ATPases (EccA and EccC) of which EccA is located in the cytosol and may be recruited to the type VII core complex upon substrate binding. Protease MycP processes the transported substrate. Once in the periplasm, the protein substrate is possibly secreted by an independent outer membrane secretion system to the extracellular space.

Due to their unambiguous role in virulence, our research will focus on the ESX-1 and ESX-5 secretion machines as models of type VII secretion systems in pathogenic mycobacteria. Our goal is to determine the first three-dimensional structures of these two type VII secretion machines by state-of-the-art structural methods such as cryo-electron tomography, cryo-electron microscopy (in collaboration with the Baumeister group at the Max Planck Institute for Biochemistry, Munich) and X-ray crystallography, permitting functional studies to generate a model of the type VII secretion mechanism.

Overall, this project will improve our understanding of the pathogen-host communication in tuberculosis and has the potential to provide the structural basis for the development of new therapeutics against tuberculosis.

SELECTED PUBLICATIONS

Geibel S, Waksman G (2013) *The molecular dissection of the chaperone-usher pathway.* **Biochemica Biophysica Acta** Oct 16. pii: S0167-4889

Geibel S, Procko E, Hultgren SJ, Baker D, Waksman G (2013) *Structural and energetic basis of folded-protein transport by the FimD usher.* **Nature** 496: 243-246

Rego AT, Johnson JG, **Geibel S**, Enguita FJ, Clegg S, Waksman G (2012) *Crystal structure of the MrkD1P receptor binding domain of Klebsiella pneumoniae and identification of the human collagen V binding interface.* **Molecular Microbiology** 86: 882-893

INSTITUTE FOR MOLECULAR
INFECTION BIOLOGY

INSTITUTE FOR HYGIENE AND MICROBIOLOGY

INSTITUTE FOR VIROLOGY AND
IMMUNOBIOLOGY – CHAIR OF IMMUNOBIOLOGY

INSTITUTE FOR VIROLOGY AND
IMMUNOBIOLOGY – CHAIR OF VIROLOGY

DEPARTMENT OF MICROBIOLOGY,
THEODOR BOVERI INSTITUTE, BIOCENTER

DEPARTMENT OF INTERNAL MEDICINE II

03

INSTITUTIONS OF THE RESEARCH CENTER FOR INFECTIOUS DISEASES

03.1

Institute for Molecular Infection Biology

JÖRG VOGEL

HEIDRUN MOLL

JOACHIM MORSCHHÄUSER

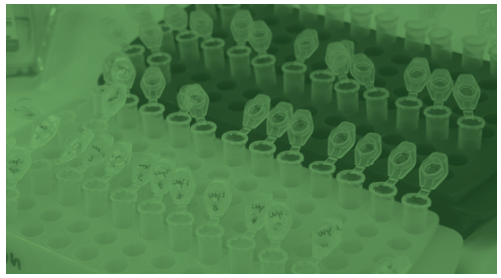
TOBIAS ÖLSCHLÄGER

KNUT OHLSEN

WILMA ZIEBUHR

The Institute for Molecular Infection Biology (IMIB) is an interdisciplinary research institution within the Medical Faculty of the University of Würzburg, with strong links to the Faculty of Biology. IMIB was founded in 1993 and has been chaired by Prof. Dr. Jörg Vogel since 2009.

Members of the institute investigate fundamental biological problems and molecular mechanisms, with a focus on pathogens and infectious disease processes. IMIB research involves the study of bacteria, parasites and fungi, as well as their eukaryotic host, and ranges from bacterial and eukaryotic cell biology and immunology to fundamental aspects of gene regulation and RNA biology. Furthermore, the institute is home to the groups of the prestigious ZINF Young Investigator program of the interfaculty Research Center for Infectious Diseases Research (ZINF). IMIB researchers lecture university seminars and practical courses to biology, medical and dental students.



3.1. INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

3.1.1. RNA BIOLOGY

Non-coding RNAs play important regulatory roles in all domains of life. The group aims to understand the role of ncRNAs and RNA-binding proteins in the host and pathogen in the context of bacterial infections.

PROF. JÖRG VOGEL
INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3182576
M joerg.vogel@uni-wuerzburg.de

INTRODUCTION

The combination of genetic, biochemical and genomic approaches has led to the discovery of a large number of non-coding RNAs with regulatory potential. These molecules include the small regulatory RNAs (sRNAs) of bacteria and numerous classes of small (microRNAs, siRNAs, piRNAs, snoRNAs) and long non-coding RNAs (lncRNAs) in eukaryotes many of whose functions are currently only beginning to be understood. It is now well-established that non-coding RNAs regulate key cellular processes and have a major impact on the outcome of chronic and infectious diseases.

Interestingly, many of the pathogens responsible for the leading number of infectious diseases also contain regulatory RNAs that play an important role in virulence and stress responses involved in pathogenesis. For example, bacterial pathogens are known to express a number of different subclasses of small RNAs including cis-antisense RNAs and the trans-encoded Hfq-dependent sRNAs. The latter sRNAs associate with the RNA chaperone Hfq, which facilitates binding to their target mRNAs by imprecise base-pairing regulating their translation or stability. Importantly, pathogens such as *Salmonella* Typhimurium encode in excess of a hundred sRNAs some of which regulate transcription factors or virulence genes required for pathogenesis.

Mammalian cells are also well established to utilise non-coding RNAs, such as miRNAs, as part of their anti-bacterial response. In addition to miRNAs, recently lncRNAs have also been implicated in infections by regulating the ability of mice to clear persistent bacterial infection. RNA molecules do not exist alone in the cell but soon after synthesis the RNA transcripts associate with a variety of RNA binding proteins (RBP), often to form more complex ribonucleoprotein particles (RNPs). The associated proteins regulate different aspects of RNA metabolism and influence the function and fate of the RNA molecules, or may themselves be the regulated target of a noncoding RNA. In bacteria, the major RNA binding proteins Hfq and CsrA have been shown to be important for virulence, by facilitating the action of small RNAs or being regulated by noncoding RNA antagonists, respectively

RESEARCH HIGHLIGHTS

During the last few years we have been using high-throughput sequencing technology to understand the full complement of coding and non-coding RNA transcripts in bacterial pathogens such as *Salmonella* Typhimurium. This has included using a modified RNA-seq protocol which has enabled the identification of almost all mRNA promoters and also 140 sRNAs in Salmonella, which has increased our ability to functionally annotate the genome. Moreover, an in-depth characterisation of sRNAs that associate with the RNA chaperone Hfq identified dozens of sRNAs that are likely generated for the 3'UTRs of genes, suggesting a potential pathway by which gene expression and sRNA biogenesis may be coupled.

The majority of bacterial sRNAs were thought to regulate the expression of their targets by influencing their translation or enhancing the turnover of the transcript. However, we have described for the first time two examples whereby sRNAs in Salmonella, SgrS and RydC can bind to the turnover products of a transcript and thereby protecting the transcript from further degradation and increasing its steady state level leading to enhanced protein levels and impacting key metabolic processes. Other important findings with *Salmonella* sRNAs, for example, SgrS and DapZ, include unexpected links between metabolic control and virulence gene expression.

Beyond Hfq-associated sRNAs, we are interested in the roles of RNA in the bacterial genome defense system, CRISPR, which confers adaptive, sequence-based immunity against viruses and plasmids using CRISPR-derived RNAs (crRNAs). We have discovered a novel crRNA biogenesis pathway in *Neisseria meningitidis*.

We have also studied the functions of host ncRNAs, where we have described different functions for the co-expressed miR146 and miR-155 in immune sensing. To obtain a deeper understanding of the changes in gene expression during infection, we have been developing a novel approach termed Dual RNA-seq in which we are sequencing the total RNA of infected cells to simultaneously determine the host and pathogen transcriptomes from the initial times during infection to the later stages.

FUTURE DIRECTIONS

In the next few years we are aiming to use Dual RNA-seq to gain a new perspective on RNA expression patterns in infected tissues and single cells. Characterisation of differentially regulated ncRNAs will provide new insight into the molecular interplay between the host and pathogen.

In addition, almost all RNAs in cells are associated with RNA-binding proteins, which affect their stability and function. To understand their role, RNA-seq will be combined with *in vivo* cross-linking methods and proteomics for the de novo discovery of RNA interacting molecules and targets on a global level and with unprecedented resolution. Overall, we aim to obtain a comprehensive view of the role of ncRNAs and RNA binding proteins in bacterial infections.

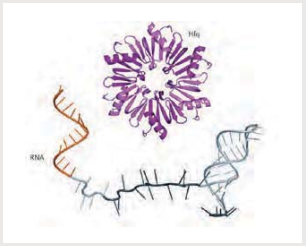


Fig. 1: Hfq and RybB sRNAs.

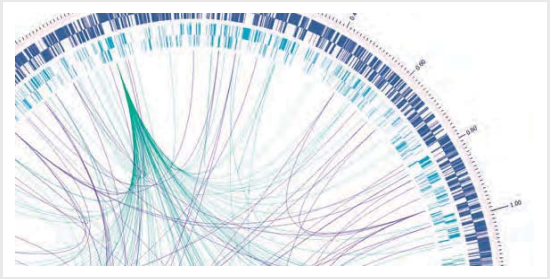


Fig. 2: Interaction network of sRNAs, mRNAs and proteins in *Salmonella* Typhimurium.

SELECTED PUBLICATIONS

Papenfort K, Sun Y, Miyakoshi M, Vanderpool CK, **Vogel J** (2013) *Small RNA-mediated activation of sugar phosphatase mRNA regulates glucose homeostasis.* **Cell** 153:426–437

Fröhlich KS, Papenfort K, Fekete A, **Vogel J** (2013) *A small RNA activates CFA synthase by isoform-specific mRNA stabilization.* **EMBO Journal** 32:2963-79

Zhang Y, Heidrich N, Ampattu BJ, Gunderson CW, Seifert HS, Schoen C, **Vogel J**, Sontheimer EJ (2013) *Processing-Independent CRISPR RNAs Limit Natural Transformation in Neisseria meningitides.* **Molecular Cell** 50:488-503

Papenfort K, Podkaminski D, Hinton JC, **Vogel J** (2012) *The ancestral SgrS RNA discriminates horizontally acquired Salmonella mRNAs through a single G-U wobble pair.* **PNAS** 109:E757-64

Westermann AJ, Gorski SA, **Vogel J** (2012) *Dual RNA-seq of pathogen and host.* **Nature Reviews Microbiology** 10:618-30

PRIZES AND AWARDS

2013 Elected to the German National Academy of Sciences – Leopoldina

2013 Elected to the American Academy of Microbiology

in the mutant mice. In contrast, the macrophage/neutrophil-specific IL-10-deficient mice did not show any altered phenotype. Thus, IL-10 secretion by T cells has an influence on immune activation early after infection and is sufficient to render mice susceptible to *L. major* infection.

Currently, few drugs are available for the treatment of human leishmaniasis. Antimonials, the main chemotherapeutic tool, cause serious side effects and promote chemoresistance. Thus, alternative drugs against leishmaniasis are urgently needed. To this end, our laboratory collaborates with different groups of the Faculty of Chemistry and Pharmacy of the University of Würzburg within the Collaborative Research Center 630 (www.sfb630.uni-wuerzburg.de).

The best approach to identify leishmanicidal compounds is the use of amastigotes residing in macrophages. Reporter gene-based assays are rather new tools in the search for drugs against protozoa, permitting the development of faster, more automated assays. We have established a new rapid screening assay by generating a luciferase-transgenic (Luc-tg) *L. major* strain to be used for infection of macrophages (BMDM). Amastigote-infected BMDM were treated with different compounds. Cells were lysed with a luciferin-containing buffer and the resulting luminescence was measured to determine the half-maximal inhibitory concentration (IC_{50} value). To validate this new amastigote screening assay, a library of a new class of quinolinium salts was synthesized and tested for leishmanicidal activity. Some of the quinolinium salts showed very promising activities, with IC_{50} values and selectivity indices that matched the WHO criteria.

FUTURE DIRECTIONS

In the next few years, we are aiming to test the newly identified immunoprophylactic and immunotherapeutic strategies *in vivo* for their potential to protect mammalian hosts from parasite infections. Furthermore, the molecular mechanisms underlying their effects will be characterized.

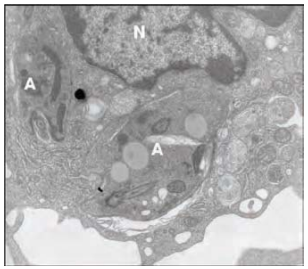


Fig. 1: Transmission electron microscopic image of *Leishmania* amastigotes (A) residing in a macrophage (N, nucleus of macrophage).

SELECTED PUBLICATIONS

Bringmann G, Thomale K, Bischof S, Schneider C, Schultheis M, Schwarz T, **Moll H,** Schurigt U (2013) *A novel Leishmania major amastigote assay in 96-well format for rapid drug screening and its use for the discovery and evaluation of a new class of leishmanicidal quinolinium salts.* **Antimicrobial Agents and Chemotherapy** 57: 3003-3011

Schwarz T, Remer KA, Nahrendorf W, Masic A, Siewe L, Müller W, Roers A, **Moll H** (2013) *T cell-derived IL-10 determines leishmaniasis disease outcome and is suppressed by a dendritic cell-based vaccine.* **PLoS Pathogens** 9: e1003476

Schurigt U, Masic A, **Moll H (2013)** *Interaction of Leishmania parasites with host cells and its functional consequences.* In Trypanosomatid Diseases - Molecular Routes to Drug Discovery, Jäger T, Koch O, Flohé L (eds) pp 105-119. **Wiley-VCH** Weinheim, Germany

Masic A, Hurdayal R, Nieuwenhuizen N E, Brombacher F, **Moll H** (2012) *Dendritic cell-mediated vaccination relies on Interleukin-4 Receptor signaling to avoid tissue damage after Leishmania major infection of BALB/c mice.* **PLoS Neglected Tropical Diseases** 6: e1721

HONOURS, PRIZES AND AWARDS

President of the German Society of Parasitology *(until the end of 2012)*

3.1. INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

3.1.2. INFECTION IMMUNOLOGY

Infectious diseases caused by parasites represent a major worldwide disease burden. The group aims to analyse the interaction between parasites and the host's immune system, to develop novel vaccination strategies, identify new anti-parasitic compounds and to characterize their mode of action.

PROF. HEIDRUN MOLL
INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3182627
M heidrun.moll@uni-wuerzburg.de

INTRODUCTION

Leishmaniasis is considered a tropical affliction that is included in the WHO/Tropical Disease Research list of the six most important diseases, with a sharp increase in the number of recorded cases in recent years. There is a growing interest in leishmaniasis in industrialized countries, due to the importance of travel medicine and the rising incidence of HIV and *Leishmania* co-infections. In addition to these clinical aspects, leishmaniasis represents one of the most important models to define the factors controlling the outcome of infectious diseases by cell-mediated immunity. This experimental system has provided a wealth of information on the immunological mechanisms leading to the restriction or facilitation of pathogen growth, with implications not only for infectious diseases but also for general aspects of immunoregulation.

In contrast to viral and bacterial infections, no vaccines are available to protect humans from parasitic diseases including leishmaniasis and, therefore, control measures rely exclusively on chemotherapy. The current treatments for leishmaniasis are unsatisfactory due to their toxic side effects, expense and the increasing problems with drug resistance. Thus, there is an urgent need to develop novel strategies for the prevention and the treatment of leishmaniasis and other parasite infections.

RESEARCH HIGHLIGHTS

Dendritic cells (DC) are critical determinants of the type of immune response against microbial pathogens. The rapidly expanding knowledge of DC immunobiology has offered new perspectives for the development of vaccines against infectious diseases. Studies in our laboratory have provided the first proof of principle that several distinct DC populations, once properly conditioned *ex vivo*, are indeed able to induce complete protection against *Leishmania major* as a model microbial pathogen. Recently, we investigated the role of interleukin 4 in DC-induced protection. Thus, wild-type (wt) mice or DC-specific IL-4 receptor α (IL-4Rα)-deficient ($CD11c^{cre}$IL-$4R\alpha^{/lox}$) mice were injected with either wt or IL-4Rα-deficient *Leishmania* antigen (LmAg)-loaded bone marrow-derived DC exposed or not to CpG oligodeoxynucleotides (CpG ODN) prior to infection with *L. major* promastigotes. The results show that IL4/IL-4Rα-mediated signaling in the vaccinating DC is required to prevent tissue damage at the site of *L. major* inoculation, as properly conditioned wt DC but not IL-4Rα-deficient DC were able to confer resistance. Furthermore, uncontrolled *L. major* expansion was observed in $CD11c^{cre}$IL-$4R\alpha^{/lox}$ mice immunized with CpG ODN-exposed LmAg-loaded IL-4Rα-deficient DC, indicating the influence of IL-4Rα-mediated signaling in host DC to control parasite replication.

Furthermore, since the immunosuppressive effects of IL-10 have been ascribed a crucial role in the development of the different clinical correlates of *Leishmania* infection in humans, we have compared leishmaniasis disease progression in T cell-specific, macrophage/neutrophil-specific and complete IL-10-deficient mice. As early as two weeks after infection of these mice with *L. major*, T cell-specific and complete IL-10-deficient animals showed significantly increased lesion development accompanied by a markedly elevated secretion of IFN-γ or IFN-γ and IL-4 in the lymph nodes that drain the lesions

result in constitutive overexpression of their target genes. By constructing strains containing such mutations in all possible combinations, we have demonstrated that each of these mechanisms contributes to drug resistance, that resistance is increased after loss of heterozygosity for a mutated allele, and that different resistance mechanisms have additive effects and their combination results in highly resistant strains, a phenomenon that explains how clinically relevant resistance evolves. On the other hand, we have also shown that the deregulated gene expression in strains with constitutively active transcription factors resulted in decreased fitness in the absence of the drug. In a mouse model of gastrointestinal colonization, a strain with hyperactive forms of Upc2, Tac1, and Mrr1 was rapidly outcompeted by an isogenic wild-type strain, while the reverse pattern was seen when the mice were treated with fluconazole (Fig. 2).

Upc2, Tac1, and Mrr1 are members of the zinc cluster transcription factor family, which is unique to fungi. We have devised a method for the artificial activation of these transcription factors and generated a library of *C. albicans* strains expressing all of its zinc cluster transcription factors in a potentially hyperactive form. By screening this library we have discovered previously unknown transcription factors that induce the transition to invasive hyphal growth or confer increased resistance to drugs, immune defence mechanisms, and other stress conditions that are relevant within the host. As *C. albicans* frequently acquires natural gain-of-function mutations in these transcriptional regulators under selective pressure, our library of strains containing hyperactive zinc cluster transcription factors can be used to probe the evolutionary potential of this fungus when it faces novel environmental challenges.

FUTURE DIRECTIONS

We are currently investigating how specific transcription factors and signaling pathways promote morphological transitions and resistance to adverse conditions. In addition, we will investigate if *C. albicans* uses sexual recombination to combine different resistance mechanisms and if and how drug-resistant *C. albicans* strains can overcome the costs of resistance by further evolution.

SELECTED PUBLICATIONS

Ramírez-Zavala B, Weyler M, Gildor T, Schmauch C, Kornitzer D, Arkowitz R, **Morschhäuser J** (2013) *Activation of the Cph1-dependent MAP kinase signaling pathway induces white-opaque switching in Candida albicans.* **PLoS Pathogens** 9:e1003696

Schillig R, **Morschhäuser J** (2013) *Analysis of a fungus-specific transcription factor family, the Candida albicans zinc cluster proteins, by artificial activation.* **Molecular Microbiology** 89:1003-1017

Dunkel N, Hertlein T, Franz R, Reuß O, Sasse C, Schäfer T, Ohlsen K, **Morschhäuser J** (2013) *Role of different peptide transporters in nutrient acquisition in Candida albicans.* **Eukaryotic Cell** 12:520-528

Sasse C, Hasenberg M, Weyler M, Gunzer M, **Morschhäuser J** (2013) *White-opaque switching of Candida albicans allows immune evasion in an environment-dependent fashion.* **Eukaryotic Cell** 12:50-58

Sasse C, Dunkel N, Schäfer T, Schneider S, Dierolf F, Ohlsen K, **Morschhäuser J** (2012) *The stepwise acquisition of fluconazole resistance mutations causes a gradual loss of fitness in Candida albicans.* **Molecular Microbiology** 86:539-556

Fig. 1: The white and opaque forms of *C. albicans*. The left picture shows the appearance of colonies formed by white and opaque cells on agar plates. Opaque colonies are selectively stained pink by the dye phloxine B. The fluorescence micrograph on the right shows a mixture of white and opaque cells of a strain that expresses *RFP* from a white-phase-specific promoter (white cells appear red) and *GFP* from an opaque-phase-specific promoter (opaque cells appear green).

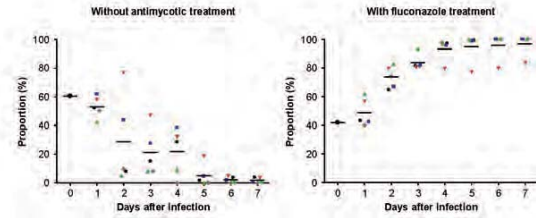


Fig. 2: Relative in vivo fitness of a strain that contains gain-of-function mutations in *UPC2, TAC1,* and *MRR1* in competition with its wild-type parental strain. In vivo competition experiments were performed in mice which were treated (right panel) or not treated (left panel) with fluconazole. The proportion of the drug-resistant strain in the inoculum (time point 0, empty circle) and in samples recovered at the indicated times from the feces of orally infected animals is given. Each individual mouse in a group is represented by a different symbol. The horizontal bars represent the mean values for each group of mice and day.

3.1. INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

3.1.3. MYCOLOGY

The yeast *Candida albicans* is one of the most important fungal human pathogens. Our group studies the regulation of gene expression, switching between different cell morphologies, and genomic alterations to understand how *C. albicans* adapts to different host environments.

PROF. JOACHIM MORSCHHÄUSER
INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3182152
M joachim.morschhaeuser@uni-wuerzburg.de

INTRODUCTION

Infections by opportunistic pathogenic fungi have become a major medical problem in the past few decades, due to the increasing number of immunocompromised patients who are highly susceptible to such infections. The yeast *Candida albicans* is a harmless commensal in most healthy people, but it can also cause mucosal as well as life-threatening systemic infections. A variety of virulence-associated characteristics contribute to the capacity of *C. albicans* to colonize and infect many different body locations, including the switching between different morphologies and the metabolic adaptation to the nutritional requirements in diverse host niches. In addition, *C. albicans* can generate genetic variants that are better adapted to permanent alterations in the host environment, as exemplified by the emergence of drug-resistant strains during antimycotic therapy. Within our group we are studying the regulation of morphogenesis and other virulence traits, the role of nutrient sensing and acquisition systems in pathogenicity, and the evolution of drug resistance in *C. albicans* to better understand how this important human fungal pathogen adapts to different niches and altered environmental conditions during colonizaton and infection.

RESEARCH HIGHLIGHTS

C. albicans can switch from the normal yeast morphology (white) to an elongated cell type (opaque), which is the mating-competent form of this fungus (Fig. 1). Opaque cells are less virulent than white cells, but they can also avoid recognition by macrophages and neutrophils under conditions in which white cells are efficiently phagocytosed. Therefore, switching to the opaque phase not only results in the acquisition of mating competence but may also allow escape from the immune system. White-opaque switching occurs spontaneously at a relatively low frequency, but it can also be induced by certain environmental signals. To identify signaling pathways that stimulate white-opaque switching, we have generated comprehensive tetracycline-inducible gene expression libraries encompassing all transcription factors, protein kinases, and phosphatases encoded in the *C. albicans* genome. Screening of these libraries resulted in the identification of novel regulators of white-opaque switching, which provided important clues about conditions that induce the transition to a mating-competent state in *C. albicans* to allow the exchange of genetic information.

C. albicans can develop resistance to the widely used antifungal drug fluconazole, which inhibits ergosterol biosynthesis. Fluconazole resistance is caused by different mechanisms, including mutations in the *ERG11* gene encoding the drug target enzyme, overexpression of *ERG11* and other ergosterol biosynthesis genes, and upregulation of multidrug efflux pumps that transport fluconazole and other toxic compounds out of the cell. The transcription factors Upc2, Tac1, and Mrr1, which regulate the expression of ergosterol biosynthesis genes and different drug transporters, respectively, play a key role in the development of drug resistance. Many fluconazole-resistant, clinical *C. albicans* isolates have acquired gain-of-function mutations in these transcription factors that

Finally, we have been investigating if EcN is able to provide protection against all classical EHEC serotypes, the so called 'Big Five', which are EHECs of serotypes O157:H7, O26:H11, O103:H2, O111:H-, O145:H4 and also the rather new serotype O104:H4 (EAHEC). EcN inhibits the growth of all the EHECs/EAHEC tested, although to a different degree depending on the serotype. EcN also inhibits the adhesion of the "Big Five" and of EAHEC O104:H4 to non-mucus producing Caco-2 cells and mucus producing LS174T cells. Most importantly, Stx production of all EHEC and EAHEC strains tested was significantly reduced in the presence of EcN. These anti-EHEC/EAHEC antagonistic activities are independent of live host cells, because EHEC/EAHEC adhesion to epithelial cells is also reduced to glutaraldehyde-fixed epithelial cells and growth and Stx-production is inhibited in the absence of any host cells.

FUTURE DIRECTIONS

We are aiming to identify the factors involved in the antagonistic activity of EcN against EHEC/EAHEC and elucidating their mechanisms of action. We are using a comparative transcriptome approach to identify these factors. As a prerequisite, we have recently sequenced the genome of EcN in collaboration with GenXPro. Once the most highly regulated genes in EcN in the presence of EHEC/EAHEC are identified, we will use a variety of genetic and molecular biology based approaches to understand the function of the respective protein.

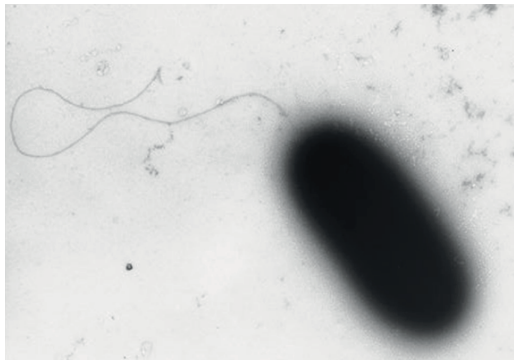


Fig. 1: Transmission electron micrograph of the monotrichously flagellated *E. coli* Nissle 1917.

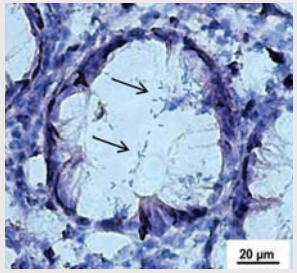


Fig. 2: Light microscopic image of a human gut biopsy cryosection with *E. coli* Nissle 1917 bacteria adhering to a cross-section of a crypt. Arrows point to adhering bacteria.

SELECTED PUBLICATIONS

Rund SA, Rohde H, Sonnenborn U, **Oelschlaeger TA** (2013) *Antagonistic effects of probiotic Escherichia coli Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7.* **International Journal of Medical Microbiology** 303:1-8

Becker S, **Oelschlaeger TA**, Wullaert A, Vlantis K, Pasparakis M, Wehkamp J, Stange EF, Gersemann M (2013) *Bacteria regulate intestinal epithelial cell differentiation factors both in vitro and in vivo*. **PLoS One** 8:e55620

Troge A, Scheppach W, Schroeder BO, Rund SA, Heuner K, Wehkamp J, Stange EF, **Oelschlaeger TA** (2012) *More than a marine propeller - the flagellum of the probiotic Escherichia coli strain Nissle 1917 is the major adhesin mediating binding to human mucus.* **International Journal of Medical Microbiology** 302:304-14

Seo EJ, Weibel S, Wehkamp J, **Oelschlaeger TA** (2012) *Construction of recombinant E. coli Nissle 1917 (EcN) strains for the expression and secretion of defensins.* **International Journal of Medical Microbiology** 302:276-87

3.1. INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

3.1.4. ENTEROBACTERIA

The species *Escherichia coli* contains pathogenic, commensal as well as probiotic strains. The group aims to understand the molecular basis of the *E. coli* strain Nissle 1917's (EcN) anti-bacterial properties by studying the interaction between EcN and pathogenic bacteria such as enterohaemorrhagic *E. coli* (EHEC).

DR. TOBIAS ÖLSCHLÄGER
INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3182150
M t.oelschlaeger@uni-wuerzburg.de

INTRODUCTION

The species *Escherichia coli* contains not only commensal but also highly pathogenic and even probiotic strains. All *E. coli* share a common core genome, which facilitates the identification of genes that are specific to pathogenic or probiotic *E. coli* strains. The significance of *E. coli* as a pathogen was again highlighted in 2011 by the outbreak in Germany of an epidemic caused by shiga toxin (Stx) producing enteroaggregative *E. coli* (EHEC) of serotype O104:H4. This outbreak resulted in about 2800 O104:H4 infected people and left more than 50 patients dead. Unfortunately, there is no specific therapy available to treat infections caused by EHEC. Antibiotic treatment is not recommended, because this has been shown to increase the production and release of Stx, which exacerbates the symptoms of the patient. An effective antibiotic-independent therapy is therefore urgently needed. One such therapy could be provided by the probiotic EcN which is already licensed as a pharmaceutical under the trade-name Mutaflor and used for the treatment of ulcerative colitis and diarrhoea. Mutaflor has been in use for many decades and has achieved GRAS (generally recognized as safe) status. However, its mode of action is still not well understood. Its effectiveness in keeping patients with ulcerative colitis in remission seems to be dependent on its ability to induce human alpha-defensin 2 production in human enterocytes via its flagellin FliC. In contrast, EcN is not effective in the treatment of Crohn's Disease patients. While initial reports of *EnN's* antagonistic effects against the classical EHEC serotype O157:H7 appeared in 2008, it remained unclear, if EcN might also be able to interfere with growth and adhesion of and, most importantly, with Stx expression in the relatively newly evolved Stx-producing enteroaggregative *E. coli* (EHEC) of serotype O104:H4, also named EAHEC.

RESEARCH HIGHLIGHTS

Previously, in collaboration with the group of Klaus Fellermann, we have shown that EcN uses its flagellum not only for mobility but also to induce beta-defensin production in human enterocytes. Recently, we have described another role for the EcN's flagellum by revealing its functions as an adhesin mediating adhesion to cryosections of human gut biopsies, porcine mucin 2 and human but not mouse mucus. This adherence is independent of type 1, F1C and curli fimbriae but involves the EcN's flagellum, which recognises gluconate. Surprisingly, the domain D3 of flagellin is not the domain responsible for binding to gluconate, even though this domain is the most surface exposed. Most likely it is domain D2, which is normally covered by D3 in the wild-type FliC that binds to gluconate, since a D3-deletion mutant, which would uncover D2 binds to gluconate with higher affinity than wild-type flagella.

The fact that EcN is not therapeutically effective in Crohn's Disease patients seems to be caused by the inability of the patients to produce enough defensins due to genetic deficiencies. We have therefore constructed a recombinant EcN strain, which can be induced to synthesize and secrete biologically active human alpha-defensin 2. This strain represents a first important step in the development of a recombinant EcN strain for the treatment of Crohn's Disease patients.

role of these enzymes. The analysis of knock-out mutants and kinase/phosphatase over-expressing strains has uncovered important functions of PknB/Stk and Stp as modulators of cell wall structure and susceptibility to cell wall-acting antibiotics such as certain β-lactams (Fig. 1). Furthermore, transcriptional profiling and phosphoproteome studies have revealed an important role of PknB/Stk in regulating the expression of genes associated with basic metabolic processes. Interestingly, PknB is able to phosphorylate WalR, the response regulator of a classical two-component signal transduction system.

Moreover, we are developing *in vivo* imaging technologies to visualize the dynamics of staphylococcal infections. In order to monitor phagocytic immune cells spreading to the site of infection in a native context we are collaborating with the Physics department to establish novel *in vivo* imaging techniques based on nanoparticles as contrast agents (Fig. 2). In addition, targeted fluorescent imaging (FLI) has been recently used for the first time to detect severe invasive *S. aureus* bacterial infections on medical implants.

FUTURE DIRECTIONS

We are extending our research to understand the role of PknB-dependent phosphorylation during infection. Quantitative phosphoproteomics will be used to detect infection-related changes of the host phosphoprotein networks to learn more about manipulation of host signaling pathways by the pathogen. Furthermore, several *in vivo* imaging techniques will be applied to answer questions related to the spread of *S. aureus* in different disease models and the specific response of the host immune system by tracking the migration of immune cells. Finally, we aim to develop antibody-based immunotherapy for clinical application by combining several monoclonal antibodies with different modes of action.

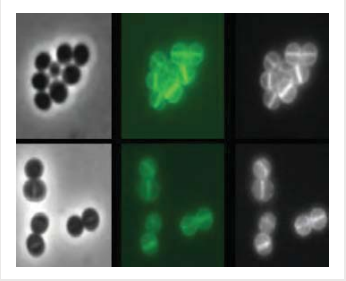


Fig. 1: Fluorescence micrograph of GFP-labelled PknB (upper part) and WalR (lower part) shows localization of PknB and WalR near the septum.

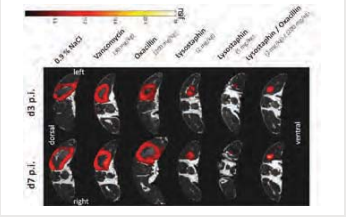


Fig. 2: Two-dimensional 19F magnetic resonance (MR) overlays on anatomical 1H MR images indicate site of *S. aureus* infection and treatment efficacy of different antibiotics. Mice received a single intravenous injection of perfluorocarbon emulsion at day 2 post-infection, and three-dimensional MR measurements were performed on day 3 (top row) and day 7 (bottom row) post-infection.

SELECTED PUBLICATIONS

van Oosten M, Schäfer T, Gazendam JA, **Ohlsen K**, Tsompanidou E, de Goffau MC, Harmsen HJ, Crane LM, Lim E, Francis KP, Cheung L, Olive M, Ntziachristos V, van Dijl JM, van Dam GM (2013) *Real-time in vivo imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labelled vancomycin.* **Nature Communications** 4:2584

Hertlein, T, Sturm V, Jakob P, **Ohlsen K** (2013) *19F Magnetic Resonance Imaging of perfluorocarbons for the evaluation of response to antibiotic therapy in a Staphylococcus aureus infection model.* **PLoS One** 8(5):e64440

Yu W, Leibig M, Schäfer T, Bertram R, **Ohlsen K**, Götz F (2013) *Mevalonate auxotrophic mutant of Staphylococcus aureus can adapt to mevalonate depletion.* **Antimicrobial Agents and Chemotherapy** 57:5710-3

Rakette S, Donat S, **Ohlsen K**, Stehle T (2012) *Structural Analysis of Staphylococcus aureus Serine/Threonine Kinase PknB.* **PLoS One** 7:e39136

Tischer M, Pradel G, **Ohlsen K**, Holzgrabe U (2012) *Quaternary ammonium salts and their antimicrobial potential: targets or nonspecific interactions?* **ChemMedChem** 2;7:22-31

3.1. INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

3.1.5. GRAM-POSITIVE COCCI

Gram-positive pathogens are the leading cause of hospital-acquired infections. The aim of our work is to unravel the regulatory processes that lead to pathogenicity, to visualize the dynamics of infections and to develop novel therapeutic strategies against *Staphylococcus aureus.*

DR. KNUT OHLSEN
INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3182155
M knut.ohlsen@uni-wuerzburg.de

INTRODUCTION

Staphylococci are the most common causative agents of nosocomial infections. In particular, *Staphylococcus aureus* is able to cause a broad spectrum of diseases ranging from the formation of mild abscess to life threatening infections such as septicemia, endocarditis, pneumonia and osteomyelitis. This pathogen expresses an extraordinary number of virulence traits including leukotoxins, hemolysins, adhesins and degradation enzymes. Importantly, the difficulty in eradicating *S. aureus* infections is compounded by the bacterium's ability to acquire new antibiotic resistance determinants that favour its survival in the highly competitive hospital environment and the decline in therapeutic options due to the emergence of new types of multiple resistant strains. Methicillin-resistant *S. aureus* (MRSA) has emerged as serious threat during the past two decades in both health care facilities and the community at large. As a consequence it is likely that new strategies will be required to combat the pathogen. We have focused our research on the factors and processes that are associated with the pathogenesis of staphylococcal diseases and contribute to the establishment of these bacteria in the hospital environment. A major goal of our group is the development of immunotherapies and the identification of new potential antibiotics in order to combat staphylococcal infections, this also includes the identification of novel targets for antibiotic therapy. Moreover, we are developing in vivo imaging technologies to visualize the dynamics of staphylococcal infections and the efficacy of novel antistaphylococcal agents.

RESEARCH HIGHLIGHTS

The emergence of multiple antibiotic resistant staphylococcal strains coupled with the severe associated clinical outcomes provide a strong rationale for the development of immunoglobulin-based therapeutic strategies. Traditionally, novel immunological approaches against bacterial pathogens require the generation of antibodies directed against cell surface exposed virulence-associated epitopes or toxins. However, while monoclonal antibodies targeting immunodominant antigens expressed *in vivo* during infection triggered the patient`s immune system to produce antibodies, the amount of those antibodies was not sufficient to clear the infection. Therefore, we have developed a passive immunotherapy strategy based on immunodominant antigens. One of the target antigens is a soluble lytic transglycosylase of *S. aureus.* We have developed a humanized antibody which recognizes all *S. aureus* strains tested including hospital-acquired and community-acquired methicillin-resistant *S. aureus* strains. The therapeutic efficacy of this strategy has been validated in experimental mouse infection models. Importantly, the monoclonal antibody activates professional phagocytes and induces highly microbicidal reactive oxygen metabolites in a dose-dependent manner, resulting in bacterial killing also in patients who are prone to staphylococcal infections.

In addition we are interested in regulation of cellular functions by eukaryotic like Serine/Threonine kinases and phosphatases. A major focus of our work, as part of the transregional collobarative research center 34 (TR-SFB34) is to understand the cellular

3.1. INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

3.1.6. NOSOCOMIAL INFECTIONS BY STAPHYLOCOCCI

Staphylococci are common causes of hospital-acquired (nosocomial) infections. The research of the group focuses on the factors and processes that contribute to the establishment of these bacteria as pathogens in the hospital environment and beyond.

DR. WILMA ZIEBUHR

INSTITUTE FOR MOLECULAR INFECTION BIOLOGY

Institut für Molekulare Infektionsbiologie
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3182154
M w.ziebuhr@mail.uni-wuerzburg.de

INTRODUCTION

Staphylococci are primarily commensals residing on the skin and mucosa of humans and animals; but they also represent important pathogens causing a broad variety of diseases. The most prominent species in this respect is *S. aureus* which is armed with an array of highly potent virulence factors and which causes infections in both the hospital setting and in the community. In contrast, the large group of coagulase-negative staphylococci (CoNS) has generally a lower pathogenic potential and endangers mainly immunocompromised individuals in hospitals. CoNS are typical opportunistic pathogens associated with medical progress as the vast majority of infections are linked to the use of indwelling medical implants on which these bacteria form biofilms.

Antibiotic resistance is a common theme both in *S. aureus* and CoNS. Thus, methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant CoNS (MR-CoNS) are widespread. As a matter of concern, methicillin-resistance is often accompanied by antibiotic resistance to other, unrelated antibiotic groups and classes, significantly limiting our opportunities to fight such infections. Health authorities have identified multiresistant staphylococci as a serious threat to the public. While in the recent past MRSA occurrence was restricted to the health care setting, specific MRSA lineages are now also present in the community. Most recently, a novel MRSA clone emerged which is adapted to animals and spreads rapidly in livestock and pets.

Recent progress in genome research has provided exciting novel insights into the biology of staphylococci, demonstrating that these versatile microorganisms can adapt very rapidly to changing environmental conditions. We have a strong interest in teaming basic research with public health aiming at an in-depth understanding of staphylococcal infections and laying the basis for future innovative prevention and treatment strategies. Currently, we focus on regulatory networks linking staphylococcal metabolism and virulence. Also, we investigate the mechanisms of biofilm formation on medical devices, which significantly contributes to therapy recalcitrance of CoNS infections; and finally, we study the role of CoNS as reservoirs for the evolution and spread of novel antibiotic resistance genes.

RESEARCH HIGHLIGHTS

Bacterial metabolism is key to understanding the lifestyle of microorganisms that balance between commensalism and virulence. In a recent project, we set out to characterize the so far poorly understood regulation of de novo methionine biosynthesis in staphylococci. N-formyl methionine is the universal N-terminal amino acid of prokaryotic proteins, making methionine indispensable for bacterial growth. We identified a unique hierarchical control pathway integrating complex metabolic circuits with a so-called T-box riboswitch and RNA decay. The T-box family of riboswitches represent transcription termination control systems which bind cognate tRNAs as effector molecules. Interestingly, the T-box riboswitch residing upstream of the staphylococcal methionine biosynthesis operon specifically interacts with uncharged initiator $tRNA_i^{fMet}$, an unexpected finding

whose exact biological function remains to be determined. We see our results in the light of the increasing antibiotic resistance development in staphylococci and hypothesize that methionine biosynthesis might represent a promising future antibiotic target.

Life in biofilms is another typical hallmark of staphylococci, notably of CoNS species such as *Staphylococcus epidermidis*. Biofilms are highly organised structures consisting of bacterial communities engulfed in a self-produced extracellular matrix. Biofilms have been proposed to functionally resemble a multicellular organism in terms of heterogeneous gene expression patterns. In a changing environment, this heterogeneity is likely to facilitate persistence and survival of the population as a whole, but may also support division of labour and maintenance of the biofilm structure. In various projects, we study the regulatory networks controlling biofilm formation as well as factors that trigger metabolic heterogeneity and genetic adaptation. Through dual RNA-sequencing approaches, we have identified differentially expressed metabolic genes and regulators. Currently, we are studying the co-existence as well as the spatial and quantitative distribution of these variants in *S. epidermidis* biofilms by confocal laser scanning microscopy.

FUTURE DIRECTIONS

In the near future we will continue and focus our research on the association between bacterial metabolism and virulence. By combining various 'omics' approaches with genetic and structural studies we will specifically address methionine biosynthesis regulation and its putative role in protein translation initiation control. In addition, we will analyze the biological significance of metabolic heterogeneity on long-term survival and stress resistance. With these data we hope to underpin theoretical models for describing phenotypic variation and differentiation in bacterial communities. Finally, we will investigate the influence of antibiotic resistant CoNS species on the persistence and dissemination of livestock-associated MRSA in animal husbandry.

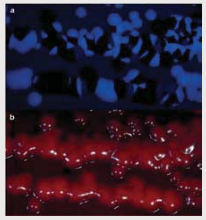


Fig. 1: Heterogeneous expression of a regulatory RNA in *S. epidermidis* colonies. (a) The effect is visualized by a promoter fusion of the regulator with the blue-fluorescent protein Cerulean. (b) Phenotype of the colonies on Congo red agar.

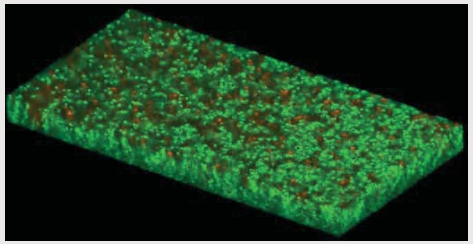


Fig. 2: Confocal laser microscopy and 3D-reconstruction of a *S. epidermidis* biofilm after live/dead staining. Live cells appear in green and dead bacteria in red.

SELECTED PUBLICATIONS

Schoenfelder SMK, Marincola G, Geiger T, Goerke C, Wolz C, **Ziebuhr W** (2013) *Methionine Biosynthesis in Staphylococcus aureus is Tightly Controlled by a Hierarchical Network Involving an Initiator tRNA-specific T-box Riboswitch.* **PloS Pathogens** 9:e003606

Makgotlho PE, Marincola G, Schäfer D, Liu Q, Bae T, Geiger T, Wasserman E, Wolz C, **Ziebuhr W,** Sinha B (2013) *SDS Interferes with SaeS Signaling of Staphylococcus aureus Independently of SaePQ.* **PLoS One** 8:e71644. doi: 10.1371

Hilmi D, Parcina M, Bode K, Ostrop J, Schuett S, Heeg K, **Ziebuhr W**, Sommerburg O, Bekeredjian-Ding I (2013) *Functional variation reflects intra-strain diversity of Staphylococcus aureus small colony variants in the host-pathogen interaction.* **International Journal of Medical Microbiology** 303:61-9

Papenfort K, Corcoran CP, Gupta SK, Miyakoshi M, Heidrich N, Chao Y, Fröhlich KS, Sharma CM, Ziebuhr W, Böhm A, Vogel J (2013) *Regulatory Mechanisms of Special Significance: The role of sRNAs in Virulence.* In: **Regulation of Bacterial Virulence** (eds. Darwin AJ, Vasil ML), ASM Press, pp493-527

Ziebuhr W, Flückiger U (2012) *Coagulase-negative Staphylococci.* In: **Hospital Epidemiology and Infection Control**, Lippincott Williams & Wilkins, Philadelphia, PA p443-462

03.2

Institute for Hygiene and Microbiology

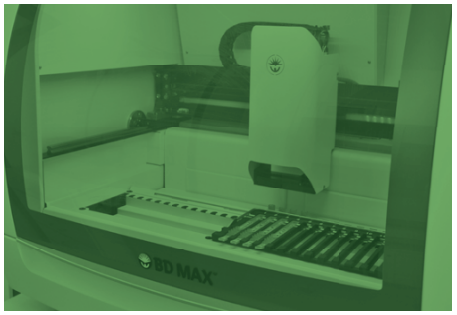
MATTHIAS FROSCH

KLAUS BREHM

CHRISTOPH SCHOEN

ALEXANDRA SCHUBERT-UNKMEIR

ULRICH VOGEL



The Institute for Hygiene and Microbiology (IHM) is part of the Medical Faculty at the University of Würzburg. The institute has been chaired by Prof. Dr. Matthias Frosch since 1996.

The IHM is responsible for the diagnosis of infectious diseases caused by bacteria, fungi and parasites, in addition to advising clinicians on the treatment and prevention of these diseases. Research activity within IHM focuses on the molecular mechanisms involved in the pathogenesis of various infectious diseases. The institute houses the National Reference Centre for Meningococci (HRZM) and coordinates the European Centre for Disease Control (ECDC) program "Laboratory surveillance and external quality assurance of invasive bacterial diseases in EU – IBD-labnet". The latter represents an EU wide network monitoring *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* infections. IHM also hosts the National Reference laboratories for meningococci and *Haemophilus influenzae* and the consiliary laboratory for echinococcosis. Teaching includes students of medicine, dentistry and related subjects.

3.2. INSTITUTE FOR HYGIENE AND MICROBIOLOGY

3.2.1. MOLECULAR SURVEILLANCE OF INVASIVE BACTERIAL INFECTIONS

Matthias Frosch is the chair of the Institute for Hygiene and Microbiology (IHM). The main tasks of the IHM are (i) research on infectious diseases and their causative agents, (ii) the laboratory diagnosis of infectious diseases caused by bacteria, fungi and parasites, (iii) the provision of advice to clinicians with respect to diagnosis, therapy and prevention of infectious diseases, (iv) hospital hygiene as well as (v) providing teaching and training for students of medicine, dentistry and related subjects.

PROF. MATTHIAS FROSCH
INSTITUTE FOR HYGIENE AND MICROBIOLOGY
BIOLOGY

Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/E1
D-97080 Würzburg
T +49 (0)931-3146160
M mfrosch@hygiene.uni-wuerzburg.de

INTRODUCTION

The genomic and phenotypic diversity of bacterial isolates within a species is the focus of many research activities at the IHM. There is increasing evidence, that this diversity of pathogenic bacteria is key to understanding the complex interplay between pathogen and host and the correlations between certain bacterial finetypes and the clinical presentation, progression and outcome of infectious diseases.

Neisseria meningitidis, the meningococcus, is a paradigm of a commensal and pathogenic bacterium, many variants of which colonise the human nasopharynx. However, only a relatively small number of these variants, known as hypervirulent and hyperinvasive types, are associated with severe invasive and often lethal disease. Several groups at the IHM focus on deciphering the basis for virulence, analysing the complex interaction of commensal and virulent meningococci with the human host and developing tools for their identification and typing. The reports of the ZINF members Ulrich Vogel, Alexandra Schubert-Unkmeir and Christoph Schoen, working at the IHM, illustrate these research projects in more detail. Their research is stimulated by access to meningococcal strains collected and typed at the National Reference laboratory for Meningococci and *Haemophilus influenzae* (NRZMHi), which is hosted by the institute, and also access to clinical specimens from the IHM diagnostic laboratories that are involved in the diagnosis of infectious diseases in patients at the University Hospital and other local hospitals. Vice versa, these diagnostic laboratories also benefit from the achievements of the research groups, such as when new methods for pathogen detection and typing become available. This is especially true for the hospital hygiene laboratory, where an increasing number of molecular and genome-based detection and typing methods have been introduced for nosocomial pathogens, for the analysis of transmission traits and in the surveillance of pathogens, providing early warning systems for the prevention of outbreaks.

RESEARCH HIGHLIGHTS

In addition to the work in the diagnostic laboratories and especially in the National Reference laboratory for meningococci and *Haemophilus influenzae*, which includes the identification and molecular typing of infectious agents, providing advice to public health institutions in case management, there is also a great interest in continuously improving laboratory surveillance of the diseases in collaboration with public health authorities at the European level. This has resulted in the development of new typing tools as well as in scientific studies on the population biology of the infectious agents.

Transnational collaborations between European Reference laboratories have been further promoted on behalf of the ECDC (European Centre for Disease Prevention and Control). Within this framework a laboratory network (IBD-labnet) comprising of all Reference laboratories on *Neisseria meningitidis, Haemophilus influenzae* and *Streptococcus pneumoniae* in the EU Member States is coordinated by the IHM and aims to harmonize laboratory surveillance of invasive bacterial diseases. The goal is to improve laboratory capacities to accurately characterize these invasive bacterial isolates. To fulfill this task, the IHM assists the participating National Reference laboratories to continuously improve their laboratory performance on the identification and characterization of *N. meningitidis, H. influenzae* and *S. pneumoniae* as well as the implementation of new techniques for routine diagnosis and typing. Moreover, and as a joint effort of all participating European National Reference laboratories for meningococci, the IHM together with the Norwegian Institute of Public Health (NIPH) has contributed to the establishment of a centralised European Meningoccal Strain Collection (EMSC). The EMSC currently comprises roughly 1200 meningococcal invasive isolates obtained from 17 European countries. The purpose of the EMSC is to provide a sustainable infrastructure for the comprehensive laboratory surveillance and control of invasive meningococcal disease in the EU and for the validation and implementation of new detection and typing tools. As an example, the genome sequence of more than 800 meningococcal disease isolates are currently being determined by using Illumina HiSeq2000 technology in a joint endeavour between IHM, NIPH and the University of Oxford within the framework of IBD-labnet. This aims to form a basis for the introduction of whole-genome sequencing as a next generation typing tool for meningococci, to provide public health services with information on the diversity of European disease associated meningococci and to provide a genomic baseline for interpreting possible effects of novel MenB vaccines within routine vaccination schedules in the EU Member States.

FUTURE DIRECTIONS

Due to its strong commitment to continuously optimize patient care, the IHM is engaged in improving and developing diagnostic test systems and typing tools. In this regard, the IHM will further promote the introduction of genome sequencing techniques in the routine clinical microbiology workflow for the improvement of the diagnosis and control of infectious diseases.

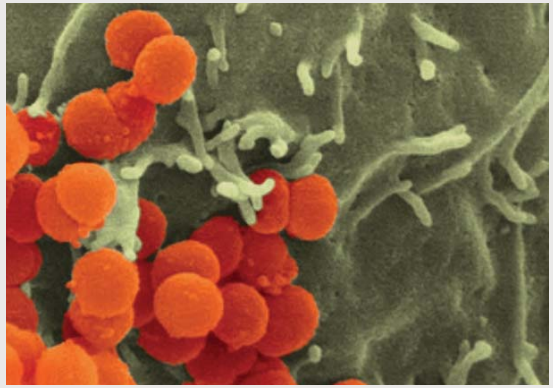


Fig. 1: Microvilli formation induced in human brain endothelial cells upon contact with *N.meningitidis.*

SELECTED PUBLICATIONS

BBrehony C, Trotter CL, Ramsay ME, Chandra M, Jolley KA, van der Ende A, Carion F, Berthelsen L, Hoffmann S, Harðardóttir H, Vazquez J, Murphy K, Toropainen M, Caniça M, Ferreira E, Diggle M, Edwards G, Taha MK, Stefanelli P, Kriz P, Gray S, Fox A, Jacobsson S, Claus H, Vogel U, Tzanakaki G, Heuberger S, Caugant DA, **Frosch M**, Maiden MC (2013) *Differential age distribution of disease-associated meningococcal lineages-Implications for vaccine development.* **Clinical and Vaccine Immunology** in press

Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, Caugant DA, Kriz P, Abad R, Bambini S, Carannante A, Deghmane AE, Fazio C, **Frosch M**, Frosi G, Gilchrist S, Giuliani MM, Hong E, Ledroit M, Lovaglio PG, Lucidarme J, Musilek M, Muzzi A, Oksnes J, Rigat F, Orlandi L, Stella M, Thompson D, Pizza M, Rappuoli R, Serruto D, Comanducci M, Boccadifuoco G, Donnelly JJ, Medini D, Borrow R (2013) *Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment.* **Lancet Infectious Diseases** 13:416-25

Maiden MC, **Frosch M** (2012) *Can we, should we eradicate the meningococcus?* **Vaccine** 30 Suppl 2:B52-6

3.2. INSTITUTE FOR HYGIENE AND MICROBIOLOGY

3.2.2. HELMINTH INFECTIONS

Parasitic flatworms are a major cause of human disease world-wide. The group aims at understanding host-parasite interaction mechanisms and parasite development using the tapeworm model system *Echinococcus multilocularis*.

PROF. KLAUS BREHM

INSTITUTE FOR HYGIENE AND MICROBIOLOGY

Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/E1
D-97080 Würzburg
T +49 (0)931-3146168
M kbrehm@hygiene.uni-wuerzburg.de

INTRODUCTION

Parasitic flatworms (mostly trematodes and cestodes) are the causative agents of severe diseases with hundreds of millions of people infected mostly in under-developed countries. A very important sub-group of these diseases are infections due to larval cestodes (tapeworms) such as alveolar (*E. multilocularis*; fox-tapeworm) and cystic (*E. granulosus;* dog-tapeworm) echinococcosis, or neurocysticercosis (*Taenia solium;* pork-tapeworm). These infections are characterized by growth and development of the parasite's metacestode or cysticercus larval stages in various host organs such as liver, lung, and brain, eventually leading to epilepsy, jaundice, organ failure, and death.

Among larval cestode infections, alveolar echinococcosis (AE) is the most dangerous disease and involves infiltrative, cancer-like growth of the metacestode within the host liver, followed by metastases formation in secondary organs (lung, brain). The infection is initiated by the oral uptake of infectious eggs that contain the first larval stage (oncosphere), which subsequently develop into the metacestode and protoscolex stages in a metamorphosis-like manner that is entirely driven by totipotent parasite stem cells. These stem cells, called germinative cells, are the most important cell types of tapeworms and account for the enormous developmental plasticity of these organisms, for asexual proliferation of cestode larvae, and for the tremendous regeneration capacity (up to immortality) of adult and larval cestodes.

Current treatment options against larval cestode infections, particularly AE, are very limited. In a few cases, surgical removal of the parasite larvae can be achieved. In the remaining cases (>80%), benzimidazole-based chemotherapy, directed against parasite beta-tubulin, has to be used. However, beta-tubulin is highly conserved between parasite and host, resulting in significant adverse side effects of benzimidazole chemotherapy. Furthermore, the drugs only act parasitostatic (not parasitocidal) and have to be taken for very long periods of time (up to the rest of the patients life).

RESEARCH HIGHLIGHTS

The group has developed important tools for studying molecular host-parasite interactions and parasite development. This includes a sophisticated in vitro cultivation systems for *Echinococcus* larvae and stem cells in which the infection of the intermediate host can be mimicked under controlled laboratory conditions. During the last couple of years, these systems have been used to study the influence of host hormones on parasite development as well as the effects of excretory/secretory parasite products on host immune cells.

Most importantly, the group has initiated a whole genome sequencing project for *E. multilocularis* and, together with groups at the Wellcome Trust Sanger Institute (Hinxton, UK), Mexico, Uruguay and Argentina, has carried out genome sequencing of four medically important tapeworm species. This was accompanied by extensive next-generation transcriptome sequencing of the entire *E. multilocularis* life-cycle. Cestode genomics revealed extensive gene loss and gene gain associated with the evolution of parasitism.

The project also revealed that several central components that regulate stem cells in all metazoan organisms, are absent or highly modified in cestodes; e.g. the absence of VASA and PIWI, which are typically involved in the maintenance of genome integrity in metazoan germline stem cells. The unique cestode stem cell system thus has undergone significant modifications in the regulatory systems that control stem cell maintenance and differentiation, which could be involved in the tremendous regeneration capacity of these organisms.

In silico analyses, genomics and transcriptomics have revealed a plethora of alternative drug targets for anti-parasitic chemotherapy. Among these are modified, parasite-specific oxygen detoxification systems and a large number of druggable kinases that are expressed in the parasite's stem cells, the most important cell type for the development of anti-*Echinococcus* drugs. We have used the in vitro cultivation systems to demonstrate the anti-*Echinococcus* activities of several drugs that are currently in clinical use against cancer (e.g. Imatinib). These drugs can serve as lead compounds for the identification of novel small molecules with high specificity for parasite kinases (leading to stem cell death) and lower effects on the corresponding host kinases (thus leading to less adverse side effects).

Finally, the group has also demonstrated that excretory/secretory products of early developing parasite larvae can induce tolerogenic phenotypes and apoptosis in host immune cells such as dendritic cells and T-cells. By these activities, the parasite is able to generate a tolerogenic immune environment that is most probably instrumental in long-term parasite persistence and development.

FUTURE DIRECTIONS

Ongoing studies aim to elucidate the regulatory mechanisms that control stem cell dynamics in *E. multilocularis*, including the stem cell specific transcriptome, how cestodes maintain genome integrity and the molecular basis for the immortality of the cestode stem cell system. This will be accompanied by in vitro and *in vivo* studies to identify small molecule compounds that specifically target and eliminate parasite stem cells. Another focus will be the identification of specific excretory/secretory parasite factors that are involved in subverting the immune response of the host towards a tolerogenic environment.

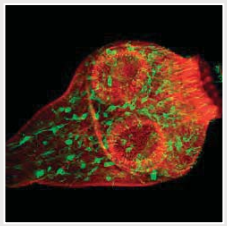


Fig. 1: E. multilocularis protoscolex after staining with phalloidin (muscles) and anti-acetylated tubulin (nervous system, flame cells).

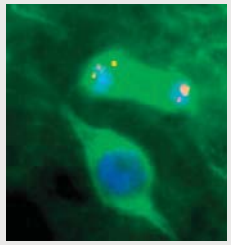


Fig. 2: E. multilocularis stem cells in interphase (below) and during mitosis (above) (staining: DAPI (blue), incorporated EdU (red), tubulin (green)).

SELECTED PUBLICATIONS

Tsai IJ, Zarowiecki M, Holroyd N, Garciarrubio A, et al., Laclette JP, **Brehm K**, Berriman M (2013) *The genomes of four tapeworm species reveal adaptations to parasitism.* **Nature** 496:57-63

Koziol U, Krohne G, **Brehm K** (2013) *Anatomy and development of the larval nervous system in Echinococcus multilocularis.* **Frontiers in Zoology** 10:24

Geyer KK, Chalmers IW, MacKintosh N, Hirst JE, Geoghegan R, Badets M, Brophy PM, **Brehm K**, Hoffmann KF (2013) *Cytosine methylation is a conserved epigenetic feature found throughout the phylum Platyhelminthes.* **BMC Genomics** 14:462

Hemer S, **Brehm K** (2012) *In vitro efficacy of the anti-cancer drug Imatinib on Echinococcus multilocularis larvae.* **International Journal of Antimicrobial Agents** 40:458-462

Nono JK, Pletinckx K, Lutz M, **Brehm K** (2012) *Excretory/secretory products of Echinococcus multilocularis larvae induce apoptosis and tolerogenic properties in dendritic cells in vitro.* **PLoS Neglected Tropical Diseases** 6:e1516

Whereas CC ST-53 *cnl* strains are frequently found in carriers but almost never in cases of IMD, ST-198 *cnl* strains have been found in two of four reported IMD cases caused by *cnl* strains. To assess the genetic basis of virulence in these cnl strains we have sequenced the genomes of the CC ST-198 *cnl* strain α704 and compared it to the genome of *cnl* strain α14 of CC ST-53. The genome of α704 was found to contain a full-length *opc* gene and a second pilC gene, which both encode (major) adhesins. This is in good agreement with the 10-fold higher adhesion and invasion rates of α704 compared to strain α14, which is likely to contribute to differences in invasive properties of between the two strains.

Natural transformation is the primary source of genetic variation in *N. meningitidis* and contributes to immune evasion and virulence in this species. Together with the group of Jörg Vogel we have studied the bacterial genome defense system, CRISPR, which confers adaptive, sequence-based immunity against foreign DNA using CRISPR-derived RNAs (crRNAs), and discovered a novel crRNA biogenesis pathway that limits natural transformation.

FUTURE DIRECTIONS

We have recently begun to use comparative transcriptomics to analyse the differences in the expression and regulation of genes common to carriage and invasive meningococcal isolates and also to investigate the role of non-coding small RNAs in meningococcal virulence. Since virulence is a complex and therefore polygenic trait we further aim to embed our experimental results in a systems biological framework to achieve a comprehensive understanding of meningococcal pathogenicity in the context of normal cell physiology.

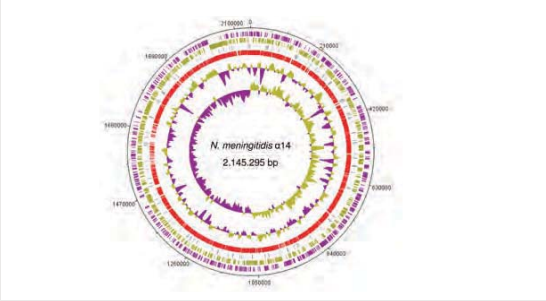


Fig. 1: Fig. 1: Comparison of the genomes of meningococcal strains α14 and α704. Circular representation of the α14 strain genome. From the outer to the inner most circle: (i) genes on the forward strand, (ii) genes on the reverse strand, (iii) pseudogenes, (iv) genes present also in α704, (v) GC content, and (vi) GC skew.

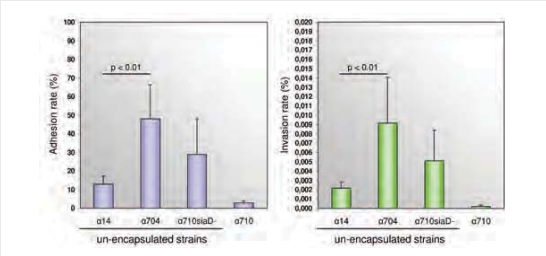


Fig. 2: Comparison of adhesion and invasion rates in a human nasopharyngeal cell line (FaDu). Depicted are the adhesion and invasion rates for the two un-encapsulated strains α14 and α704, the capsule deficient *(siaD-)* and the encapsulated isogenic (serogroup B) wild-type strain α710 that were used as controls.

SELECTED PUBLICATIONS

Zhang Y, Heidrich N, Ampattu BJ, Gunderson CW, Seifert HS, **Schoen C**, Vogel J, Sontheimer EJ (2013) *Processing-independent CRISPR RNAs limit natural transformation in Neisseria meningitidis.* **Molecular Cell** 50:488-503

Joseph B, Frosch M, **Schoen C,** Schubert-Unkmeir A (2012) *Transcriptome analyses in the interaction of Neisseria meningitidis with mammalian host cells.* **Methods in Molecular Biology** 799:267-293

Pawlik MC, Hubert K, Joseph B, Claus H, **Schoen C**, Vogel U (2012) *The zinc-responsive regulon of Neisseria meningitides comprises 17 genes under control of a Zur element.* **Journal of Bacteriology** 194:6594-6603

Schork S, Schluter A, Blom J, Schneiker-Bekel S, Puhler A, Goesmann A, Frosch M, **Schoen C** (2012) *Genome sequence of a Neisseria meningitides capsule null locus strain from the clonal complex of sequence type 198.* **Journal of Bacteriology** 194:5144-5145

3.2. INSTITUTE FOR HYGIENE AND MICROBIOLOGY –

3.2.3. EVOLUTIONARY AND FUNCTIONAL PATHOGENOMICS OF *NEISSERIA MENINGITIDIS*

The human body is host to a large variety of different microorganisms collectively called the human microbiome, which contains both commensal and potentially pathogenic species. Using *Neisseria meningitidis* as a model the group aims to better understand the genetic and genomic basis for commensal and invasive behavior in human-adapted commensal pathogens.

DR. CHRISTOPH SCHOEN
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/E1
D-97080 Würzburg
T +49 (0)931-3146162
M cschoen@hygiene.uni-wuerzburg.de

INTRODUCTION

The importance of the normal microbial populations of the human body in health and disease has received increasing attention in recent years. Although these populations are normally beneficial or neutral, some components such as facultative pathogens are responsible for more infections today than "classical" pathogens. The reality that these facultative pathogens cause serious disease has raised questions about some of the original concepts of virulence factors. As a prime example, *Neisseria meningitidis* is a commensal bacterial species that colonizes the nasopharynx of up to 30% of the healthy human population. Whereas most isolates from healthy carriers are considered non-pathogenic, a small number of strains belonging to hyperinvasive lineages can cause invasive meningococcal disease (IMD) such as acute bacterial meningitis or sepsis.

The work of our group focuses on a fundamental question regarding the genetic basis of meningococcal virulence. A basic assumption for pathogenic bacteria is that virulence is genetically determined by virulence factors encoded in the bacterial chromosome. However, the identity of these genetic factors and their evolution in meningococci remains poorly understood. In addition to differences in the gene complement between carriage and invasive strains, contrasting virulence properties could also result from altered regulation and expression of genes common to all meningococcal strains under infection-relevant conditions. However, currently little is also known about the potential differences in the transcriptomes of carriage and invasive meningococcal strains.

Since classical candidate gene-based approaches have failed to identify a set of virulence genes in *N. meningitidis,* our group is employing a variety of molecular biological and genomic techniques, including comparative whole-genome sequencing, microarrays and RNA-sequencing to search for the elusive genetic virulence determinants in *N. meningitidis.*

RESEARCH HIGHLIGHTS

In close collaboration with several partners from the ZINF we have begun to dissect the genetic basis of meningococcal virulence on a genome-wide scale.

Zinc is a bivalent cation essential for bacterial growth and metabolism. Meningococci express a homologue of the zinc uptake regulator Zur that has been postulated to repress the putative zinc uptake protein ZnuD. Together with the group of Ulrich Vogel we have elucidated for the first time the transcriptome of meningococci in response to zinc using microarrays and identified a meningococcal Zur binding consensus motif. The Zur regulon comprises of genes involved in zinc uptake, tRNA modification, ribosomal assembly as well as many genes of unknown function.

The production of a polysaccharide capsule is considered to be essential for meningococcal virulence. There have been very few reports of constitutively unencapsulated *cnl* strains causing IMD even though the prevalence of cnl strains is 16% in healthy children and young adults. Multilocus sequence typing has indicated that most cnl strains belong to only a very few clonal complexes (CCs) including, sequence type (ST)-53 and ST-198.

(RTKs) involved. Using phospho-array platforms we deciphered the RTK-dependent signaling pathways necessary for bacterial uptake. We have identified several activated RTKs, including the ErbB family receptors, epidermal growth factor receptor (EGFR), ErbB2, and ErbB4. Pharmacological inhibition and genetic ablation by RNA interference of these kinases resulted in decreased bacterial uptake. In agreement, heterologous expression of EGFR, ErbB2, or ErbB4 causes a significant increase of meningococcal invasion. We have demonstrated that phosphorylation of EGFR and ErbB4 is mediated by transactivation of their common ligand, HB-EGF. In particular, activation of three specific phosphorylation sites of EGFR, ErbB2, and ErbB4 are required for the uptake of *N. meningitidis* into the eukaryotic cell.

FUTURE DIRECTIONS

In the future we will analyse the role of lipid-rich microdomains and phospholipid dynamics during the process of meningococcal adhesion and invasion, as part of the new Forschergruppe FOR2123 (Sprecherin: S. Schneider-Schaulies). Sphingolipid-enriched membrane microdomains contribute to a variety of cellular processes, including signal transduction and vesicle trafficking. Interestingly, a number of pathogens exploit the endocytic properties of sphingolipid-enriched membrane microdomains to enter host cells. The significance of sphingolipid-enriched membrane microdomains in *N. meningitidis* pathogenesis is currently unclear. Initial experiments from our group with a serogroup B isolate are indicative for a role for the acid sphingomyelinase in the *N. meningitidis* entry process. This suggests that ceramide and ceramide-enriched membrane platforms, which are generated upon SMase activation, play a significant role in meningococcal uptake. Overall, we aim to obtain a more detailed understanding of how the changes in host membrane structures during this bacterial infection occur.

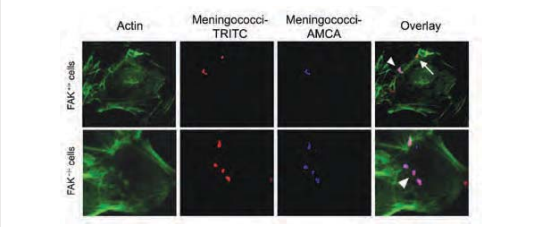


Fig. 1: FAK-deficient cells are impaired in their ability to internalise *N. meningitidis*. $FAK^{+/+}$ and $FAK^{-/-}$ fibroblasts were infected with meningococci and analyzed by immunofluorescence microscopy. Extracellular bacteria (arrowhead) stain positive with both TRITC (red fluorescence) and AMCA (blue fluorescence), whereas intracellular bacteria (arrow) are labeled with TRITC only. Cell actin was stained with Alexa Fluor® 488 phalloidin (green fluorescence).

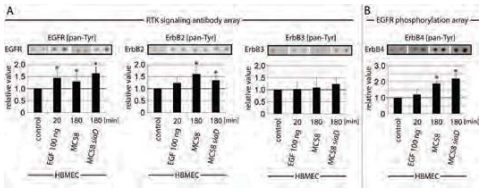


Fig. 2: N. meningitidis infection causes activation of EGFR, ErbB2 and ErbB4. HBMEC were left uninfected (control) or were incubated with EGF (positive control) or infected with *N. meningitidis* for indicated time points and cell lysates were analyzed using the PathScan RTK signaling antibody array (A) or the human EGFR phosphorylation antibody array (B).

SELECTED PUBLICATIONS

Slanina H, Mundlein S, Hebling S, **Schubert-Unkmeir A** (2013) *Role of Epidermal Growth Factor Receptor Signaling in the Interaction of Neisseria meningitides with Endothelial Cells.* **Infection and Immunity** in press

Schubert-Unkmeir A, Christodoulides M (2013) *Genome-based bacterial vaccines: current state and future outlook.* **BioDrugs** 27:419-430

Slanina H, Hebling S, Hauck CR, **Schubert-Unkmeir A** (2012) *Cell invasion by Neisseria meningitidis requires a functional interplay between the focal adhesion kinase, Src and cortactin.* **PLoS One** 7:e39613

Muller I, Freitag MH, Poggensee G, Scharnetzky E, Straube E, Schoerner C, Hlobil H, Hagedorn HJ, Stanek G, **Schubert-Unkmeir A**, Norris DE, Gensichen J, Hunfeld KP (2012) *Evaluating frequency, diagnostic quality, and cost of Lyme borreliosis testing in Germany: a retrospective model analysis.* **Clinical and Developmental immunology** 2012:595427

Joseph B, Frosch M, Schoen C, **Schubert-Unkmeir A** (2012) *Transcriptome analyses in the interaction of Neisseria meningitidis with mammalian host cells.* **Methods in Molecular Biology** 799:267-293

3.2. INSTITUTE FOR HYGIENE AND MICROBIOLOGY

3.2.4. HOST-PATHOGEN INTERACTIONS

The major interest of the group is studying the molecular interaction of *Neisseria meningitidis* with vascular endothelial cells, with a specific focus on the mechanism of brain endothelial barrier penetration.

PROF. ALEXANDRA SCHUBERT-UNKMEIR
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/E1
D-97080 Würzburg
T +49 (0)931-3146721
M aunkmeir@hygiene.uni-wuerzburg.de

INTRODUCTION

Neisseria meningitidis is an obligate commensal in humans, colonizing the nasopharyngeal mucosa usually without affecting the host, a phenomenon known as carriage. After the onset of colonization, *N. meningitidis* strains may occasionally penetrate the mucosal membrane and enter the bloodstream and cause severe septicemia. Following bacteremia, *N. meningitidis* may bind and subsequently cross the blood-cerebrospinal fluid (B-CSF) barrier to enter the subarachnoidal space, resulting in acute and purulent meningitis. The human blood-brain/blood-cerebrospinal fluid (BB/B-CSF) barrier is one of the tightest barriers of the human body. Among invasive pathogens, few are capable of invading the subarachnoideal space, thus suggesting that they have developed specific abilities to enable them to circumvent the BBB.

As is the case for other bacterial pathogens *N. meningitidis* can activate host cell signal transduction pathways to establish adhesion and induce it's uptake by the host cell. In eukaryotic cells, protein kinases play an important role in transducing extracellular signals, which can be classified on the basis of distinct substrate preferences as serine/threonine kinases and tyrosine kinases. Upon binding of meningococci to eukaryotic cells, there is an increase in tyrosine phosphorylation of host proteins, which results in cytoskeletal remodelling and subsequent engulfment of the pathogen.

The group is interested in understanding the strategies used by this microorganism to colonize the brain vasculature and to cross the BB/B-CSF barrier. To answer these main questions we are working with tissue culture based cell models including brain microvascular endothelial cells and employ a wide spectrum of innovative methods in molecular, biochemical and cell biological methods including siRNA/shRNA transfections, FACS analyses and microscopy.

RESEARCH HIGHLIGHTS

We have previously established that the non-receptor tyrosine kinases c-Src acts as an important signaling molecule downstream of integrin engagement to mediate *N. meningitidis* uptake. More recently, we have also described a role for the focal adhesion kinase (FAK) in coupling integrin-mediated signalling to the actin cytoskeleton rearrangements during the meningococcal entry process. By using specific pharmacological inhibitors, siRNA ablation and FAK deficient mice embryonic fibroblasts, we have shown that FAK activity is required for meningococcal invasion and the autophosphorylation and kinase activity of FAK are essential for the uptake of *N. meningitidis* by the host cell. In addition, meningococcal infection leads to the phosphorylation of cortactin, which is an actin-binding protein and a key regulator of actin rearrangement in response to tyrosine kinase signaling. Mutation of critical cortactin amino acids either within the domain that interacts with dynamin or within the NTA domain that activates the Arp2/3 complex support the hypothesis that both domains are critical for efficient bacterial uptake.

Based on our initial observation that *N. meningitidis* induces phosphotyrosine signalling that is necessary for invasion of infected human brain microvascular endothelial cells (HBMEC), we have begun an in depth analysis for the receptor tyrosine kinases

FUTURE DIRECTIONS

The NRZMHi will pursue the following activities in the upcoming years: (1) The introduction of next generation genome sequencing will generate DNA-sequence based typing data needed for the NRZMHi. (2) The rich finetyping and epidemiological datasets will be analysed for bacterial predictors of poor outcome of disease to generate testable hypotheses. (3) The representative surveillance of invasive *Haemophilus influenzae* disease will be further developed. This was initiated in Würzburg in 2008 and is currently generating data comparable to that of the surveillance of invasive meningococcal disease. Furthermore, the very successful collaboration with the University of Greifswald will be extended to study the effects of stimuli that occur during the adaptation of meningococci to the various compartments of the human host.

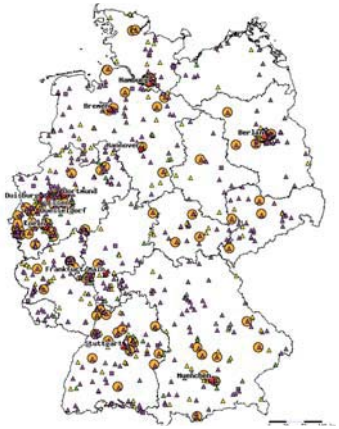


Fig. 1: Laboratory surveillance of invasive meningococcal disease by the reference laboratory. The map was generated with EpiScanGIS and shows the location of cases analysed between 2011 and 2013. The capsule serogroup information is provided. Furthermore, the most prevalent serogroup B finetype is depected by yellow circles.

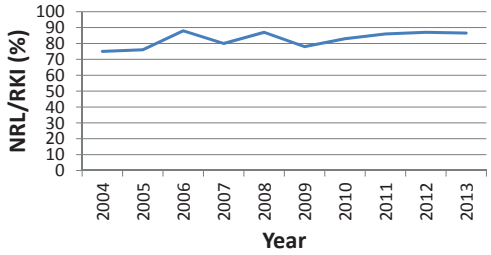


Fig. 2: The reference laboratory (NRL) dataset is representative for the statutory notification dataset compiled by the Robert Koch-Institute (RKI). The bacteria of 75-90% of the notified cases are typed at the NRL. This is achieved by close collaboration with public health offices and laboratories and continuous matching of data.

SELECTED PUBLICATIONS

Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, Caugant DA, Kriz P, Abad R, Bambini S, Carannante A, Deghmane AE, Fazio C, Frosch M, Frosi G, Gilchrist S, Giuliani MM, Hong E, Ledroit M, Lovaglio PG, Lucidarme J, Musilek M, Muzzi A, Oksnes J, Rigat F, Orlandi L, Stella M, Thompson D, Pizza M, Rappuoli R, Serruto D, Comanducci M, Boccadifuoco G, Donnelly JJ, Medini D, Borrow R (2013) *Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment.* **Lancet Infectious Diseases** 13:416-425

Lappann M, Danhof S, Guenther F, Olivares-Florez S, Mordhorst IL, **Vogel U** (2013) *In vitro resistance mechanisms of Neisseria meningitides against neutrophil extracellular traps.* **Molecular Microbiology** 89:433-449

Hellenbrand W, Elias J, Wichmann O, Dehnert M, Frosch M, **Vogel U** (2013) *Epidemiology of invasive meningococcal disease in Germany, 2002-2010, and impact of vaccination with meningococcal C conjugate vaccine.* **The Journal of Infection** 66:48-56

Pawlik MC, Hubert K, Joseph B, Claus H, Schoen C, **Vogel U** (2012) *The zinc-responsive regulon of Neisseria meningitides comprises 17 genes under control of a Zur element.* **Journal of Bacteriology** 194:6594-6603

Hubert K, Pawlik MC, Claus H, Jarva H, Meri S, **Vogel U** (2012) *Opc expression, LPS immunotype switch and pilin conversion contribute to serum resistance of unencapsulated meningococci.* **PloS One** 7:e45132

3.2. INSTITUTE FOR HYGIENE AND MICROBIOLOGY

3.2.5. INFECTION EPIDEMIOLOGY AND PATHOGENESIS OF NEISSERIA MENINGITIDIS

Neisseria meningitidis is a commensal pathogen of the human host. The group conducts infection epidemiology projects within the framework of the national reference laboratory for meningococci and *Haemophilus influenzae (NRZMHi)* and researches various aspects of meningococcus pathogenesis.

PROF. ULRICH VOGEL
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

Institut für Hygiene und Mikrobiologie
Josef-Schneider-Str. 2/E1
D-97080 Würzburg
T +49 (0)931-3146802
M vogel_u@ukw.de

INTRODUCTION

Neisseria meningitidis colonizes the human host and is considered to be a commensal species that accidentally causes severe invasive disease in children and adolescents. The NRZMHi, a reference laboratory funded by the Robert Koch-Institute and headed by Ulrich Vogel and Matthias Frosch for more than 10 years, provides the public health system with laboratory surveillance data of invasive meningococcal disease. The data are continuously matched with statutory notification data to generate a robust and reliable dataset. This dataset is important for following national epidemiological trends and to address specific questions such the effect of recommendations of vaccinations.

Meningococci, albeit being restricted to the human host, undergo a variety of adaptations as a result of their particular lifestyle. This includes the adaptation to a new host after droplet transmission, a process involving biofilm or microcolony formation. Within biofilms, but also during replication in the bloodstream in the rare event of invasive disease, the spontaneous release outer membrane proteins are observed. These particles are of interest in understanding the interaction of the pathogen with the host, the frightening speed of sepsis development, and as vaccine components to fight meningococcus B disease. Meningococci adapt to changes in their environment by regulating their gene expression profiles, for example to changes in metal ion levels, and by phase variation, which rapidly and stochastically alter the surface composition of the bacteria.

RESEARCH HIGHLIGHTS

The reference laboratory together with an international consortium have used a novel assay to assess the strain coverage of the meningococcus B vaccine BexseroTM, which has been available in Germany since December 2013. The study made use of the reference laboratory surveillance and strain collection from all over Germany. The data have been important in guiding vaccine recommendations on the international level.

The excellent collaboration between the reference laboratory and the Robert Koch-Institute was highlighted by a joint publication on the effects of the serogroup C conjugate vaccination schedule recommended in Germany since 2006. This detailed assessment of disease incidence stratified for age groups and for regions with varying vaccine uptake provided a first thorough evaluation of the population effects obtained.

A collaboration with the group of Dörte Becher (Greifswald) exploited a new quantitative metabolic labelling approach to quantify proteomic differences between the outer membrane of Neisseria and of spontaneously released outer membrane vesicles. This suggested that SOMVs are likely to be released from surface areas with a low local abundance of membrane-anchoring proteins and lytic transglycosylases.

The response of meningococci to changing zinc level changes was analysed using microarrays. The application of short term zinc exposure revealed a small regulon, which almost exclusively contained genes harbouring a Zur binding motive. A separate study into the alterations of a meningococcal population during serum stress was, in contrast to the zinc response, driven by phase variation and gene conversion affecting the adhesin Opc, LPS and Opc.

03.3

Institute for Virology and Immunobiology – Chair of Immunobiology

ZINF MEMBERS:

THOMAS HÜNIG

NIKLAS BEYERSDORF

THOMAS HERRMANN

MANFRED LUTZ

The Institute for Virology and Immunobiology is part of the Medical Faculty at the University of Würzburg. Prof. Dr. Thomas Hünig has been the Chair of Immunology since 1990.

The research interests of the individual groups focus on a broad spectrum of basic and applied immunological topics. Many of the results from basic research are translated into preclinical therapy models for infections, allergies, autoimmune diseases, transplant rejection and graft-versus-host-disease. The institute also provides diagnostic services for autoantibodies for the University Clinics. Members of the Institute provide immunology lectures for medical, biomedical, biochemistry and biology students.

cells, are functionally disabled, and developed a simple but highly effective method which resets circulating T-cells to a tissue-like status. This system has formed the basis of an extensive analysis of TGN1412 responses, identifying conditions for selective activation of Treg cells also in humans. Indeed, a recent new phase I trial strongly supported the concept of CD28SA-induced Treg activation in humans.

We had previously developed a mouse model for MS in which a model antigen is expressed in the cytoplasm of oligodendrocytes, allowing only CD8 T-cells to attack. Using CD8 T-cells from mice with a transgenic T-cell antigen receptor specific for this model antigen, we have shown that upon peripheral infection with a bacterium also expressing this antigen, CD8 T-cells routinely travel through the CNS, but are themselves destroyed (rather than being pathogenic) unless the brain is infected itself. This is a novel mechanism by which the CNS is spared from autoimmune attack and actually induces tolerance, while still being able to eradicate infectious agents in the CNS.

FUTURE DIRECTIONS

Research during the next few years is aimed at understanding in greater depth the findings described above. For example, while our protocol for resetting circulating T-cells to tissue-like conditions is very robust and useful, e.g. for immunomonitoring of cancer patients, the events leading to this re-acquisition of reactivity are only partially clear. Similarly, while we have demonstrated the importance of CD28 for the initiation of secondary T-cell responses, the influence of costimulation on the quality of those responses remains open and is currently under investigation. Finally, in a collaborative project with Martina Deckert, neuropathologist at the University of Cologne, we are analysing the cell types and signalling pathways underlying the decision of oligodendrocytes to either act as deleters of attacking CD8 T-cells, or to sacrifice themselves in order to allow the eradication of a pathogen that has invaded the brain.

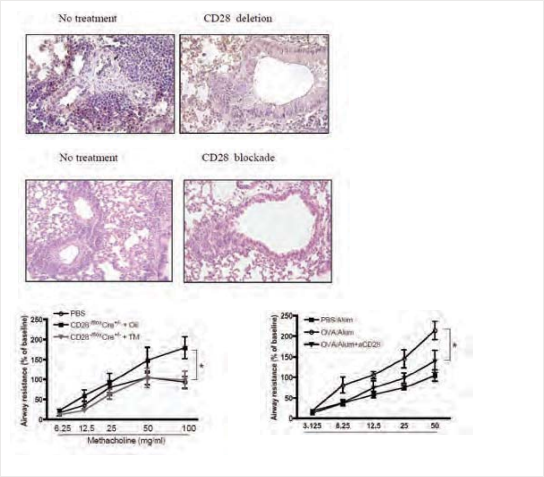


Fig. 1: CD28 deletion or CD28 blockade before allergen challenge prevents the development of airway pathology. Allergic asthma was induced by immunizing mice with the model antigen OVA in alum. Challenge was by intranasal application. In treated groups, CD28 was either deleted using tamoxifen-translocation in ER-Cre in mice with a "floxed" gene (top panels), or by applying a mouse anti-mouse monoclonal antibody which blocks the ligand binding site of the CD28 molecule (bottom panels). Shown are HE-stained sections of lung tissue (left), and airway resistance after methacholine challenge (right). From Gogishvili et al., JACI 2012.

SELECTED PUBLICATIONS

Tabares P, Berr S, Romer PS, Chuvpilo S, Matskevich AA, Tyrsin D, Fedotov Y, Einsele H, Tony HP, **Hunig T** (2013) *Human regulatory T cells are selectively activated by low-dose application of the CD28 superagonist TGN1412/TAB08.* **European Journal of Immunology** in press

Gogishvili T, Luhder F, Goebbels S, Beer-Hammer S, Pfeffer K, **Hunig T** (2013) *Cell-intrinsic and -extrinsic control of Treg-cell homeostasis and function revealed by induced CD28 deletion.* **European Journal of Immunology** 43: 188-193

Gogishvili T, Luhder F, Kirstein F, Nieuwenhuizen NE, Goebbels S, Beer-Hammer S, Pfeffer K, Reuter S, Taube C, Brombacher F, **Hunig T** (2012) *Interruption of CD28-mediated costimulation during allergen challenge protects mice from allergic airway disease.* **The Journal of Allergy and Clinical Immunology** 130: 1394-1403 e1394

Hunig T (2012) *The storm has cleared: lessons from the CD28 superagonist TGN1412 trial.* **Nature Reviews Immunology** 12: 317-318

3.3. INSTITUTE FOR FOR VIROLOGY AND IMMUNOBIOLOGY

3.3.1. IMMUNOLOGY

T-lymphocytes (T-cells) are central players of the immune system both as effectors able to destroy infected and tumor cells, and as coordinators of immune responses, including unwanted immunopathology. We are studying the rules governing the activation and effector functions of T-cells, with a focus on the key costimulatory receptor CD28.

PROF. THOMAS HÜNIG

INSTITUTE FOR VIROLOGY AND IMMUNOBIOLOGY

Institut für Virologie und Immunbiologie
Versbacher Str. 7
D-97080 Würzburg
T +49 (0)931-3189796
M huenig@vim.uni-wuerzburg.de

INTRODUCTION

Foreign and self antigens are initially presented to T-cells by dendritic cells (DC), which sense infectious agents with the help of pattern recognition receptors and instruct the T-cells if and what type of immune response they should mount. A key signal for the initiation of such responses is provided via the DC surface molecules CD80/86, which are engaged by the costimulatory receptor CD28 expressed by all resting T-cells. Antigen recognition (signal 1) and costimulation (signal 2) together induce full T-cell activation, i.e. proliferation and expression of effector functions.

Besides effector T-cells which exist as "killer" (CD8) or "helper" (CD4) cells, regulatory T-cells (Treg cells, CD4+Foxp3+) are of key importance in controlling the magnitude of immune responses and suppressing autoimmunity. Treg cells also require costimulation by CD28.

Some organs such as the brain enjoy "immune privilege", i.e. particular care is taken to avoid autoimmunity and inflammation because of the particular sensitivity of these organs to structural damage, and limited capacity for regeneration. Nevertheless, multiple sclerosis (MS), an autoimmune disease in which oligodendrocytes, the cells forming the myelin sheaths around the axons through which nerve cells communicate, are destroyed by the immune system.

In the past two years, we have extended our studies on the requirement of CD28 for effector and regulatory T-cell responses, on therapeutic strategies aimed at activating regulatory T-cells through CD28 specific monoclonal antibodies (mAb), and on the role of CD8 T-cells in the destruction of oligodendrocytes in a mouse model of MS.

RESEARCH HIGHLIGHTS

We have developed two important tools to study CD28 biology: a mouse strain in which CD28 can be inducibly deleted at any time point, and a set of monoclonal antibodies which either block or ligate CD28 in a very efficient way (CD28 superagonists; CD28SA). Using inducible CD28 deletion, we have found that costimulation by CD28 is of particular importance for regulatory T-cells, both in a cell intrinsic and in a cell extrinsic fashion (provision of the growth factor IL-2 by helper T-cells). Furthermore, we have found that contrary to previous conclusions based on less specific experimental systems, not only primary but also secondary (memory) T-cell responses are fundamentally shaped by CD28 costimulation. This includes the allergic airway response of mice, which can be greatly ameliorated by blocking CD28 interactions (Fig. 1).

In rodents, CD28SA are effective inducers of Treg expansion, and have, accordingly, been very successfully applied as experimental therapeutic agents in a broad spectrum of autoimmune and inflammatory diseases. In contrast, the phase I clinical trial of TGN1412, a human CD28SA developed by a spin-off company from the institute, induced a life-threatening cytokine release syndrome (CRS) when tested in a phase I trial in 2006. We have now investigated why cultures of peripheral blood mononuclear cells (PBMC) stimulated with TGN1412 did not predict this CRS in vitro. We found that circulating T-cells, in contrast to those residing in tissues where they physically interact with other

fumigatus or *Candida albicans*. Therefore, the group has recently started to work on the modulation of T cell immunity against *Candida albicans* by secreted fungal proteins. Our preliminary data suggest that secreted fungal proteins not only bind to T cells but also modulate their activation, potentially contributing to immune escape by the fungus.

During the first year of life we are physiologically immunocompromised making us extremely susceptible towards pathogens such as the measles virus. The underdeveloped immune system at that age even precludes successful vaccination against the measles virus. Unfortunately, in some children the measles virus persists in the brain after an acute infection leading to a lethal form of encephalitis called Subacute Sclerosing Panencephalitis (SSPE) after a few years of latency. Recent data from our group indicate that sphingolipids, i.e. a group of biomolecules found in cellular membranes and acting as second messengers for signal transduction into the cell, seem to be particularly important for the function of regulatory CD4+ T cells. As regulatory T cells control chronic infections with the measles virus we assume that manipulation of spingolipid metabolism will also influence the course of chronic measles virus infections.

FUTURE DIRECTIONS

Our future research will involve the regulation of immune responses in general including focusing on the re-programming of effector CD4+ T cells and on the role of CD28 during aGvHD development using a novel mouse model of genetic CD28 deletion. Moreover, we will work on novel therapeutic concepts for opportunistic infections by *Candida albicans* and for chronic cerebral infections with the measles virus by targeting T cell immunity either with monoclonal antibodies or small molecules.

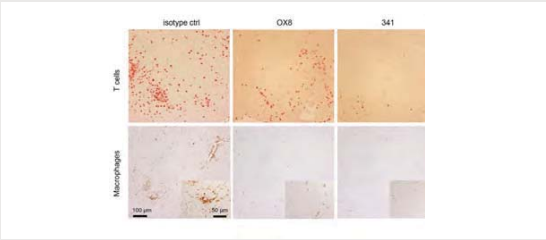


Fig. 1: The depletion of $CD8^+$ T cells using monoclonal antibodies (OX8; 341) reduces disease activity in Lewis rat EAE – an animal model for Multiple Sclerosis in humans. The reduction in clinical symptoms is associated with fewer T cells and macrophages infiltrating the CNS of these animals. $CD8^+$ T cells, thus, appear to contribute to the pathogenesis of this disease. Published in: (Camara M, et. al. (2013) Journal of Neuroimmunology 260:17-27).

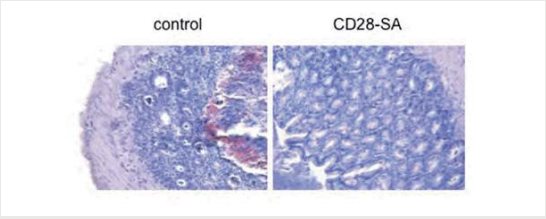


Fig. 2: Polyclonal activation and expansion of regulatory T cells with a superagonistic anti-CD28 monoclonal antibody (CD28-SA) very efficiently protects from acute Graft versus Host Disease, a major complication in leukemia patients receiving an allogeneic bone marrow or stem cell transplantation. The figure shows H&E-stained large bowel sections. Compared to T cells after control treatment, treatment with a CD28-SA prevented infiltration of T cells into the gut wall and wide-spread destruction of colonic crypts. Published in (Beyersdorf N et. al. (2009) Blood 114:4575-4582).

SELECTED PUBLICATIONS

Camara M, **Beyersdorf N**, Fischer HJ, Herold MJ, Ip CW, van den Brandt J, Toyka KV, Taurog JD, Hünig T, Herrmann T, Reichardt HM, Weishaupt A, Kerkau T (2013) *CD8+ T cell help is required for efficient induction of EAE in Lewis rats.* **Journal of Neuroimmunology** 15; 260(1-2):17–27

Kerstan A, **Beyersdorf N**, Stoevesandt J, Trautmann A (2013) *Wasp venom immunotherapy expands a subpopulation mof CD4+ CD25+forkhead box protein 3-positive regulatory T cells expressing the T-cell Receptor Vb2 and Vb5.1 chains.* **The Journal of Allergy and Clinical Immunology** 130(4):994-996

Hofmann U*, **Beyersdorf N***, Weirather J, Podolskaya A, Bauersachs J, Ertl G, Kerkau T, Frantz S (2012) *Activation of CD4+ T lymphocytes improves wound healing and survival after Experimental myocardial infarction in mice.* **Circulation** 125(13):1652-1663 * equal contribution

3.3. INSTITUTE FOR FOR VIROLOGY AND IMMUNOBIOLOGY

3.3.2. T CELL BIOLOGY

Innate and adaptive immunity interact to provide the host with a highly efficient defence against pathogenic microorganisms. T cells crucially contribute to adaptive immunity and, further, orchestrate the immune response as a whole. Apart from fighting microbial pathogens T cells also play an important role in providing immunity against cancer as well as the development of harmful autoimmunity. Therefore, our group has a long-standing interest in cell surface receptor-mediated T cell activation and how T cell responses can be modulated therapeutically.

DR. NIKLAS BEYERSDORF
INSTITUTE FOR
VIROLOGY AND
IMMUNOBIOLOGY

Institut für Virologie und Immunbiologie
Versbacher Str. 7
D-97078 Würzburg
T +49 (0)931-3181527
M niklas.beyersdorf@vim.uni-wuerzburg.de

INTRODUCTION

In order to become activated, T cells need to recognise a foreign antigen via their T cell receptor and they also need to receive a co-stimulatory signal resulting from ligation of the CD28 molecule expressed by the T cells. The requirement for co-stimulation helps to prevent inappropriate activation of T cells, which is potentially dangerous for the host as it may lead to immunopathology and autoimmune diseases. In the case of autoimmune diseases it is self-peptides, i.e. peptides derived from the normal protein spectrum of the body, which are recognised by auto-reactive T cells. A subset of T cells receiving great attention over the last 15 years consists of the CD4+ regulatory T cells. These cells are themselves highly auto-reactive but instead of inducing immunopathology they are very potent inhibitors of autoimmunity and modulators of immune responses to microorganisms.

To sufficiently protect the host from pathogens as diverse as viruses, bacteria, parasites and pathogenic fungi, the immune system needs to be able to mount very different forms of responses. The downside to this flexibility, however, is that an immune response which is appropriate to kill, for example, a parasite (Th2) may completely fail to protect against a viral infection (Th1) and vice versa. So, in order to evade destruction by the immune system pathogens can either attempt to straightforwardly inhibit immune responses or to divert them into an innocuous direction.

RESEARCH HIGHLIGHTS

A key aspect of our work over the last few years has been to better understand how one can therapeutically tackle unwanted T cell responses leading to immunopathology. Here, we found that targeting the co-stimulatory molecule CD28 with certain monoclonal antibodies preferentially provides regulatory CD4+ T cells with an advantage over conventional, including pathogenic, T cells thus enabling the regulatory T cells to suppress autoimmunity. This approach may in the future be translated into novel therapies for diseases like multiple sclerosis or rheumatoid arthritis. Moreover, we have recently shown that CD8+ T cells also contribute to the development of autoimmunity in a rat model of multiple sclerosis making them a potential target for therapeutic intervention.

In experimental models of T cell therapy for leukemia, i.e. allogeneic bone marrow transplantations, we have defined novel protocols which prevent the life-threatening acute Graft versus Host Disease (aGvHD), while not depriving the T cells of their capacity to destroy leukemia cells, referred to as the Graft versus Tumor (GvT) effect. Again, we have used anti-CD28 monoclonal antibodies to achieve this separation of the wanted GvT effect from the disastrous aGvHD. While our earlier work showed that these forms of aGvHD prevention depended on regulatory T cells we have now also defined approaches which do not require regulatory T cells. These are particularly attractive for the treatment of patients as in humans obtaining large numbers of regulatory T cells has been notoriously difficult.

Apart from aGvHD and cancer relapse, leukemia patients undergoing bone marrow transplantations are also threatened by opportunistic fungal infections by *Apergillus*

as a molecule that is mandatory for Vγ9Vδ2 TCR-mediated activation. New reporter cells have enabled us to show that the ubiquitously expressed BTN3A1 - a member of the B7-family – functions within the presenting cell and not as a co-stimulatory receptor expressed by the T cell. Currently, we are comparing Vγ9Vδ2 TCR-mediated activation by PAg with that induced by a BTN3A1 specific agonistic antibody. An initial analysis has revealed that in addition to the *BTN3A1* gene at least one other gene on human chromosome 6 is required for Vγ9Vδ2 T cell activation by PAg while activation by the agonistic antibody displays no such requirement.

Finally, we have shown co-emergence of Vγ9, Vδ2 and BTN3 genes in placental mammals and identified the first non-primate species that express these genes. Curiously, all three genes have been preserved in only a few species. Amongst them are alpaca *(Vicugna pacos)*, from which we have expressed a functional Vγ9Vδ2 TCR, and the nine-banded armadillo *(Dasypus novemcinctus)*, a natural host and model of infection for *Mycobacterium leprae*.

FUTURE DIRECTIONS

In the future we will complete the analysis of ligand recognition by rat iNKT and identify links between the iNKT TCR repertoire and iNKT cell function. We will also attempt to identify type II NKT cells in the rat and iNKT cells or other non-conventional T cells (e.g. MAIT) cells in other animal models. The goal of this will be to enable researchers to analyze the biological significance of these cells in different disease models.

We will also aim to understand the molecular determinants of TCR-mediated activation of Vγ9Vδ2 T cells. A major aim is to identify which chromosome 6 located gene(s) are necessary for PAg-presentation. Identification of such gene(s) will provide insight into the mechanisms of PAg-presentation and also be mandatory for the generation of transgenic mouse models for PAg-reactive Vγ9Vδ2 T cells in infection and cancer. Similarly, the direct identification of Vγ9Vδ2 T cells in non-primate species will help to reveal their role in naturally occurring infections and to gain insights in evolution of non-conventional T cells.

SELECTED PUBLICATIONS

Monzon-Casanova E, Paletta D, Starick L, Muller I, Sant'Angelo DB, Pyz E, **Herrmann T** (2013) *Direct identification of rat iNKT cells reveals remarkable similarities to human iNKT cells and a profound deficiency in LEW rats.* **European Journal of Immunology** 43: 404-415

Looringh van Beeck FA, Leegwater PA, **Herrmann T,** Broere F, Rutten VP, Willemse T, Van Rhijn I (2013) *Tandem repeats modify the structure of the canine CD1D gene.* **Animal Genetics** 44: 352-355

Karunakaran MM, Gobel TW, Starick L, Walter L, **Herrmann T** (2013) *Vgamma9 and Vdelta2 T cell antigen receptor genes and butyrophilin 3 (BTN3) emerged with placental mammals and are concomitantly preserved in selected species like alpaca (Vicugna pacos).* **Immunogenetics** in press

Harly C, Guillaume Y, Nedellec S, Peigne CM, Monkkonen H, Monkkonen J, Li J, Kuball J, Adams EJ, Netzer S, Dechanet-Merville J, Leger A, **Herrmann T**, Breathnach R, Olive D, Bonneville M, Scotet E (2012) *Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human gammadelta T-cell subset.* **Blood** 120: 2269-2279

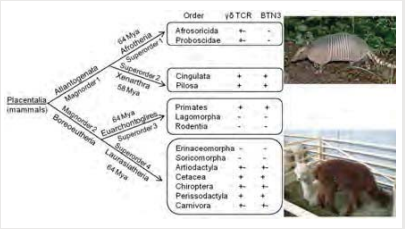


Fig. 1: Molecular determinants of the response to phosphoantigens emerged with placental mammals and are maintained in some non-primate species. Graph depicts branching of placental mammals and preservation of in situ translatable Vγ9, Vδ2 and BTN3 genes. This preservation is restricted to single plylogentic groups, which do not include species used as small animal models. Examples for species with conserved, presumably functional genes are nine banded armadillo (cingulata) and alpaca (perissodactyla). Identification and analysis of Vγ9Vδ2 T cells in these species is expected to define common determinants of Vγ9Vδ2 T cell responses.

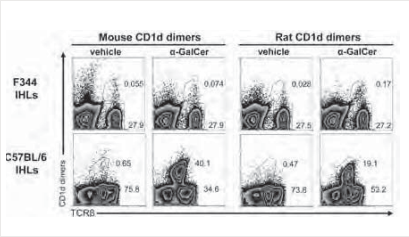


Fig. 2: Different frequencies of rat and mouse iNKT cells. Picture depicts immuno flow cytometry of intrahepatic lymphocytes (IHL) of rat (F344) and mouse (C57BL/6) . Both were stained with rat- or mouse CD1d-Ig dimers loaded with antigen (αGalCer) or vehicle (negative control) and stained against TCRβ chain. Rat CD1d-dimers bind to rat (F344 + rCD1d) but not to mouse iNKT cells (F344 + mCD1d). The rat iNKT cell frequency is very low compared to that mouse iNKT cells (C57BL/6 + mCD1d). About half of mouse iNKT cells are stained by αGalCer loaded mouse CD1d dimers (C57BL/6 + rCD1d)

3.3. INSTITUTE FOR VIROLOGY AND IMMUNOBIOLOGY

3.3.3. IMMUNOGENETICS

Non-conventional T cells recognize non-peptide antigens and act as bridge between the adaptive and innate immune systems. We aim to understand their antigen-recognition and co-evolution of antigen-receptors and antigen-presenting molecules.

PROF. THOMAS HERRMANN
INSTITUTE FOR VIROLOGY AND IMMUNOBIOLOGY

Institut für Virologie und Immunbiologie
Versbacher Str. 7
D-97078 Würzburg
T +49(0)931-3181538
M herrmann-t@vim.uni-wuerzburg.de

INTRODUCTION

The T cell antigen-receptors (TCR) of non-conventional T cells do not recognize pathogen-specific antigens but classes of pathogen-derived and stress-induced molecules and thus act as pattern recognition receptors. These features and characteristic TCR rearrangements differ from MHC-restricted TCR. Functionally, non-conventional T cells serve as bridge between adaptive and innate immunity. Our research focuses on two types of non-conventional or "innate" T cells. Both are defined by their eponymous TCR: The iNKT cells and the Vγ9Vδ2 T cells.

i(nvariant)NKT cells carry TCR with an "invariant" α chain defined by a Vα14Jα18 rearrangement. They are effectors with anti-microbial, anti-tumor and immunomodulatory activity. Their major antigens are glycolipids that associate with non-polymorphic MHC I like CD1d cell surface molecules. Their strongest antigens are bacterial sphingolipids with α-anomeric linked carbohydrates (e.g. α-Galactosyl ceramid). Such ligands bound to oligomers of CD1d can be used to directly identify iNKT.

Vδ2 T cells have so far been identified only in humans and higher primates where they are expanded in many infectious diseases. Their TCR bear characteristic rearrangements of the γ-chain (Vγ9JP), which is paired with Vδ2 containing δ-chains. They recognize pyrophosphorylated metabolites of isoprenoid synthesis, called phosphoantigens (PAg). The most potent PAg is (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) which is the immediate precursor of iso-pentenyl pyrophosphate (IPP) in the non-mevalonate pathway found in many eubacteria and in apicomplexa such as *Plasmodium spp*. HMBPP is ten-thousand-fold more potent than IPP, the central compound of either pathway of isoprenoid synthesis. Increased IPP levels in host cells also lead to Vγ9Vδ2 T cell-activation and are found in some tumors, after administration of aminobisphosphonates (e.g. zoledronate) and also upon infection.

RESEARCH HIGHLIGHTS

During the last years we have investigated iNKT cells using the rat *(Rattus norvegicus)* as a model organism. We have developed new reagents (rat CD1d dimers) that have enabled us to directly identify of rat iNKT cells. The low frequency of these cells in rats is similar to the situation in humans and in contrast to that found in mice (0.1-0.2% of intra hepatic T cells in rat compared to 30% in mice). Furthermore, Lewis rats, which are the most widely used rat model of organ-specific autoimmunity, are essentially devoid of iNKT cells. Importantly, the "invariant" α-chain of rat iNKT TCR is not truly invariant since rats have up to 9 functional Vα14 genes. We have shown that differential Vα14 usage affects TCR binding of CD1d-antigen-complexes and defined unexpected regions of the TCR-α-chains to be involved in this interaction. Finally, we are analyzing iNKT TCR, and CD1d and iNKT cells of cotton rats in collaboration with Stefan Niewiesk, Ohio State University, Columbus, Cotton rats are used as models for human viral diseases such as measles and respiratory syncytial virus and will be used to analyse iNKT cell function in viral infection and vaccination We have also studied the molecular determinants of antigen-recognition in Vγ9Vδ2 T cells. An important step was the identification of butyrophilin 3 A1 (BTN3A1)

3.3. INSTITUTE FOR FOR VIROLOGY AND IMMUNOBIOLOGY

3.3.4. IMMUNE REGULATION

Infective microbes have developed a number of strategies to avoid elimination by the host's immune system. We are investigating how different pathogens manipulate dendritic cells (DCs) and myeloid-derived supppressor cells (MDSCs) to stimulate regulatory T cells and suppress effector T cell responses.

PROF. MANFRED B. LUTZ

INSTITUTE FOR VIROLOGY AND IMMUNOBIOLOGY

Institut für Virologie und Immunbiologie
Versbacher Str. 7
D-97078 Würzburg
T +49 (0)931-3181553
M m.lutz@vim.uni-wuerzburg.de

INTRODUCTION

Under steady state conditions myeloid/monocytic precursor cells have the capacity to differentiate into neutrophils, macrophages or dendritic cells (DC). MDSC are characterized by their intermediate developmental stage between myeloid precursors and differentiated myeloid cell stages. Upon undergoing activation at this intermediate stage, myeloid precursors become MDSC, while activation of further developed DC or macrophages will result in immunogenic activated DC or macrophages.

DCs are well established as antigen presenting cells that can initiate $CD4^+$ and $CD8^+$ T cell immune responses against pathogens. DCs are not only engaged to prime T cell responses via antigen presentation (signal 1) and costimulation (signal 2) but also contribute to the further differentiation of T cells especially by the release of cytokines (signal 3). For the $CD4^+$ T cell subset this process has been termed polarization into T helper cell (Th) subsets. We are especially interested in the question which pathogen-, danger- or inflammatory signals and signaling receptors instruct DCs to induce T cell polarization into specific Th1, Th2, Th17 or $CD4^+$ regulatory T cell subtypes.

Besides these immunogenic functions it has recently become clear that immature DCs are not only resting cells waiting for pathogens, but that immature and semi-mature DC are constantly involved in mediating immune tolerance to peripheral self-antigens. While this physiological process, which prevents autoimmunity, can be exploited therapeutically to treat autoimmunity, it is also widely exploited by pathogens.

Tolerogenicity of DCs is mainly directed by their maturation stage. For every major subset in the mouse ($CD8\alpha^+$, $CD4^+$, monocyte-derived BM-DCs, plasmacytoid DCs) immature stages have been shown to act tolerogenically on T cells and only after maturation convert to immunogenic cells. We have found that DC maturation can lead to semi-mature or fully mature stages. They are distinguishable mainly by the presence or absence of proinflammatory cytokine production, while the expression of MHC II-, costimulatory molecules and the CCR7 homing receptor remain similar. Also several groups have previously shown that such semi-mature DCs appeared tolerogenic in different models of autoimmunity.

MDSC can control immune responses by directly or indirectly suppressing T cell responses against tumors and pathogens in mice and humans. The effector mechanisms by which MDSC exert their suppression include the activation of the L-arginin cleaving enzyme arginase-1 and the inducible nitric oxide synthase (iNOS) resulting in NO production and suppression of T cell function and proliferation. One way of activation occurs though the triggering of pathogen recognition receptors such as Toll-like receptors together with the recognition of IFN-γ.

RESEARCH HIGHLIGHTS

We have previously described that the Th2 instruction potential of DCs is characterized by their semi-maturation state and reflected by a genetic pattern (detected by mRNA microarrays) that encompasses only an inflammatory signature but no specific instructive

signals. In this respect TNF and selected *Trypanosoma brucei* antigens were highly overlapping in inducing gene expression changes and DC semi-maturation. In contrast, the DC maturation by LPS resulted in Th1 instruction and induced a much more extensive gene expression profile which also included the same inflammatory genes (leading to full DC maturation), including IL-12. These data indicate that quantitative differences in DC maturation can direct Th1/Th2 polarization.

Similar DC maturation signatures could be observed using different larval stages or secretory products of *Echinococcus multilocularis* on murine DCs. In another study specific antigen components of Mycobacterium tuberculosis (Mtb) were found to stimulate semi-maturation of human DCs, which also induced Th2 polarisation in T cells.

Besides the instructive roles of DCs for tolerance or immunity by T cells, overshooting effector cell responses can also be controlled by MDSC.

In collaboration with the Walzl group in South Africa we have found that Mtb patients show increased frequencies of MDSC in their blood.

FUTURE DIRECTIONS

More recent gene expression analyses of DCs upon exposure to pathogens or pathogen products have enabled us to follow not only Th1 or Th2 instructing DC-signatures but also the pathways by which DCs polarize naive T cells into Th17 responses. We are currently investigating the underlying molecular mechanisms involved in these processes.

We are also interested in understanding how Mtb targets MDSC to evade phagolysosomal degradation and activates their immunosuppressive activity. We are currently determining the role of newly identified entry receptors and specific endosomal compartments in this immune evasion process.

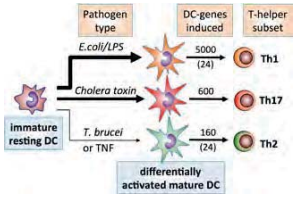


Fig. 1:Signal strength and the antigenic nature determine polarized T-helper cell responses. It is well established that antigenic nature of pathogens instructs differentially polarized $CD4^+$ T-helper cell subsets and that dendritic cells (DC) play a decisive role in this process. How DC integrate the different pathogen signals and how they transform them into instructive signals for T cells is less well understood. DC instructing Th1 and Th2 responses are characterized mainly by the number of genes that were regulated after activation.

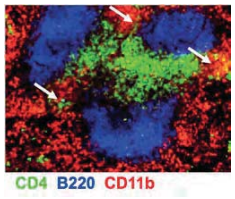


Fig. 2: Mycobacteria induce myeloid-derived suppresor cells (MDSC). Tuberculosis is characterized by an insufficient immune response in eliminating the Mycobacterium tuberculosis (Mtb). Immunization or infection with Mtb not only induces immune responses against Mtb, but also massively accumulates $CD11b^+$ cells in the bridging channels between $B220^+$ B cell follicles and the red pulp of spleens in mice, many of them identified as MDSC. These $CD11b^+$ MDSC interact with $CD4^+$ T cells (white arrows). The consequences of these interactions for immune suppresion are currently investigated.

SELECTED PUBLICATIONS

du Plessis N, Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, van Helden PD, **Lutz MB**, Walzl G (2013) *Increased Frequency of Myeloid-derived Suppressor Cells during Active Tuberculosis and after Recent Mycobacterium tuberculosis Infection Suppresses T-Cell Function.* **American Journal of Respiratory and Critcal Care Medicine** 188, 724-732

Heuer M, Behlich AS, Lee JS, Ribechini E, Jo EK, **Lutz MB** (2013) *The 30-kDa and 38-kDa antigens from Mycobacterium tuberculosis induce partial maturation of human dendritic cells shifting CD4(+) T cell responses towards IL-4 production.* **BMC Immunology** 3;14:48

Lutz MB (2013) *How quantitative differences in dendritic cell maturation can direct Th1/Th2-cell polarization.* **Oncoimmunology** 2, e22796

Lutz MB (2012) *Therapeutic potential of semi-mature dendritic cells for tolerance induction.* **Frontiers in Immunology** 18;3:123

Nono JK, Pletinckx K, **Lutz MB**, Brehm K (2012) *Excretory/Secretory-Products of Echinococcus multilocularis Larvae Induce Apoptosis and Tolerogenic Properties in Dendritic Cells In Vitro.* **PLoS Neglected Tropical Diseases** 6, e1516.86

03.4

Institute for Virology and Immunobiology – Chair of Virology

AXEL RETHWILM
JÜRGEN SCHNEIDER-SCHAULIES
SIBYLLE SCHNEIDER-SCHAULIES

The Institute for Virology and Immunobiology is part of the Medical Faculty at the University of Würzburg. Prof. Dr. Axel Rethwilm has held the Chair of Virology since 2003.

Virology-focused research at the institute centres on analysing the regulatory principles involved in viral replication and gene expression. In addition, researchers are investigating the pathogenesis of several viruses and elucidating the molecular basis for the occurrence of resistance to antiviral compounds. Research is also being conducted into the development of viral vectors to be used for gene therapy. The institute is responsible for providing virus diagnostics to the University Clinics and members provide virology lectures for medical, biomedical, biochemistry and biology students.

3.4. INSTITUTE FOR FOR VIROLOGY AND IMMUNOBIOLOGY

3.4.1. FOAMY VIRUSES

Foamy viruses (FV) are endemic in most non-human primates, cats, cattle and horses and undergo a replication pathway that distinguishes them from all other retroviruses. The group is interested in the molecular mechanism of replication and the development of resistance to antiviral compounds.

PROF. AXEL RETHWILM
INSTITUTE FOR
VIROLOGY AND
IMMUNOBIOLOGY

Institut für Virologie und Immunbiologie
Universität Würzburg
Versbacher Str. 7
D-97078 Würzburg
T +49-931-3181672
M rethwilm@vim.uni-wuerzburg.de

INTRODUCTION

The family of retroviruses has been subdivided into the orthoretrovirus subfamily (harbouring all well known retroviruses from murine leukemia virus (MLV) to the lentivirus HIV) and the subfamily of spumaretrovirus (made up only of the genus foamy viruses). Spuma- or foamy viruses (FVs) have co-evolved with their natural hosts and human infections are very rare. However, they can be detected by serological and molecular methods, sometimes decades following the primary infection by a FV from a NHP. Spread of these zoonotic infections in the human population has not been reported.

FV infection initiates upon binding to a yet unknown receptor on the target cell before being taken up by receptor-mediated endocytosis. The capsids enter the cytoplasm and traffic towards the microtubule organising center (MTOC) where they are disassembled by viral and cellular proteases. Entry of the FV preintergration complex requires nuclear membrane breakdown and the FV genes are subsequently expressed upon establishment of the proviral state. The processed viral RNAs are exported form the nucleus and translated in the cytoplasm, preassembly of capsids occurs at the MTOC. Unlike the orthoretroviruses, FVs reverse transcribe their packaged genome after capsid assembly, and the release of viral like particles is dependent on the cognate viral Env glycoprotein.

MicroRNAs (miRNAs) are small RNAs that are on average 21nts in length and expressed by many eukaryotes to post-transcriptionally regulate gene expression. They have been shown to play an important role in many important physiological processes such as development and the immune response. Viruses also encode miRNAs within their genomes and to date over 300 viral miRNAs have been identified, although for the vast majority their functional role is unclear. The characterised miRNAs have been shown to play important role in the latent-lytic switch, promoting viral replication, influencing host cell survival and modulating the immune response. Most viral miRNAs have been identified in DNA viruses.

RESEARCH HIGHLIGHTS

During the last few years we have been investigating several aspects of FV biology. Although the cellular receptor for FV is unknown, the large number of permissive cell types originating from different species suggests that it must be an almost ubiquitous factor. We have shown that permissivity of cells lines positively correlates with the levels of cell surface heparin sulfate. Removal of heparin sulfate significantly decreased permissivity while addition of soluble heparin sulfate inhibited infection.

We have also been interested in understanding how FV assembly and maturation differ from the orthoretroviruses. The FV Gag gene that encodes the viral structural genes is processed by the viral protease. We have shown that this Gag processing is essential for full-length cDNA synthesis.

Recently, in collaboration with the group of Christopher Sullivan (Texas, USA) we have identified clusters of transcribed miRNA genes in the viral long terminal repeats (LTRs) of several FV. These miRNAs undergo an unconventional biogenesis process since while they are transcribed by RNA pol III they are processed by Drosha. Characterisation of two of the candidate miRNAs revealed that they are expressed during viral infection and display sequence similarity to several human miRNAs including the lymphoproliferative miR-155 and the IFN-suppressive miR-132. These miRNAs provide new insight into understanding the biology of FV and their use as vectors.

Retroviruses such as HIV-1, are popular targets for the development of antiviral compounds, but they are known to quickly develop resistance against these drugs. The exact mechanisms by which retroviruses develop resistance are, however, often not known. To elucidate how this is achieved on a molecular level we are performing functional and structural investigations using isolates of both HIV and FV.

In cooperation with Prof. Stich from the Medical Mission Hospital, Würzburg and Dr. Majinge and Dr. Kalluvya from Bugando Medical Center in Mwanza, Tanzania, we are undertaking large clinical trials targeting HIV infection in Sub-Saharan Africa. In a randomized interventional clinical study ("ProCort1") we are studying the effects of low-dose prednisolone treatment on the progression of HIV infection in antiretroviral therapy-naïve HIV patients. We are also investigating the spread of HIV drug-resistance into the therapy-naïve population and the prevalence of HIV-associated neurological disorders ("HAND1-study") in Tanzania and its association with different viral subtypes. These collaborations with East Africa benefit from the logistic and scientific background provided by the DFG-funded international research-training group (IRTG 1522) between the Universities of Stellenbosch and Cape Town (South Africa) and the University of Würzburg.

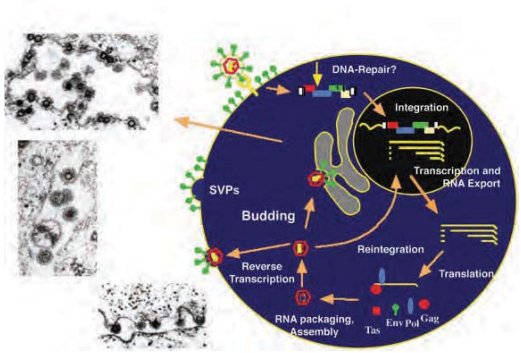


Fig. 1: The Foamy virus replication cycle

SELECTED PUBLICATIONS

Rethwilm A, Lindemann, D (2013) *Foamy Viruses.* In: **Fields Virology**. Edited by Knipe DM, Howley PM; vol 6th ed.

Kincaid RP, Chen Y, Cox JE, **Rethwilm A** Sullivan CS (2013) *Non-canonical miRNA biogenesis gives rise to retroviral mimics of lymphoproliferative and immunosuppressive host miRNAs.* **mBio** in press

mutations as caused by the viral RNA-dependent RNA polymerase (vRdRp) in the absence of A3G. These findings suggest that A3G (directly or indirectly) impairs the activity and fidelity of the viral RNA polymerase.

It has been unclear if CDV can use the human MV uptake receptors (CD150, nectin-4), or if the virus must adapt and acquire mutations in the viral envelope proteins before it can use them. We have found that human nectin-4 can be used by CDV without any adaptive mutations, and human CD150 can be used after selection of a single mutation in the haemagglutinin (H) of CDV at position 540 Asp→Gly (D540G). Modelling of the structure of CDV-H with the interacting receptor CD150 (based on the crystal structure for MV-H), revealed that this interaction occurs in a region that differs between human CD150 and canine CD150 (Fig. 2). These findings nicely demonstrate how mutation and selection respond to a certain molecular force to achieve functional interaction between a virus and its cellular receptor.

FUTURE DIRECTIONS

We will further investigate the role of host cell factors in morbillivirus infections to identify critical steps that can be targeted for therapeutic interventions. This includes analysing individual CDV proteins in human cells to understand the basis of species specificity of their interactions with factors of the intrinsic and innate immune system. In addition, we will investigate the role of specific membrane lipids, and the enzymes that process them such as sphingomyelinases, during viral infections and the immune response. This will be performed both in tissue culture and using the mouse model of persistent CNS infection within the framework of the DFG-supported research unit (Forschergruppe; FOR 2123) investigating "Sphingolipid dynamics in infection control".

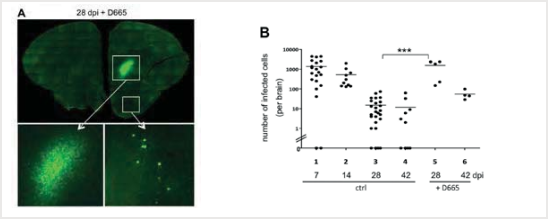


Fig. 1: Expansion of regulatory T lymphocytes upon treatment with the superagonistic CD28 antibody D665 enhances virus replication and spread. Coronal brain sections from complete mouse cerebra infected with an enhanced GFP expressing recombinant MV (MV-eGFP). (A) The MV-eGFP infected neurons (green) can be seen in cerebra at day 28 post infection. (B) The numbers of infected eGFP+ neurons per brain increased upon expansion of regulatory T lymphocytes after the acute infection (between day 21 and 26; compare the number of infected cells between lane 3 and lane 5; Reuter et al., 2012).

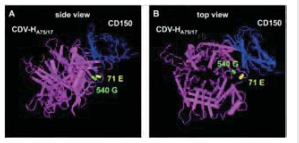


Fig. 2: Structural model of the CDV-H-CD150 interaction. Side (A) and top (B) view of the globular head of CDV-H (amino acids 188 to 602) presented in magenta with highlighted amino acid 540 G (green), and of the interacting first V-like domain of human CD150 (amino acids 32 to 140) shown in blue with highlighted amino acid 71 E (yellow; Bieringer et al., 2013). The structures were modelled based on the crystal structure of MV-H bound to CD150 as published by Hashiguchi et al., PNAS 104:19535-19540, 2007, using the program Phyre2 and protein structure prediction on the web, and 3DLigandSite (Imperial College, London).

SELECTED PUBLICATIONS

Ader N, Brindley M, Sanchez MA, **Örvell** C, Horvat B, Hiltensperger G, **Schneider-Schaulies J,** Vandevelde M, Zurbriggen A, Plemper RK, Plattet P (2013) *Mechanism for active membrane fusion triggering by morbillivirus attachment protein.* **Journal of Virology** 87:314-326

Bieringer M, Han WJ, Kendl S, Khosravi M, Plattet P, **Schneider-Schaulies J** (2013) *Experimental adaptation of canine distemper virus (CDV) to the human entry receptor CD150.* **PLoS One** 8: e57488

Rasbach A, Abel T, Münch RC, Boller K, **Schneider-Schaulies J**, Buchholz CJ (2013) *Measles virus cell entry: Receptor attachment and fusion-helper function can be separated to enhance titers of pseudotyped lentiviral vectors.* **Journal of Virology** 87:6246-56

Fehrholz M, Kendl S, Prifert C, Weissbrich B, Lemon K, Rennick L, Duprex PW, Rima BK, Holmes RK, Malim MH, **Schneider-Schaulies J** (2012) *The innate antiviral factor APOBEC3G targets replication of measles, mumps, and respiratory syncytial virus.* **Journal of General Virology** 93:565-576

Reuter D, Sparwasser T, Hünig T, **Schneider-Schaulies J** (2012) *Foxp3+ regulatory T cells control persistence of viral CNS infection.* **PLoS One** 7: e33989

3.4. INSTITUTE FOR FOR VIROLOGY AND IMMUNOBIOLOGY

3.4.2. MORBILLIVIRUS PATHOGENESIS

Morbilliviruses, which include the human pathogenic measles virus, cause devastating diseases in their specific hosts. The group is interested in the mechanisms of virus uptake, the function of host cell factors, the species specificity of infections, and the corresponding immune response.

PROF. JÜRGEN SCHNEIDER-SCHAULIES
INSTITUTE FOR VIROLOGY AND IMMUNOBIOLOGY

Institut für Virologie und Immunbiologie
Versbacher Str. 7
D-97078 Würzburg
T +49 (0)931-3181566
M jss@vim.uni-wuerzburg.de

INTRODUCTION

Measles virus (MV) and canine distemper virus (CDV) belong to the genus *Morbillivirus* of the family Paramyxoviridae. Measles is not a „simple" children's disease, but can cause a number of complications such as diarrhoea, pneumonia, blindness, and various forms of encephalitis. Worldwide, more than 100,000 children still die every year due to acute measles infections. In addition, after the acute infection of predominantly very young children the virus may persist for longer times in the host, which occurs with a frequency of approximately 1: 1,000 – 10,000 cases. After an average 6 to 12 years of persistence MV may replicate and spread in the brain and cause the lethal disease subacute sclerosing panencephalitis (SSPE). Vaccination against measles not only protects from the acute disease, but also from SSPE.

Since an effective vaccine is available, the World Health Organisation (WHO) has declared the aim of eradicating measles before 2020, however, due to socioeconomic problems this target will not be easily achieved. If the measles eradication programme is successful, there remains the threat that other animal pathogenic morbillivirusessuch as CDV could cross the species barrier and adapt to humans and establish a new human pathogenic morbillivirus. Therefore, one of our aims is to investigate the mechanisms that determine the species specificity of infection.

A mouse model of persistent measles virus infection of the CNS, which partially models the human disease SSPE, has been established and has the potential to be used to analyse the antiviral immune response and also the effects of antiviral substances on persistent infection. This approach promises to provide new insight into the mechanisms that influence the infection such as alterations in the cell membrane and intracellular host factors as intrinsic factors or as part of the innate immune response.

RESEARCH HIGHLIGHTS

The group has been investigating the role of the adaptive immune response and regulatory T cells (Treg) in persistent brain infections. This has revealed that the expansion of regulatory T cells after the acute phase of the infection by superagonistic anti-CD28 antibodies (D665) leads to an enhancement of MV infection of the CNS (Fig. 1). In agreement with this, the depletion of Treg cells by diphtheria toxin treatment (in DEREG-mice expressing a human diphtheria toxin receptor-GFP fusion protein under the control of the Foxp3 promoter) reduced CNS infection. This clearly demonstrates that the persistent viral CNS infection is under sustained immunological control and that this control is itself modulated by Treg cells. It further demonstrates that this mouse model can be used to investigate and characterize antiviral compounds.

Another focus of the group is to investigate the role of an intrinsic antiviral factor, the cytidine deaminase APOBEC3G (A3G), which has previously been implicated in interfering with HIV infection. Interestingly, in the presence of A3G MV transcription and protein expression was reduced by 50-70%, and there was an increase in the mutation rate of the viral genome. However, in contrast to HIV infections, A3G-specific hypermutations were not observed. The pattern of detected mutations was similar to the pattern of background

partment to the cell surface together with the acid sphingomyelinase. We are currently investigating if sphingomyelinase activation and formation of ceramide enriched membrane domains support viral fusion and/or sorting in specific compartments.

MV infection also induced the activation of neutral and acid sphingomyelinase in T cells, which almost entirely accounted for the observed inhibition of actin cytoskeletal dynamics in these cells. Based on two genetic screening approaches we have identified candidate receptors that potentially mediate T cell silencing upon MV interaction and we are investigating their coupling to sphingomyelinase activation. Furthermore, we are studying the molecular targets of receptor-mediated sphingomyelin breakdown in T cells, especially those crucially involved in regulating cytoskeletal and microcluster dynamics, as well as their roles in T cell motility, ability to conjugate to DCs and to expand. We hypothesise that when inappropriately catalyzed by viral interaction, ceramide release interferes with the activity of the immune synapse and we are therefore investigating whether sphingolipid dynamics per se might regulate the threshold of T cell activation.

FUTURE DIRECTIONS

During the next few years we will investigate the role of virally induced sphingomyelin breakdown on pattern recognition receptor crosstalk, downstream signaling and innate defenses in APCs. In T cells, we will explore the hypothesis that efficiency and patterning of ceramide release acts to modulate immune synapse activity and identify molecular targets there. On a broader level, the impact of sphingolipid dynamics on the uptake and trafficking of pathogens other than MV and its role in their cell autonomous, innate and adaptive immune control will be investigated in collaboration with the Research Unit FOR2123 Sphingolipid dynamics in infection control.

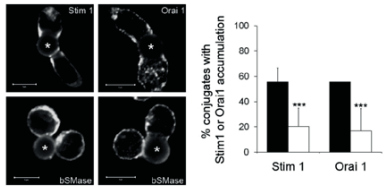


Fig. 1: T cells pretreated with bacterial sphingomyelinase (bSMase) were conjugated to costimulatory beads (indicated by asterisks) and translocation of the ER Ca2+ sensor Stim1 and the CRAC channel component Orai I to the stimulatory interface was analyzed.

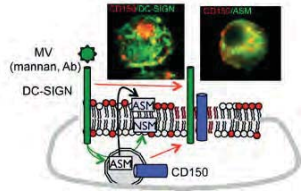


Fig. 2: DC-SIGN ligation activates acid sphingomyelinase (ASM) in DCs, which promotes surface display of the enzyme and formation of ceramide enriched platforms. There, DC-SIGN colocalizes with CD150 which is co-transported with ASM from an intracellular compartment.

SELECTED PUBLICATIONS

Riedel A, Mofoto B, Avota E, **Schneider-Schaulies S**, Meijntjes A, Mulder N, Kneitz S (2013) *Accumulation of splice variants and transcripts in response to PI3K inhibition in T cells: potential role of their gene products in cell silencing.* **PLoS One** 8:e50695

Avota E, Koethe S, **Schneider-Schaulies S** (2013) *Membrane dynamics and interactions in measles virus dendritic cell infections.* **Cellular Microbiology** 15:161-169

Koethe S, Avota E , **Schneider-Schaulies S** (2012) *Measles virus transmission from dendritic cells to T cells: formation of synapse like interfaces concentrating viral and cellular components.* **Journal of Virology** 86:9773-9781

Schneider-Schaulies J, **Schneider-Schaulies S** (2012) *Viral Infections and sphingolipids.* In: **Sphingolipids in Disease**. Gulbins E, Petrache I eds. pp. 321-340

3.4. INSTITUTE FOR FOR VIROLOGY AND IMMUNOBIOLOGY

3.4.3. VIRAL IMMUNOMODULATION

Viruses can modulate the host immune response to facilitate their survival and replication. Using measles virus (MV) as a model system, the group aims to decipher how viruses take advantage of membrane receptors to interfere with the activation of antigen presenting and T cells.

PROF. SIBYLLE SCHNEIDER-SCHAULIES

INSTITUTE FOR VIROLOGY AND IMMUNOBIOLOGY

Institut für Virologie und Immunbiologie
Versbacher Str. 7
D-97078 Würzburg
T +49 (0)931-3181566
M s-s-s@vim.uni-wuerzburg.de

INTRODUCTION

Most viruses have evolved strategies to evade immune surveillance in order to survive and spread in their hosts. Many of them do so by encoding non-structural proteins that confer resistance to cell autonomous or innate immunity in their respective host cells. In addition to this strategy, a few human pathogenic viruses, including measles virus (MV), induce general immunosuppression, which in addition to dampening virus-specific immune responses favors the establishment of secondary or chronic infections.

Viral interactions with host cell receptors substantially contribute to MV induced immunosuppression such as lymphopenia, cytokine imbalance and the failure of patient-isolated T cells to expand upon stimulation. Since antigen-presenting cells, especially dendritic cells (DCs), are early targets for viral infection, receptors mediating the attachment to (DC-SIGN) and entry into (CD150) these cells critically determine viral transport to secondary lymphatic tissues and subsequent transmission to lymphocytes, which is important for viral dissemination. Furthermore, MV interactions with pattern recognition receptors such as TLR2 and DC-SIGN are also crucial for triggering DC maturation, mobilisation, subsequent activation and shaping of T cell responses. As evident from the efficient induction of antiviral immunity, processes that are required for its priming are not grossly affected by MV, while the virus alters the T cell response such as the suppression of IL-12 production. Although CD150 also mediates infection of T cells at the synaptic interface with infected DCs, other receptors are involved in promoting apoptosis or silencing of T cells.

The silencing of T cells by MV has been attributed to the interaction of the viral glycoprotein (gp) complex expressed on the surface of DCs and an unknown receptor on T cells. As a result, the T cell receptor (TCR) signaling required for expansion is inefficiently relayed together with a marked paralysis of actin dynamics in these cells which are largely devoid of actin based protrusions and unable to undergo actin reorganization. TCR dependent activation of PI3K and its downstream effectors have been identified as a major target of MV induced T cell silencing, which is compatible with the inability of these cells to exit the G1 phase.

RESEARCH HIGHLIGHTS

During the last few years we have focused on the functional consequences of dynamic membrane alterations induced by the interaction of MV with its receptors on DCs and T cells. As major components of the cell membrane, sphingolipids, especially sphingomyelin and its metabolite ceramide, have been implicated in the differential segregation of receptors and their associated signalosomes into membrane microdomains, membrane curvature and the formation of protrusions.

We have identified DC-SIGN as a sphingomyelinase activator that catalyses sphingomyelin breakdown and ceramide release in DCs. This is important not only for membrane proximal signaling and modulation of NF- B activation, but also the enhancement of MV entry into DCs by this receptor. Rather than enhancing MV binding, sphingomyelinase activation by DC-SIGN promoted the vertical sorting of CD150 from an intracellular com-

03.5

Department of Microbiology, Theodor Boveri Institute, Biocenter

THOMAS RUDEL
MARTIN FRAUNHOLZ
ROY GROSS
VERA KOZJAK-PAVLOVIC

The Department of Microbiology is part of the Biology Faculty at the University of Würzburg. Prof. Dr. Thomas Rudel has chaired the department since 2008.

The research activities at the department centre on the pathogenicity mechanisms of different microorganisms, including the manipulation of various signalling cascades, non-coding RNAs and cellular processes such as the cell death pathways in the host. In this context the infection biology of obligate intracellular bacteria such as *Chlamydia* is a major focus. Groups are also investigating the molecular basis of disseminating gonococcal infections and the host cell death induced by *Staphylococcus aureus* as well as the intracellular lifestyle of this bacterium. In addition, there is also great interest in understanding the role of (co-)infections in the onset of ovarian cancer and the signaling pathways involved. Members of the department provide microbiology and infection biology lectures and practical courses for medical, biomedical, biochemistry and biology students.

and signaling pathways that depend on caveolin-1-Y14 phosphorylation (Cav1-pY14). We have identified the p85 regulatory subunit of PI3 kinase (PI3K) and phospholipase C 1 as new, exclusive and essential interaction partners for Cav1-pY14 during the course of Por-B_{IA}-induced invasion. Activated PI3K induces the uptake of gonococci via a new invasion pathway involving protein kinase D1. Thus, we have described a novel route of bacterial entry into epithelial cells and offer the first mechanistic insight into the switch from local to invasive gonococcal infection.

We have also shown that co-infection of *C. trachomatis* and human herpesvirus-6 (HHV-6) promotes chlamydial persistence and increases viral uptake in an *in vitro* cell culture model. Furthermore, we have investigated *C. trachomatis*-induced HHV-6 activation in cell lines and fresh blood samples from patients with Chromosomally integrated HHV-6 (CiHHV-6). We observed the activation of latent HHV-6 DNA replication in CiHHV-6 cell lines and fresh blood cells without the formation of viral particles. Interestingly, HHV-6 DNA was detected in blood as well as cervical swabs from *C. trachomatis*-infected females. Low virus titers correlated with high *C. trachomatis* load and vice versa, demonstrating a potentially significant interaction between these pathogens in blood cells and in the cervix of infected patients. Our data suggest a thus far underestimated interference between HHV-6 and *C. trachomatis* with a likely impact on the outcome of the disease as consequence of co-infection.

FUTURE DIRECTIONS

In the future we will continue to investigate pathogenicity mechanisms of different bacteria. Within the research of the infection biology of obligate intracellular bacteria the function of non-coding RNA will be of special interest. Furthermore, we will continue to pursue the molecular basis of disseminating gonococcal infections and host cell death induced by *S. aureus* as well as phagosomal escape mechanisms of these bacteria. In addition, it is our goal to elucidate the significance of infections in the emergence and progression of cancer. Therefore, we aim to investigate the contribution of (co-)infections to the onset of ovarian cancer and the signaling pathways involved using suitable *in vitro* and *in vivo* models for malignant transformation.

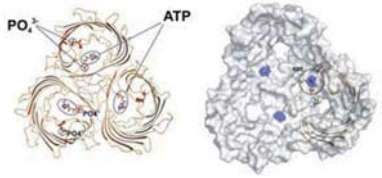


Fig. 1: Surface accessibility of phosphate-binding sites in $PorB_{IA}$.

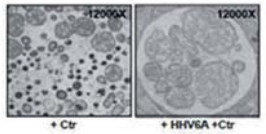


Fig. 2: Transmission electron microscopy images of chlamydial inclusions of *Chlamydia* single infected (left) and HHV6A co-infected cells(right).

SELECTED PUBLICATIONS

Prusty BK, Krohne G, **Rudel T** (2013) *Reactivation of chromosomally integrated human herpesvirus-6 by telomeric circle formation.* **PLoS Genetics** 9:e1004033

Faulstich M, Böttcher JP, Meyer TF, Fraunholz M, **Rudel T** (2013) *Pilus phase variation switches gonococcal adherence to invasion by caveolin-1-dependent host cell signaling.* **PLoS Pathogens** 9:e1003373

Prusty BK, Siegl C, Hauck P, Hain J, Korhonen SJ, Hiltunen-Back E, Puolakkainen M, **Rudel T** (2013) *Chlamydia trachomatis infection induces replication of latent HHV-6.* **PLoS One** 8:e61400

Zeth K, Kozjak-Pavlovic V, Faulstich M, Fraunholz M, Hurwitz R, Kepp O, **Rudel T** (2013) *Structure and function of the PorB porin from disseminating Neisseria gonorrhoeae.* **Biochemical Journal** 449:631-42

Prusty BK, Böhme L, Bergmann B, Siegl C, Krause E, Mehlitz A, **Rudel T** (2012) *Imbalanced oxidative stress causes chlamydial persistence during non-productive human herpes virus co-infection.* **PLoS One** 7:e47427

3.5. DEPARTMENT OF MICROBIOLOGY

3.5.1. INFECTION BIOLOGY OF BACTERIA

The group investigates pathogenicity mechanisms of the major human pathogens *Chlamydia, Neisseria gonorrhoeae,* and *Staphylococcus aureus*. Furthermore, there is a focus on bacterial and viral co-infections, particularly *Chlamydia* and herpes virus, and their impact on human diseases such as cancer.

PROF. THOMAS RUDEL
DEPARTMENT OF
MICROBIOLOGY

Lehrstuhl für Mikrobiologie
Biozentrum
Am Hubland
D-97074 Würzburg
T +49 (0)931-3184401
M thomas.rudel@biozentrum.uni-wuerzburg.de

INTRODUCTION

During the course of infection bacterial pathogens can dramatically alter host cell function to overcome innate and acquired immune responses and to inhabit their preferred niches. This requires intimate contact between host and pathogen and many bacterial pathogens have evolved strategies to directly interact with their host. In this context our group investigates (i) the infection biology of obligate intracellular bacteria *(Chlamydia, Simkania)*, (ii) the bacterial factors required for dissemination and adaptation as well as the host cell response to *Neisseria gonorrhoeae,* (iii) phagosomal escape mechanisms and host cell death induced by *Staphylococcus aureus,* and (iv) the import of proteins into mammalian mitochondria.

Many pathogens have acquired the ability to manipulate key biological processes within the host to facilitate their replication and dissemination. This includes membrane trafficking and signaling pathways as well as cell survival. The group is interested in understanding the signaling cascades that lead to inhibition or induction of apoptosis. In relation to this we are aiming to understand the role of bacterial infections in cancer, since *Chlamydia trachomatis* has been associated with cervical and ovarian cancer. For example, similar deregulated pathways are observed in ovarian cancer cells and epithelial cells after infection with *Chlamydia* and antibodies against *Chlamydia* are observed in a disproportionately large percentage of ovarian cancer patients. Furthermore, epidemiological studies have also connected human herpes virus' (HHVs) and *Chlamydia* in several conditions suggesting that co-infections may play a role in several human diseases. For instance, human herpesvirus-6 (HHV-6) DNA is frequently detected in different grades of cervical lesions. *C. trachomatis* infection has also been separately linked to cervical cancer but it is unclear, whether both these pathogens contribute to the development of the disease. HHV-6 exists in a latent form either as a nuclear episome or integrated into human chromosomes in more than 90% of healthy individuals without causing clinical symptoms. Immunosuppression and stress conditions can reactivate HHV-6 replication, which is associated with clinical complications and even death. Therefore, a main goal of our research is to understand chlamydial co-infections and how they correlate with important human diseases.

RESEARCH HIGHLIGHTS

Many pathogenic bacteria cause local infections but occasionally they also enter the blood stream, often with fatal outcome. Little is known about the mechanisms underlying the switch from local to invasive infections. In the case of *Neisseria gonorrhoeae,* phase variable type 4 pili (T4P) stabilize local infections by mediating microcolony formation and inducing anti-invasive signals. The outer membrane porin $PorB_{IA,}$ in contrast, is associated with disseminated infection and facilitates the efficient invasion of gonococci into host cells. We have demonstrated that loss of pili by natural pilus phase variation is a prerequisite for the transition from local to invasive infections. Unexpectedly, both T4P-mediated inhibition of invasion and $PorB_{IA}$-triggered invasion utilize membrane rafts

3.5. DEPARTMENT OF MICROBIOLOGY

3.5.2. CELLULAR MICROBIOLOGY

Staphylococcus aureus is endocytosed by human cells, however, some strains such as certain methicillin-resistant S. aureus (MRSA) readily escape from the phagosome thereby avoiding eradication. The group focuses on the identification of bacterial virulence factors involved in phagosomal escape and other aspects of the enigmatic intracellular life style of *S. aureus*.

DR. MARTIN FRAUNHOLZ
DEPARTMENT OF MICROBIOLOGY

Lehrstuhl für Mikrobiologie
Biozentrum
Am Hubland
D-97074 Würzburg
T +49 (0)931-3183242
M martin.fraunholz@uni-wuerzburg.de

INTRODUCTION

S. aureus causes a variety of diseases ranging from abscesses to endocarditis and sepsis. Treatment options are rapidly dwindling due to the emergence of methicillin-resistant *S. aureus* (MRSA), which are resistant to most beta-lactam antibiotics. The ability of the bacterium to withstand harsh environmental conditions combined with its resistance to a broad spectrum of antibiotics makes *S. aureus* an important healthcare-associated pathogen. However, recently epidemic MRSA has increased in prevalence, and has begun to spread among and cause disease in previously healthy people that are not associated with predisposing risk factors. Most of the clinical manifestations of *S. aureus* are due to extracellular bacteria; however, *S. aureus* is also able to survive within host cells and thus constitutes a facultative intracellular pathogen. The bacteria are taken up by professional phagocytes such as neutrophils and macrophages but they are also able to enter epithelial or endothelial cells. This process is generally mediated by bacterial adhesins that are covalently anchored to the staphylococcal cell wall. After phagocytosis the *S. aureus*-containing vesicles fuse with lysosomes. These phagolysosomal organelles release a cocktail of antibacterial compounds to clear internalised pathogens. *S. aureus* not only persists inside its host cell but is also able to escape from the phagosome. We have previously excluded a role for the suspected pore-forming-hemolysin in phagosomal escape and described that only a limited number of clinical strains are able to evade lysosomal killing. We therefore sought to identify the factors involved in phagosomal escape of *S. aureus*.

RESEARCH HIGHLIGHTS

During the last few years we have demonstrated that membrane-active peptides, the phenol-soluble modulins (PSM) are involved in phagosomal escape.The expression of PSMs is regulated by the quorum-sensing system and further boosted by the stringent response. PSM production is thus activated in densely populated or spatially limited volumes such as in a phagosome. Due to their amphiphilic nature the PSMs have detergent-like properties and thus are capable of interfering with the integrity of host cell membranes. *S. aureus* strains that produce high levels of PSMs translocate efficiently from the phagosome to the cytoplasm of their respective host cells. This process commences approximately 2-3 hours after phagocytosis of the pathogen. The bacteria then change their transcriptional response upon escape and switch off the quorum sensing dependent production of virulence factors. Instead the bacteria start to grow intracellularly. We have observed phagosomal escape not only in epithelial and endothelial cells, but also in macrophages. Macrophages are part of the cellular (innate) immune system and part of the natural defense against infections. Therefore, our results can explain the increased virulence of *S. aureus* strains that express high levels of PSMs, such as the epidemic methicillin-resistant S. aureus strain LAC.

FUTURE DIRECTIONS

We are currently employing genome-wide deep sequencing approaches to identify all virulence factors involved in phagosomal escape and aim to link this process to host cell death and survival pathways. We will also analyze phagosomal escape of *S. aureus in vivo* to understand how this immune evasive pathogen strategy contributes to infection.

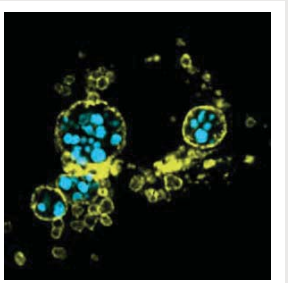


Fig. 1: S. aureus (cyan) is readily endocytosed by a lung epithelial cell and resides in late endosomes (yellow).

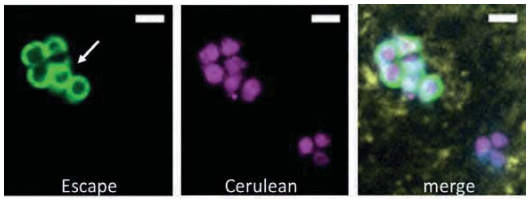


Fig. 2: S. aureus (magenta) escapes from phagosomes of a recombinant endothelial cell line. A reporter protein (green) is recruited to the bacterial cell wall only once *S. aureus* translocated to the cytoplasm of its host cell. The arrow indicates escaped *S. aureus*. Bar: 2 µm.

SELECTED PUBLICATIONS

Grosz M, Kolter J, Paprotka K, Winkler AC, Schafer D, Chatterjee SS, Geiger T, Wolz C, Ohlsen K, Otto M, Rudel T, Sinha B, **Fraunholz M** (2013) *Cytoplasmic replication of Staphylococcus aureus upon phagosomal escape triggered by phenolsoluble modulin.* **Cellular Microbiology** in press

Faulstich M, Böttcher JP, Meyer TF, **Fraunholz M**, Rudel T (2013) *Pilus phase variation switches gonococcal adherence to invasion by caveolin-1-dependent host cell signaling.* **PLoS Pathogens** 9:e1003373

Geiger T, Francois P, Liebeke M, **Fraunholz M**, Goerke C, Krismer B, Schrenzel J, Lalk M, Wolz C (2012) *The Stringent Response of Staphylococcus aureus and Its Impact on Survival after Phagocytosis through the Induction of Intracellular PSMs Expression.* **PloS Pathogens** 8:3016-3016

Fraunholz M, Sinha B (2012) *Intracellular Staphylococcus aureus: Live-in and let die.* **Frontiers in Cellular and Infection Microbiology** 24;2:43

To further investigate the role of these PRRs we have begun to establish RNA interference technology in different life stages of ants. This has proven to be difficult since injection of dsRNA leads to a strong immune response in these animals. This was overcome by feeding ants dsRNA, which successfully reduced gene expression in adult animals. However, a major problem in transferring this protocol to larvae has been that they accept food exclusively from caretaker animals that feed the brood via their crop content. Thus, we have developed a protocol that produces high concentrations of dsRNA in the crop of the caretaker animals. However, despite the massive transfer of the dsRNA from the workers to the larvae, we have been unable to alter gene expression in the larvae, potentially due to the presence of large amounts of nucleases in their digestive tract. We are currently attempting other approaches to manipulate RNA expression in this important stage of development.

FUTURE DIRECTIONS

The group aims to elucidate the molecular mechanisms involved in how insects tolerate chronic infections by mutualistic bacteria while at the same time they are able to eradicate pathogenic bacteria. To address this the ant`s immune system will be further characterized, e.g. a detailed analysis of the hemolymph by mass spectrometry. In addition, the project aims to identify and characterize (immune) factors involved in the control of the endosymbionts present in the ovaries of adult animals. The ovaries are of special interest, since removing worker ants from the nest leads to the maturation of the ovaries including the expansion of the endosymbiont population, which resembles the events in the midgut during pupation.

Fig. 1: A Camponotus species on a leaf (photograph by H. Feldhaar).

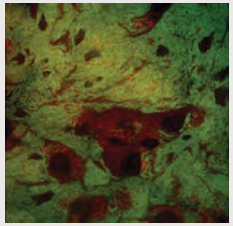


Fig. 2: Confocal image of the midgut tissue of a *Camponotus floridanus* pupa. The bacteria are labelled in green (photograph by Sascha Stoll).

SELECTED PUBLICATIONS

Ratzka C, **Gross R**, Feldhaar H (2013) *Gene expression analysis of the endosymbiontbearing midgut tissue during ontogeny of the carpenter ant Camponotus floridanus.* **Journal of Insect Physiology** 59: 611-623

Ratzka C, **Gross R**, H. Feldhaar H (2013*) Systemic gene knockdown in Camponotus floridanus workers by feeding of dsRNA.* **Insectes sociaux** 60:475-484.

Ratzka C, Förster F, Liang C, Kupper M, Dandekar T, Feldhaar H, **Gross R** (2012) *Molecular characterization of antimicrobial peptide genes of the carpenter ant Camponotus floridanus.* **PLoS One** 7: e43036

Gross R, Beier D (2012) *Two-component systems in bacteria.* **Caister Academic Press**, Norfolk, United Kingdom, ISBN: 978-1-908230-08-9

3.5. DEPARTMENT OF MICROBIOLOGY

3.5.3. ENDOSYMBIONT BIOLOGY

Bacterial endosymbiosis is a major driving force in the evolution of life on earth. Endosymbiotic bacteria are extremely widespread in insects and have significantly contributed to the evolutionary success of this major group of animals. Thus, these animals must have evolved mechanisms to tolerate chronic infection by such beneficial bacteria while discriminating and eradicating pathogenic bacteria.

PROF. ROY GROSS
DEPARTMENT OF MICROBIOLOGY

Lehrstuhl für Mikrobiologie
Biozentrum
Am Hubland
D-97074 Würzburg
T +49 (0)931-3184403
M roy@biozentrum.uni-wuerzburg.de

INTRODUCTION

More than 120 years ago F. Blochmann described for the first time the presence of obligate mutualistic intracellular bacteria in an animal. These uncultered bacteria (*Candidatus* Blochmannia) are found in bacteriocytes in the midgut and ovaries of ants of the genus *Camponotus* and transmitted vertically to the offspring. This bacteria-host association has existed for at least 50 Mio years. Previous work from our group has revealed a nutritional basis of the symbiosis, since the bacteria produce essential amino acids for the host and are involved in nitrogen recycling. The replication of the bacteria within the midgut is tightly coupled to the developmental cycle of the holometabolous host. They massively multiply only during pupation, while in adult animals the number of endosymbionts decreases rapidly.

Recently, we have been interested in understanding how these bacteria are tolerated by the host despite the fact that they are still recognized by the host immune system due to their Gram-negative molecular patterns (MAMPs) such as peptidoglycan. To address this question we have begun to characterize the immune system of the ants and to develop methods to genetically manipulate these social insects.

RESEARCH HIGHLIGHTS

The antimicrobial repertoire of social insects has been actively discussed for many years. Since these insects live in huge colonies they adopt alternative measures on the colony level to overcome pathogen infestation, a phenomenon called social immunity. It has therefore been suggested that the existence of social immunity may have resulted in the reduction of a cost-intensive immune system in such animals. Nevertheless, the genome sequence of the ant *Camponotus floridanus* together with our recent transcriptome analysis and re-annotation efforts revealed the presence of typical immune pathways found in other insects such as *Drosophila* in addition to a large repertoire of antimicrobial effectors. Of particular interest was the gene encoding the antimicrobial peptide hymenoptaecin. This gene is strongly induced after immune challenge and encodes a large precursor protein with several repeated hymenoptaecin domains, which after proteolytic maturation amplify the immune response in pathogen challenged animals. Interestingly, the overall structure of this gene is conserved in all ants and appears to be of prime importance for their immune response.

By correlating host gene expression with the massive endosymbiont multiplication in the midgut during pupation we discovered two pattern recognition receptors (PRRs) that are strongly upregulated in pupae, but only in their endosymbiont bearing midgut tissue. These PRRs are unusual, since they are endowed with an amidase activity that cleaves peptidoglycan fragments thus eliminating a major MAMP. These data suggest a down-modulation of the immune response in the midgut during pupation, thus allowing the expansion of the endosymbiont population. This assumption is strongly supported by experimental evidence showing that after infection the immune response of the midgut tissue but not of other tissues is strongly dampened during pupation. Thus, these amidase PRRs appear to be key players in endosymbiont tolerance in *Camponotus floridanus.*

cal function in the OM integration of porins. It remains to be seen if this function of the SAM machinery has been recently acquired during evolution, or if something similar was present in the bacterial ancestors of mitochondria.

We have also purified, crystallized and solved the crystal structure of $PorB_{IA}$. This has provided us with information regarding the functional sites in the protein, including those potentially involved in the interaction of $PorB_{IA}$ with the cell surface receptor SREC-I.

FUTURE DIRECTIONS

We aim to further study mitochondrial transport routes of proteins from viruses and bacteria, with a hope that this will reveal novel insights into how proteins can enter mitochondria. Likewise, we will investigate the submitochondrial localisation of pathogenic bacterial proteins and their interaction partners. Using bioinformatics, we plan to search bacterial genomes for proteins that have a potential to be targeted to mitochondria and we will work on establishing the role of such proteins in infection. Currently, we are also involved in the testing of new molecules that are effective against bacterial infection by *C. trachomatis* and *N. gonorrhoeae*. Some of these compounds are similar to the inhibitors of the MIP protein, and we are establishing the relevant assays to analyze the potential of MIP inhibition as a way of controlling bacterial infection.

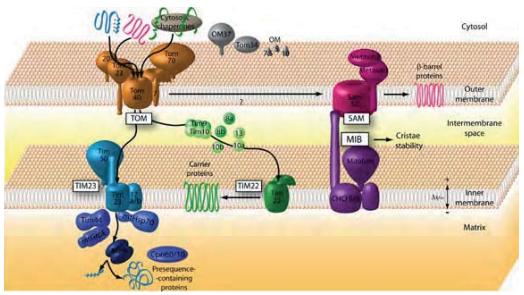


Fig. 1: Transport machineries of human mitochondria

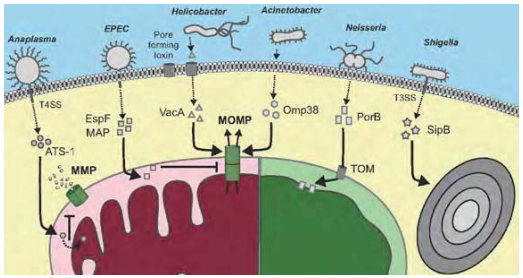


Fig. 2: Mitochondria as targets of bacterial pathogenicity factors.

SELECTED PUBLICATIONS

Kato H, Lu Q, Rapaport D, **Kozjak-Pavlovic V** (2013) *Tom70 is essential for PINK1 import into mitochondria.* **PLoS One** 8:e58435

Ott C, Utech M, Goetz M, Rudel T, **Kozjak-Pavlovic V** (2013) *The requirements for the import of neisserial Omp85 into the outer membrane of human mitochondria.* **Bioscience Report** 33:e00028

Zeth K, **Kozjak-Pavlovic V**, Faulstich M, Fraunholz M, Hurwitz R, Kepp O, Rudel T (2013) *Structure and function of the PorB porin from disseminating N. gonorrhoeae.* **Biochemical Journal** 449:631-642

Ott C, Ross K, Straub S, Thiede B, Götz M, Goosmann C, Krischke M, Mueller MJ, Krohne G, Rudel T, **Kozjak-Pavlovic V** (2012) *Sam50 functions in mitochondrial intermembrane space bridging and biogenesis of respiratory complexes.* **Molecular and Cellular Biology** 32:1173-1188

3.5. DEPARTMENT OF MICROBIOLOGY

3.5.4. PROTEIN IMPORT INTO MITOCHONDRIA

Targeting of bacterial or viral proteins to mitochondria plays an important role in infection. The group aims to understand the interaction of pathogenic microorganisms with mitochondria, as well as additional roles of the outer membrane proteins of *Neisseria gonorrhoeae* during infection.

DR. VERA KOZJAK-PAVLOVIC
DEPARTMENT OF MICROBIOLOGY

Lehrstuhl für Mikrobiologie
Biozentrum
Am Hubland
D-97074 Würzburg
T +49 (0)931-3188061
M vera.kozjak@uni-wuerzburg.de

INTRODUCTION

Mitochondria are organelles that play a central role in important cellular processes such as cell signalling and apoptosis. In many cases, mitochondrial function is affected during infection by pathogenic microorganisms. For example, changes in metabolism, energy production and calcium transport are observed, as well as loss of mitochondrial membrane potential and compromised mitochondrial integrity. In addition, some intracellular-residing bacteria and parasites are known to recruit mitochondria to the surface of their infection vacuole, although the purpose of this remains to be determined.

Viral proteins, such as vMIA from cytomegalovirus, target mitochondria during infection and mostly inhibit apoptosis, ensuring the survival of the infected cell and their own replication. Specific effector proteins from pathogenic bacteria, such as the SipB protein from *Salmonella typhimurium*, VacA from *Helicobacter pylori* and Ats-1 from *Anaplasma phagocytophilum*, translocate to the mitochondria and induce mitophagy, dissipate membrane potential or inhibit apoptosis, respectively. In the case of *Neisseria gonorrhoeae*, an outer membrane protein $PorB_{IA}$ is transported into mitochondria, where it induces mitochondrial fragmentation and membrane potential loss, contributing to cell death.

In addition to its mitochondrial targeting, $PorB_{IA}$ of *N. gonorrhoeae* also interacts with the surface receptor SREC-I of epithelial cells and is involved in neisserial internalisation, as well as dissemination during neisserial infections. Another important pathogenicity factor of *N. gonorrhoeae* is the macrophage infectivity potentiator (MIP) homolog, which is required for neisserial survival and proliferation within human macrophages. Both proteins present attractive targets for novel antimicrobial substances.

RESEARCH HIGHLIGHTS

To study the translocation of bacterial and viral proteins into mitochondria, we have created a collection of inducible, siRNA-based cell lines in which the transport machineries of the outer (OM) and the inner (IM) mitochondrial membrane are downregulated. Using these cell lines we have investigated the transport pathway of $PorB_{IA}$ from *N. gonorrhoeae*, and established that this protein is not integrated into the mitochondrial OM as expected, but is mistargeted to the intermembrane space, which causes its adverse effect on mitochondria. The reason for this is that despite similarities with the neisserial machinery for $PorB_{IA}$ outer membrane integration, the mitochondrial sorting and assembly (SAM) machinery does not recognize the membrane-targeting signal of PorBIA. We have therefore analyzed the differences between bacterial and mitochondrial systems for integration of porins into the outer membranes and found that the main component of the bacterial machinery, Omp85, can also integrate and function in the mitochondrial OM. Once Omp85 is present in mitochondria, $PorB_{IA}$ is transported into the mitochondrial OM and the mitochondria remained undamaged.

The knockdown cell lines have also been used to analyze the importance of the protein translocation machineries in mitochondrial membrane biogenesis. We have described a role for the SAM machinery in stabilising IM cristae structures, in addition to its canoni-

03.6

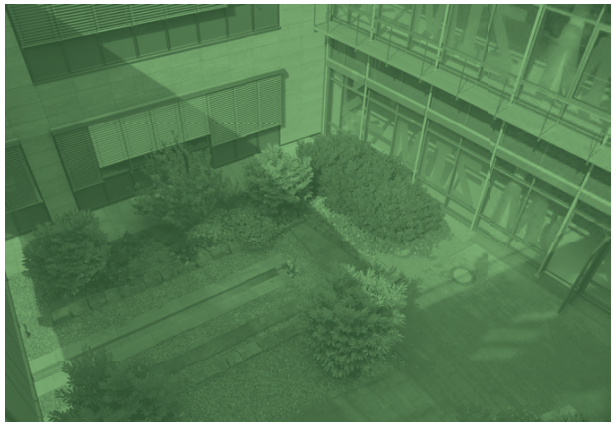
Department of Internal Medicine II

HERMANN EINSELE
ANDREAS BEILHACK
HARTWIG KLINKER
JÜRGEN LÖFFLER
ANDREW ULLMANN

The Department of Internal Medicine II at the University Hospital Clinics is part of the Medical Faculty of the University of Würzburg. Since 2004 it has been under the directorship of Prof. Dr. Hermann Einsele.

The department contains six research divisions, which include Hematology and Medical Oncology, Infectious Diseases, Gastroenterology, Hepatology, Clinical Immunology and Psychosomatics. Excellent conditions for clinical research, teaching, and patient care exist due to close interdisciplinary interactions with the Center of Internal Medicine and Center of Operative Medicine.

It contains a new and state-of-the-art stem cell transplantation unit and the University Hospital Würzburg runs the second largest stem cell transplantation program in Germany implementing many novel strategies. The division of infectious diseases has been certified as one of the first Centers of Infectiology in Germany. The clinical focuses of the division are HIV-infections, chronic virus hepatitis and opportunistic infections in immunocompromised patients.



University and Hans Knöll Institute, Jena and the Research Center for Infectious Diseases in Würzburg, research groups are characterising infection-relevant networks of *A. fumigatus* and host cells in response to the pathogen. To obtain a better understanding of IA, the groups are systematically investigating all levels of infection biology starting with the pathogen, via its interaction with single cell types (epithelial cells, DCs, alveolar macrophages, neutrophils, natural killer (NK) cells) and more complex infection models involving several cell types at the same time before moving to mouse models and clinical samples. These approaches will enable the elucidation of the regulatory circuits in both the pathogen and the host cells using functional genomics. The relevance of single genes / proteins in this process will be further studied by applying functional analyses (generation of knock-out mutants, biochemical analysis, cell culture and animal models, RNAi). Finally, based on these data, patient material will be analysed to provide the clinical relevance of experimental (primary cells, cell cultures, animal models) and computational models.

FUTURE DIRECTIONS

In the next few years we not only aim to provide new insight into the pathogenicity mechanisms of *Aspergillus fumigatus,* but also identify diagnostic biomarkers and potential targets for new antimycotic approaches, including the development of protocols for GMP-grade generation of DCs, NK and Treg cells suitable for clinical use.

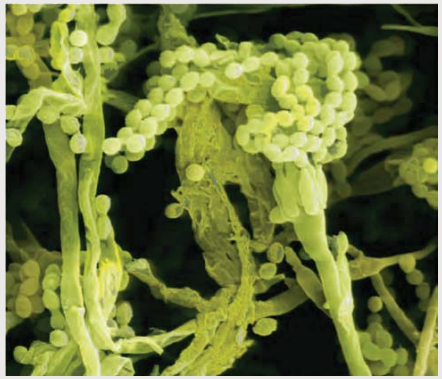


Fig. 1: Aspergillus fumigatus spores.

SELECTED PUBLICATIONS

Rogers TR, Morton CO, Springer J, Conneally E, Heinz W, Kenny C, Frost S, **Einsele H,** Loeffler J (2013) *Combined real-time PCR and galactomannan surveillance improves diagnosis of invasive aspergillosis in high-risk patients with haematological malignancies.* **British Journal of Haematology** 161:517-24

Springer J, Morton CO, Perry M, Heinz WJ, Paholcsek M, Alzheimer M, Rogers TR, Barnes RA, **Einsele H,** Loeffler J, White PL (2013) *Multicenter comparison of serum and whole-blood specimens for detection of Aspergillus DNA in high-risk hematological patients.* **Journal Clinical Microbiology** 51:1445-50

Mueller-Loebnitz C, Ostermann H, Franzke A, Loeffler J**,** Uharek L, Topp M, **Einsele H** (2013) *Immunological aspects of Candida and Aspergillus systemic fungal infections***. Interdisciplinary Perspectives on Infectious Diseases** 102934

Brede C, Friedrich M, von Krosigk V, Jordán-Garrote AL, Riedel SS, Bäuerlein CA, Heinze K, Bopp T, Schulz S, Mottok A, Rosenwald A, **Einsele H,** Negrin RS, Harms GS, Beilhack A (2012) *Mapping immune processes in intact tissues at cellular resolution.* **Journal of Clinical Investigations** 122: 4439-4446

Springer J, Schloßnagel H, Heinz W, Doedt T, Soeller R, **Einsele H**, Loeffler J (2012) *A novel extraction method combining plasma with a whole-blood fraction shows excellent sensitivity and reproducibility for patients at high risk for invasive aspergillosis.* **Journal of Clinical Microbiology** 50:2585-91

PRIZES AND AWARDS

2012 Nobel Lecture Stem Cell Biology/Transplantation, Nobel Forum, Karolinksa Institute

3.6. DEPARTMENT OF INTERNAL MEDICINE II

3.6.1. INTERACTION OF IMMUNE EFFECTOR CELLS WITH *ASPERGILLUS FUMIGATUS*

Aspergillus fumigatus is a ubiquitious fungus that has the ability to initiate invasive infections in immunocompromised patients. The group aims to understand the pathophysiology of invasive aspergillosis and develop new strategies to improve diagnosis.

PROF. HERMANN EINSELE

DEPARTMENT OF INTERNAL MEDICINE II

Medizinische Klinik und Poliklinik II
Oberdürrbacher Str. 6
D-97080 Würzburg
T +49 (0)931-20140001
M Einsele_h@klinik.uni-wuerzburg.de

INTRODUCTION

Aspergillus fumigatus is a saprophytic fungus that is found ubiquitously in the environment, where it plays an important role in the recycling of carbon and nitrogen. However, within the last two decades it has become one of the most important fungal pathogens. While inhaled conidia are efficiently cleared by the innate immune system of healthy humans, they can cause severe invasive disease in immunocompromised patients. In these immunocompromised hosts, the inhaled conidia are internalized by airway epithelial cells or pulmonary macrophages before undergoing germination and hyphal growth, leading to invasive aspergillosis (IA), with the primary site of infection being the lungs. Patients with neutropenia, T cell depletion, CD34-selected stem cell products, corticosteroid therapy, and cytomegalovirus infections are especially at risk of developing IA. Currently, there are a lack of reliable diagnostic tools and effective treatments, resulting in a high mortality rate of between 40 and 90 % in high-risk populations.

RESEARCH HIGHLIGHTS

Invasive aspergillosis (IA) is the most detrimental infection in patients with haematological malignancies. Although, IA may be perceived to be an uncommon disease with an incidence of 10,000 annual cases in Europe, there is increasing evidence that it is affecting a broader range of patients. In addition, IA is the most expensive opportunistic infection in immunosuppressed patients, with the annual cost in Europe being >100 million Euro. The major infectious diseases related interests of groups within the Dept. of Internal Medicine II are founded within the framework of two international and national research consortia.

A major problem in the management of IA is the poor diagnosis. Therefore, within the framework of the EU FP7 ERA-NET pathogenomics program (Invasive aspergillosis: Biomarkers for prevention, diagnosis and treatment response (aspBIOmics)), groups within the Dept. of Internal Medicine are developing and evaluating a battery of *in vitro* assays for a comprehensive multimodal analysis. This includes combining the detection of *Aspergillus fumigatus* elements (DNA, RNA, polysaccharides, proteins), host factors and the individual genetic susceptibility of the patients. The major benefit of this combined approach is the availability of a panel of biomarkers incorporated into rapid and sensitive *ex vivo* assays. This means that for the first time, a multi-parameter diagnostic strategy is being undertaken to target IA. This strategy has the potential to identify patients who are at highest risk of IA before the infection occurs. As a consequence, effective tailored prophylaxis can be provided and the success of antifungal therapy can be monitored.

The CRC/Transregio 124 (Pathogenic fungi and their human host: Networks of interaction) aims to combine state-of-the-art research in mycology and immunology to gain novel insights into the pathophysiology of invasive mycoses. The explicit goal of this initiative is to use modern sophisticated high-throughput approaches in basic research to generate data that can be used to improve diagnosis and treatment of these infections. As part of this national consortium, which includes groups from the Friedrich Schiller

3.6. DEPARTMENT OF INTERNAL MEDICINE II

3.6.2. EXPERIMENTAL STEM CELL TRANSPLANTATION

Allogeneic hematopoietic cell transplantation can be a life-saving therapy for patients with high-risk malignant diseases. The group investigates beneficial and detrimental immune effector mechanisms - particularly anti-tumor effects, protection from opportunistic infections and graft-versus-host disease.

DR. ANDREAS BEILHACK

DEPARTMENT OF
INTERNAL MEDICINE II

Medizinische Klinik und Poliklinik II
Zentrum für Experimentelle Molekulare Medizin
Zinklesweg 10
D-97080 Würzburg
T +49 (0)931-20144040
M beilhack_a@ukw.de

INTRODUCTION

After allogeneic hematopoietic cell transplantation or in other patient situations when the immune system is perturbed, the fungus *Aspergillus fumigatus* can cause life-threatening fungal infections. Imbalances in the immune system and barrier disruptions can result in invasive pulmonary aspergillosis. Clearance of infections depends on immune effector cells from the innate and adaptive immune system. Until now, only limited information is available concerning the time-resolved progression and spatial distribution of *Aspergillus fumigatus* during infection and its dependence on the underlying predisposing condition. Furthermore, the dynamics of immune cell recruitment and the way in which they act in concert is not well understood. Our group employs various *in vivo* and *ex vivo* imaging techniques to visualise disease progression, immune cell recruitment and the physical interactions among immune cells and the pathogen under *in vivo* conditions. By modulating the immune system we are analysing the changes in interaction patterns and its effect on the outcome of fungal infections. We aim to elucidate the interplay of host and pathogen under *in vivo* conditions to develop novel strategies to improve disease outcome.

RESEARCH HIGHLIGHTS

During the last few years we have been developing microscopy and imaging techniques to investigate complex immune processes *in vivo*. Together with Sven Krappmann (former ZINF Young Investigator, now at the University Hospital in Erlangen) we have developed systems to non-invasively visualize luminescent *Aspergillus fumigatus* infection *in vivo*. Together with the group of Gregory Harms (Rudolf Virchow Center, Würzburg) we developed a high-resolution multicolour light-sheet fluorescence microscopy (LSFM) technique to monitor dynamic immune responses in intact organs of mice or in biopsies from patients. This method has the advantage of being able to visualize and quantify single cell interactions within their three-dimensional tissue environment. Together with Katrin Heinze (Rudolf Virchow Center, Würzburg) we are continuing this endeavour and have built a next-generation LSFM that enables the concomitant detection of four colour channels of mesocopic tissue specimens of up to 1cm in diameter. We have applied this approach to our *in vivo* animal models and elucidated the function of signalling pathways and essential transcription factors of defined T cell subsets after allogeneic hematopoietic cell transplantation. Non-invasive bioluminescence imaging in combination with multicolour fluorescence microscopy enabled us to pinpoint critical events after allogeneic transplantation. Using mouse models we have identified a time period of two weeks of massive alloreactive donor T cell migration in the blood after allogeneic hematopoietic cell transplantation before clinical aGVHD symptoms appeared. Based on these observations we were able to define a collection of diagnostic markers to timely predict acute graft-versus-host disease. We could also identify potential therapeutic markers to foster transplantation tolerance without impairing desired effector functions such as the immune control of cancer cells and infections.

FUTURE DIRECTIONS

As members of the Collaborative Research Centre TRR124 *Pathogenic fungi and their human host: Networks of interaction* we will further develop and continue to employ our imaging and microscopy platform to investigate dynamic immune-pathogen interactions *in vivo*. Also we will take advantage of the strong bioinformatics core and our close ties with the clinics to elucidate critical host-pathogen interactions to improve diagnostics and therapeutic options for patients undergoing hematopoietic cell transplantation and/or suffering from chronic opportunistic infections.

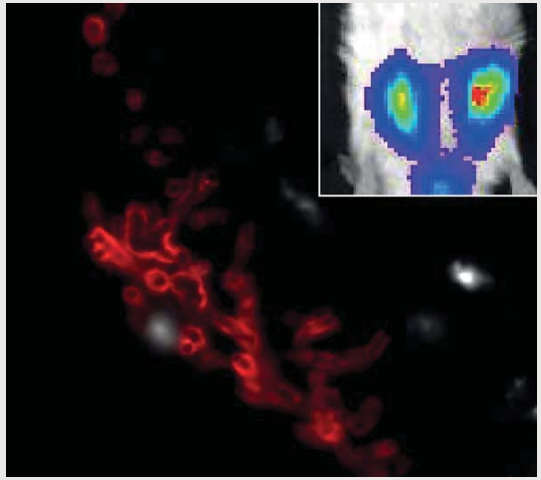


Fig. 1:Aspergillus fumigatus (red) infection of the lung visualized by microscopy or by non-invasive bioluminescence imaging (picture insert).

SELECTED PUBLICATIONS

Bäuerlein CA, Riedel SS, Baker J, Brede, C, Jordán Garrote AL, Ritz M, Schulz S, Grether M, ChopraM, Beilhack GF, Zeiser R, Schlegel PG, Einsele H, Negrin RS, **Beilhack A** (2013) *A diagnostic window for the treatment of acute GVHD prior to visible clinical symptoms in amurine model.* **BMC Medicine** 11: 134

Ophir E, Or-Geva N, Gurevich I, Tal O, Eidelstein Y, Shezen E, Margalit R, Lask A, Shakhar G, Hagin D, Bachar-Lustig E, Reich-Zeliger S, **Beilhack A**, Negrin R, Reisner Y (2013) *Murine anti-3rd-party central-memory CD8+T-cells promote hematopoietic chimerism under mild conditioning: Lymph-node sequestration and deletion of anti-donor T-cells.* **Blood** 121: 1220-1228

Brede C, Friedrich M, von Krosigk V, Jordán-Garrote AL, Riedel SS, Bäuerlein CA, Heinze K, Bopp T, Schulz S, Mottok A, Rosenwald A, Einsele H, Negrin RS, Harms GS, **Beilhack A** (2012) *Mapping immune processes in intact tissues at cellular resolution.* **Journal of Clinical Investigations** 122: 4439-4446

Riedel SS, Mottok A, Brede C, Bäuerlein CA, Jordán Garrote AL, Ritz M, Mattenheimer K, Rosenwald A, Einsele, H, Bogen B, **Beilhack A** (2012) *Non-invasive imaging provides spatiotemporal information on disease progression and response to therapy in a murine model of multiple myeloma.* **PLoS One** 7: e52398

Donat S, Hasenberg M, Schäfer T, Ohlsen K, Gunzer M, Einsele H, Löffler J, **Beilhack A**, Krappmann S (2012) *Surface display of Gaussia princeps luciferase allows sensitive fungal pathogen detection during cutaneous aspergillosis*. **Virulence** 3: 50-61

macokinetic studies, we have developed a combined HPLC-assay for the determination of serum concentrations of both triazoles.

For hepatitis C, antiviral treatment is dramatically changing. The division of infectious diseases is a study center for many international phase II and III studies with investigational anti-HCV drugs (protease inhibitors, polymerase inhibitors) such as ABT-267, ABT-333, ABT-450, Asunaprevir, Daclatasvir, Danoprevir, Faldaprevir, GS-5885, GS-9451, GSK2336805, Simeprevir, Sofosbuvir, and Tegobuvir.

Clinical phase III studies in patients with haematological malignancies are mainly focused on fungal infections. In an investigator-initiated study (CASPHYLAX, initiated by Dr. Werner Heinz, Würzburg), we are evaluating the pharmacokinetics and efficacy of caspofungin treatment for primary prophylaxis.

From 2011 to 2014, we have participated in a joint project with the Institute for Virology (Axel Rethwilm) to investigate the pharmacokinetics and drug monitoring of new direct-acting anti-HIV and anti-HCV antivirals. In addition, with respect to HIV we have participated in the NIH-sponsored worldwide START-study, one of the most important strategic HIV-studies, to evaluate the optimal timing for beginning antiretroviral therapy. Within an International Research Training Group (IRTG1522) several pharmacokinetic investigations were performed together with the universities of Cape Town and Stellenbosch (South Africa) on HIV-infected patients under high active antiretroviral therapy in South Africa. Eight medical students have been in Cape Town/Stellenbosch for a period of 4-5 months to work on this project and their medical thesis.

FUTURE DIRECTIONS

During the the course of the IRTG project, numerous clinical and pharmacokinetic data were collected from HIV-patients with different co-morbidities. Together with the project partners, we are planning a detailed analysis of these data, including a population study of the pharmacokinetics of antiretroviral drugs.

In the next few years, we will focus on the development of methods for the quantification of new anti-HIV- and HCV-agents. The determination of plasma concentrations of these drugs will provide insight into the individual pharmacokinetics of antiviral treatments in different patient groups and will contribute to improving the safety of long term treatment.

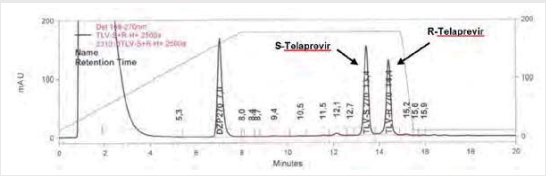


Fig. 1: HPLC illustration of telaprevir plasma concentration (S-Telaprevir 2.457 ng/ml, R-Telaprevir 2.414 ng/ml)

SELECTED PUBLICATIONS

Heinz WJ, Einsele H, Helle-Beyersdorf A, Zirkel J, Grau A, Schirmer D, Lenker U, **Klinker H** (2013) *Posaconazole concentrations after allogeneic hematopoietic stem cell transplantation.* **Transplant Infectious Disease** 15:449-56

Gunda DW, Kasang C, Kidenya BR, Kabangila R, Mshana SE, Kidola J, Kalluvya SE, Kongola GW, **Klinker H** (2013) *Plasma concentrations of efavirenz and nevirapine among HIV-infected patients with immunological failure attending a tertiary hospital in Northwestern Tanzania.* **PLoS One** 8:e75118

Mauss S, **Klinker H** (2013) *Drug-drug interactions in the treatment of HCV among people who inject drugs* **Clinical Infectious Disease** 57 Suppl 2:S125-8

Zeuzem S, Buggisch P, Agarwal K, Marcellin P, Sereni D, **Klinker H**, Moreno C, Zarski JP, Horsmans Y, Mo H, Arterburn S, Knox S, Oldach D, McHutchison JG, Manns MP, Foster GR (2012) *The protease inhibitor, GS-9256, and non-nucleoside polymerase inhibitor tegobuvir alone, with ribavirin, or pegylated interferon plus ribavirin in hepatitis C.* **Hepatology** 55:749-58

Heinz WJ, Grau A, Ulrich A, Helle-Beyersdorf A, Zirkel J, Schirmer D, Lenker U, Einsele H, **Klinker H** (2012) *Impact of benzodiazepines on posaconazole serum concentrations. A population-based pharmacokinetic study on drug interaction.* **Current Medical Research Opinion** 28:551-7

3.6. DEPARTMENT OF INTERNAL MEDICINE II

3.6.3. DIVISION OF INFECTIOUS DISEASES

The laboratory and clinical research of the group is focused on innovative antiinfective strategies in the fields of HIV-infection, chronic hepatitis B/C, and opportunistic infections in immunocompromised hosts. The pharmacokinetic analyses center on the detection and quantification of different antiviral and antifungal agents.

PROF. HARTWIG KLINKER
DEPARTMENT OF
INTERNAL MEDICINE II

Medizinische Klinik und Poliklinik II
ZIM (Zentrum Innere Medizin)
Oberdürrbacher Straße 6, Haus A4
D-97080 Würzburg
T +49 (0)931-20140043
M klinker_h@ukw.de

INTRODUCTION

Worldwide, approximately 33 million people are living with HIV/AIDS. The availability of potent antiretroviral drugs to inhibit HIV replication has resulted in an important clinical benefit for many patients. However, a lifelong treatment regime with complex medication places great demands on both patients and their physicians. Major problems associated with long-term antiretroviral therapy are adherence to antiretroviral treatments, drug resistance, toxicity, pharmacokinetics and pharmacological interactions.

Chronic hepatitis C is among the most frequent infections in the world (about 170 million cases) and is often complicated by liver cirrhosis and hepatocellular carcinoma. Recently, antiviral treatment has dramatically improved but it remains difficult to perform and is associated with many side effects. Data on adequate drug exposure are widely missing.

Invasive fungal infections are a life threatening complication in patients with haematological malignancies, especially those with acute myeloic leukemia or who are undergoing allogeneic stem cell transplantation. These patients often receive a number of different drugs for the underlying disease and for prophylaxis or treatment of complications. Therefore, drug interactions are a relevant problem in daily medical care.

The section of Infectious Diseases is a clinical center within the German Competence Network on HIV/AIDS and the German Competence Network on Hepatitis, sponsored by the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF). Since 2005, the study-center has qualified for the worldwide study-network for strategical HIV-studies INSIGHT (International Network for Strategic Initiatives in Global HIV Trials) sponsored by the National Institutes of Health/USA (http://www.insighttrials.org).

RESEARCH HIGHLIGHTS

The laboratory specializes in developing and implementing methods for evaluating the pharmacokinetics and therapeutic drug monitoring of virostatic and antifungal agents.

One major focus is the pharmacokinetic evaluation of HIV protease inhibitors (PI) and non nucleoside reverse transcriptase inhibitors (NNRTI) during highly active antiretroviral therapy (HAART) in patients with HIV-infection. We have developed high pressure liquid chromatographic (HPLC) methods for determination of plasma levels of HIV-1 PI saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir, atazanavir, darunavir and tipranavir, the integrase inhibitor raltegravir, and for the NNRTI efavirenz, etravirine, and rilpivirine. Concentrations of nevirapine, another NNRTI, are investigated by a gas chromatographic setup with nitrogen-phosphorous detection. In 2013, we established a new method to determine the levels of telaprevir, one of the first HCV-protease inhibitors (see figure).

The antifungal triazoles voriconazole and posaconazole are broadly used for either treatment or prophylaxis of invasive fungal infections Voriconazole is metabolized by the CYP P450-system, while posaconazole inhibits the cytochrome P450 enzymes. For phar-

been documented that the antigen Aspf1 plays a significant role in the maturation and activation of immature DCs. In our work with monocytes and with DCs we have used genome-wide arrays and RNAi to demonstrate the relevance of these cells for the immune response against *A. fumigatus*. Our group has been the first to show that human DCs recognize *A. fumigatus* by dectin-1. The immune response in DCs is regulated by the glycogen synthase kinase-3 pathway, which modulates mainly the release of IL-10. During the interaction of the fungus with polymorphonuclear leukocytes (PMN), we could identify AfYap1 as the main fungal factor directed against reactive oxygen species.

Recently, we extended our research activities to analyse the interaction of human NK cells with different morphologies of *A. fumigatus*, this revealed that human NK cells interact with *A. fumigatus* germlings, which induce the release of Th1 like cytokines and cause significant fungal killing. These data showed for the first time that IFN-γ, released by NK cells, is a soluble molecule mediating direct fungicidal activity.

In addition, genotyping of a large DNA bank was performed to identify single nucleotide polymorphisms, potentially associated with the occurrence of *Aspergillus* infections. Three markers in the *chemokine (C-X-C motif) ligand 10 gene* were found to be significantly associated with an increased risk of developing IA.

FUTURE DIRECTIONS

During the next few years we will focus our research on pathway analyses in different innate immune cells (macrophages, DC, NK cells) exposed to various *A. fumigatus* morphologies. By parallel deep sequencing analyses of the fungus and the host, with and without siRNA knockdown of selected target genes, we are aiming to define specific immune-relevant pathways in aspergillosis. We will also correlate mRNA and miRNA profiles and investigate the direct effects of rIFN- on the fungus. Finally, we will complete our Genome Wide Association Study (AspIRS) to further define genetic markers, which are significantly associated with IA. Our overall aim is the development of patient-specific risk profiles and individual management strategies for IA.

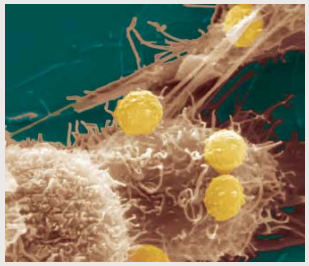


Fig. 1: Aspergillus fumigatus and monocyte-derived dendritic cells

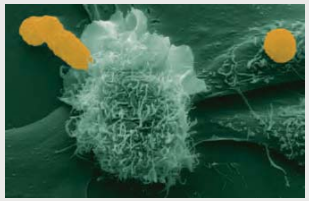


Fig. 2: Human dendritic cell interacting with an *A. fumigatus* spore and germling

SELECTED PUBLICATIONS

Rogers TR, Morton CO, Springer J, Conneally E, Heinz W, Kenny C, Frost S, Einsele H, **Loeffler J** (2013) *Combined real-time PCR and galactomannan surveillance improves diagnosis of invasive aspergillosis in high-risk patients with haematological malignancies.* **British Journal of Haematology** 161:517-24

Springer J, Morton CO, Perry M, Heinz WJ, Paholcsek M, Alzheimer M, Rogers TR, Barnes RA, Einsele H, **Loeffler J**, White PL (2013) *Multicenter comparison of serum and whole-blood specimens for detection of Aspergillus DNA in high-risk hematological patients.* **Journal Clinical Microbiology** 51:1445-50

Maschmeyer G., Heinz W., Hertenstein B., Horst A., Requadt C., Wagner T., Cornely O., **Loeffler J.**, Ruhnke M (2013) *Immediate versus deferred empirical antifungal (IDEA) therapy in high-risk aptients with febrile neutropenia: a randomized, double-blind, placebo-controlled multicentre study.* **European Journal of Clinical Microbiology and Infectious Diseases** 32: 679 - 689

Mueller-Loebnitz C, Ostermann H, Franzke A, **Loeffler J,** Uharek L, Topp M, Einsele H (2013) *Immunological aspects of Candida and Aspergillus systemic fungal infections*. **Interdisciplinary Perspectives on Infectious Diseases** 102934

Springer J, Schloßnagel H, Heinz W, Doedt T, Soeller R, Einsele H, **Loeffler J** (2012) *A novel extraction method combining plasma with a whole-blood fraction shows excellent sensitivity and reproducibility for patients at high risk for invasive aspergillosis.* **Journal of Clinical Microbiology** 50:2585-91

3.6. DEPARTMENT OF INTERNAL MEDICINE II

3.6.4. IMMUNITY AGAINST *ASPERGILLUS* SPP.

Aspergillus fumigatus is a major cause of morbidity and mortality in different cohorts of immunocompromised patients. Our group aims to better understand the interaction of *A. fumigatus* with the innate and adaptive immune system and to characterize genetic susceptibility to the fungus.

PROF. JÜRGEN LÖFFLER
DEPARTMENT OF
INTERNAL MEDICINE II

Medizinische Klinik II
Forschungslabore Haus C11
Josef-Schneider-Str. 2
D-97080 Würzburg
T +49 (0)931-20136412
M juergen.loeffler@ukw.de

INTRODUCTION

Aspergillus spp. are ubiquitous moulds present as saprophytes in air, soil, and water. Therefore, humans are constantly exposed to the omnipresent spores. Spores are inhaled daily and the exposure to the fungus can be considerable depending on circumstances. In patients with a compromised immune system, deposition of the conidia on mucous membranes in the upper and lower respiratory tract enables their germination and the penetration of tissue barriers. Major groups of patients who are predisposed to invasive fungal infections are leukemia patients and patients after allogeneic stem cell and solid organ transplantation.

A. fumigatus is the predominant human pathogenic species that induces invasive aspergillosis (IA). The clinical features of IA are often non-specific, imaging and mycological investigations rarely confirm the diagnosis, making discrimination between IA and other fungal infections particularly difficult. Medical progress and thus longer survival times have expanded the number of immunocompromised patients, and consequently the rate of IA has increased 14-fold in Europe within the last years, with an annual incidence of 10,000 cases in Europe. Lethality is around 85% and falls to 50% if patients are treated with antimycotic drugs. Furthermore, IA is the most expensive opportunistic infection in immunosuppressed patients with annual treating costs in Europe of approximately € 100 million. In-hospital stays due to complications arising from IA cause additional costs of € 75,000 per patient.

In contrast to most bacterial pathogens, *A. fumigatus* undergoes major morphological changes during the early phase of infection, resulting in different fungal surface structures. Spores are constantly inhaled and reach the lung alveoli. Cells of the innate immune system recognize the fungus and its different morphologies by distinct pattern recognition receptors (PRR), which induce cell specific as well as general defense mechanisms. The most important cells of the initial innate immune system are alveolar epithelia, alveolar macrophages, as well as dendritic cells (DC). To-date, interactions between the fungus and cells of the innate immune system, such as natural killer cells (NK-cells) and granulocytes, during invasion into the blood vessels has not been well characterized.

RESEARCH HIGHLIGHTS

The research of my group is targeted towards immune recognition of *A. fumigatus*, genetic susceptibility to this fungus, and the molecular diagnosis of invasive fungal infections. My group has extensively characterized the interaction of *A. fumigatus* with the human and murine innate and adaptive immune systems, including the interaction with different Toll like receptors and dectin-1.

Using monocyte-derived immature dendritic cells (moDC) as well as murine bone marrow-derived dendritic cells from wild-type, TLR4-deficient, TLR2 knockout, and TLR2/TLR4 double-knockout mice, we have shown that activation of these cells is mediated via TLR2 and TLR4. Furthermore, we have analysed the influence of recombinant antigens on the immune response of moDCs and neutrophils to *A. fumigatus*. It has

FUTURE DIRECTIONS

This new group is in the process of being established and once completed will focus on translational research bringing results from bench science to the patients' bedside. One project will have a closer look at the relative expression of TNF-alpha, interleukins IL1ß, IL10 and IL12 in PBMCs during fungal infection. An animal infection model has been established and the development of antifungal agents will be actively followed by clinically relevant results. This model will be further modified to assess the immunology of fungal infections especially that of mucormycosis in vivo. With a new investigator initiated trial for the administration of *Aspergillus*-specific T-cells in allogeneic haematopoietic stem cell transplant recipients, the group will bring forward a major phase I-II trial for the immunological treatment of invasive aspergillosis (supported by BayImmuNet). These trial and research projects have the potential to further close the gap between immunological research and its use in the clinics for the therapy of fungal diseases. Furthermore, novel antiviral agents and vaccines against Herpesviridae will be another clinical research area.

SELECTED PUBLICATIONS

Chemaly RF*, **Ullmann AJ***, Stoelben S, Richard MP, Bornhäuser M, Groth C, Einsele H, Silverman M, Mullane KM, Brown J, Nowak H, Kölling K, Stobernack HP, Lischka P, Zimmermann H, Rübsamen-Schaeff H, Champlin RE, Ehninger G; AIC246 Study Team *Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation.* **New England Journal of Medicine** in press

Cornely OA, Cuenca-Estrella M, Meis JF, **Ullmann AJ** (2013) *European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Fungal Infection Study Group (EFISG) and European Confederation of Medical Mycology (ECMM) 2013 joint guidelines on diagnosis and management of rare and emerging fungal diseases.* **Clinical Microbiology and Infection** Suppl 3:1-4

Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, Lanternier F, Pagano L, Skiada A, Akova M, Arendrup MC, Boekhout T, Chowdhary A, Cuenca-Estrella M, Freiberger T, Guinea J, Guarro J, de Hoog S, Hope W, Johnson E, Kathuria S, Lackner M, Lass-Flörl C, Lortholary O, Meis JF, Meletiadis J, Muñoz P, Richardson M, Roilides E, Tortorano AM, **Ullmann AJ**, van Diepeningen A, Verweij P, Petrikkos G (2013) *ESCMID and ECMM joint clinical guidelines for the diagnosis and management of mucormycosiS:* **Clinical Microbiology and Infection** Suppl 3:5-26

Leroux S, **Ullmann AJ** (2013) *Management and diagnostic guidelines for fungal diseases in infectious diseases and clinical microbiology: critical appraisal.* **Clinical Microbiology and Infection** 19:1115-21

3.6. DEPARTMENT OF INTERNAL MEDICINE II

3.6.5. CLINICAL INFECTIOUS DISEASES

Andrew Ullmann is the new appointed head of the Infectious Diseases division, whose main clinical focus is research into and the treatment of HIV disease, infectious hepatitis, various viral infections and resistance development in microbes. A main focus of research is the complications arising from filamentous fungal infections, which are associated with high mortality rates due to difficulties in diagnosis and treatment. Therefore, the group aims to develop new biomarkers and gain new insight into the immune pathogenesis of rare filamentous fungi.

PROF. ANDREW J. ULLMANN
DEPARTMENT OF
INTERNAL MEDICINE II

Medizinische Klinik und Poliklinik II
Oberdürrbacher Str. 6
D-97080 Würzburg
T +49 (0) 931-20140115
M ullmann_a@ukw.de

INTRODUCTION

During the last decades invasive fungal infections have emerged as an important cause of life threatening infections. This not only occurs in the context of immunosuppression but is also frequently noted in non-neutropenic patients, especially in those requiring treatment in intensive care units (ICU). Despite the growing body of evidence and knowledge in this field the diagnosis and management of these complex infections remains challenging. In comparison to the incidence rate of other invasive fungal diseases, mucormycosis is rarely diagnosed. Although the treatment of aspergillosis has improved, only a few antifungal agents depict activity against mucormycosis species, which remains associated with a relatively high mortality rate. As for invasive aspergillosis, diagnostic procedures are challenging, however, in contrast to invasive aspergillosis, there is currently no recognized biomarkers available for the diagnosis of mucormycosis.

RESEARCH HIGHLIGHTS

The group has been recently established in the University Hospital Clinics. However, one of the major undertaken tasks has been the publication of new European guidelines for the diagnosis and management of fungal diseases. Invasive fungal infections (IFI) are life-threatening conditions that require rapid diagnostic and optimal management to mitigate their high morbidity and mortality rates. They are also associated with a high economic burden owing to the prolonged hospitalisation, need of intensive supportive care, and costs associated with expensive new antifungal therapy. To address these issues the ESCMID has published guidelines on the management of IFI. Andrew Ullmann is the chair of the fungal study group within ESCMID (EFISG) which was responsible for publication of the guidelines.

Another area of concern in the immunocompromised is CMV infections. Human cytomegalovirus (HCMV) infection is a leading viral cause of morbidity and mortality in allogeneic hematopoietic cell transplant (HCT) recipients. Available treatments are restricted by significant toxicities of and resistance to current medication. Letermovir (previously known as AIC246) is a new highly potent anti-HCMV agent in vitro, with a novel mechanism of action targeting the viral terminase subunit pUL56, a component of the terminase complex involved in viral DNA cleavage and packaging. Since the target is not present in human cells, letermovir provides a safe new treatment option for patients infected with HCMV strains that are resistant to approved antiviral drugs. Initial clinical data relating to letermovir use in a patient infected with a multi-resistant HCMV strain and multi-organ HCMV disease appear to support in vitro observations based on letermovir's novel mechanism of action. The group has performed a multicentered study on the effect of letermovir in allogeneic HCT recipients. The results of this successful trial have been recently published in the New England Journal of Medicine.

GERHARD BRINGMANN

THOMAS DANDEKAR

MARKUS ENGSTLER

UTE HENTSCHEL-HUMEIDA

ULRIKE HOLZGRABE

CAROLINE KISKER

AUGUST STICH

HEIKE WALLES

04

ZINF MEMBERS ASSOCIATED WITH OTHER INSTITUTES

Trypanosoma spp., or *Leishmania* spp.). These SAR studies finally paved the way for a systematic optimization of the activity-cytotoxicity ratio, e.g. by QSAR calculations, resulting in the preparation of highly active and biocompatible anti-infective agents. This synthetic work furthermore helped provide sufficient quantities of the most active compounds for further biotesting *in vitro* and *in vivo*, and preparing synthetic analogs with simplified structures and improved activities and ADMET parameters.

Our key methodology for the spectroscopy-guided search for structurally rewarding secondary metabolites like naphthylisoquinoline alkaloids and related compounds from tropical plants is the analytical triad HPLC coupled to MS, NMR, and CD (= circular dichroism). This triad permits recognition and structural assignment of novel-type compounds from the peak in the chromatogram, including the full absolute stereostructure. The LC-CD option is even more valuable in combination with quantum chemical CD calculations, which permits a secure interpretation of the spectra independent from any empirical rules. This combination of hyphenated analytical methods with computational investigations is unique in natural products chemistry. By using this concept, we have succeeded in establishing a large number of different stereostructures, with stereogenic centers or axes or with planar chirality, in many cases, our analytical method was the only possibility to assign the absolute configuration of complex chiral natural products. A convincing example is the discovery of the mbandakamines A and B, dimeric naphthylisoquinoline alkaloids with three consecutive chiral axes from a Congolese *Ancistrocladus* plant by this hyphenated analysis. These bioactive dimers possess the highest number of consecutive stereogenic biaryl axes ever found not only in naphthylisoquinolines, but also in natural products in general.

FUTURE DIRECTIONS

We are continuously aiming to improve the activities of the naphthylisoquinoline alkaloids compounds for further drug development. This requires the elucidation of the mode of action of the drugs and, in particular, the identification of the target protein, which we are pursuing by photo-affinity labelling studies, together with our cooperation partners within the SFB 630 network.

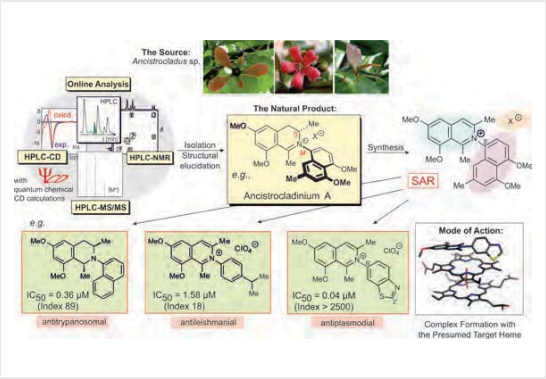


Fig. 1: Identification of ancistrocladinium A, a representative of a new subclass of N,C-coupled naphthylisoquinoline alkaloids, i.e., linked via an unprecedented $N_{iminium}$-C_{aryl} axis: structural elucidation online, by HPlC-MS/MS-NMR-CD assisted by quantum chemical CD calculations, atroposelective total synthesis; synthesis of simplified structural analogs with high and specific antitrypanosomal, antileishmanial, or antiplasmodial activities – just depending on the individual structure, and with reduced toxicities; further improvement i.a., by the search for the mode of action and the target molecules, together with our cooperation partners within the SFB 630 network.

SELECTED PUBLICATIONS

Bringmann G, Zhang G, Büttner T, Bauckmann G, Kupfer T, Braunschweig H, Brun R and Mudogo V (2013) *Jozimine A2: The First Dioncophyllaceae-Type Naphthylisoquinoline Alkaloid, with Three Chiral Axes and High Antiplasmodial Activity.* **Chemistry - A European Journal** 19: 916-923

Bringmann G, Lombe BK, Steinert C, Ndjoko Ioset K, Brun R, Turini F, Heubl G and Mudogo V (2013) *Mbandakamines A and B, the First Unsymmetrically Coupled Dimeric Naphthylisoquinoline Alkaloids, from a Congolese Ancistrocladus Species.* **Organic Letters** 15: 2590-2593

Bringmann G, Zhang G, Ölschläger T, Stich A, Wu J, Chatterjee M and Brun R (2013) *Highly Selective Antiplasmodial Naphthylisoquinoline Alkaloids from Ancistrocladus tectorius.* **Phytochemistry** 91: 220-228.

Bringmann G, Thomale K, Bischof S, Schneider C, Schultheis M, Schwarz T, Moll H and Schurigt U (2013) *A Novel Leishmania major Amastigote Assay in 96-Well Format for Rapid Drug Screening and Its Use for Discovery and Evaluation of a New Class of Leishmanicidal Quinolinium Salts.* **Antimicrobial Agents and Chemotherapy** 57: 3003- 3011

Loedige M, Lewis MD, Paulsen ES, Esch HL, Pradel G, Lehmann L, Brun R, **Bringmann G**, Mueller AK (2013) *A Primaquine-Chloroquine Hybrid with Dual Activity against Plasmodium Liver and Blood Stages.* **International Journal of Medical Microbiology** 303: 539-547

4. ZINF MEMBERS ASSOCIATE WITH OTHER INSTITUTES

4.1. NATURAL PRODUCTS CHEMISTRY

Natural products are a rich source of bioactive molecules. The aim of the group is the isolation, structural elucidation, total synthesis, biosynthesis, and pharmaceutical development of anti-infective and anti-tumoral agents from nature.

PROF. GERHARD BRINGMANN

ORGANIC CHEMISTRY I

Lehrstuhl für Organische Chemie I
Am Hubland
97074 Würzburg
T +49 (0)931-85323
M bringman@chemie.uni-wuerzburg.de

INTRODUCTION

We consider natural products chemistry as multidisciplinary per se and therefore try to approach this topic in a broad, highly interdisciplinary way, applying novel efficient methods of analytical, synthetic, computational, and medicinal chemistry. More specifically, we select rewarding sources for novel natural products – among them, i.a., tropical plants (e.g., from promising families) and search for new compounds. This is not only done in a bioassay-guided way, but, even more efficiently, in a structure-oriented manner, by using our analytical triad HPLC-MS/MS-NMR-CD, assisted by quantum chemical circular dichroism calculations. This approach permits the 'early' recognition of novel-type molecules and the online elucidation of their full absolute stereostructures. The compounds are then isolated for structural confirmation and pharmacological investigation; these focus mainly on anti-infectious properties (antiplasmodial, antitrypanosomal, antileishmanial, anti-*Candida*, anti-biofilm, etc.), but also include antitumoral activities. We elaborate synthetic pathways to the most rewarding metabolites using biomimetic or merely synthetic strategies; for this purpose, we also develop novel synthetic methodology like, e.g., the lactone method for the atropo-selective construction of even highly hindered biaryl and hetero biaryl systems of any desired (and predictable) axial configuration.

RESEARCH HIGHLIGHTS

Naphthylisoquinoline alkaloids from tropical Ancistrocladaceae and Dioncophyllaceae plants are remarkable in many respects: biosynthetically because of their unprecedented origin of isoquinoline alkaloids from acetate units (and not from the usual amino acids), structurally because of the presence of stereogenic centers and rotationally hindered biaryl axes, and last, but not least, pharmacologically because of their promising anti-infective bioactivities.

We have been mainly focusing on the isolation, structural elucidation, and enantio- and atroposelective synthesis of structurally novel representatives of C,C- and N,C-coupled mono- and dimeric naphthylisoquinoline alkaloids, and on the detailed investigation of their bioactivity potential as active agents against the pathogens of infectious diseases. In this large project, which is part of our collaborative research centre "Recognition, Preparation, and Functional Analysis of Agents against Infectious Diseases" (SFB 630), we have, as an example, elaborated a highly convergent and stereoselective first access to the alkaloids ancistrocladinium A and B, which possess an unprecedented iminium-aryl axis, via a short sequence of eight linear steps. Since ancistrocladinium A and related compounds were found to exhibit very good anti-infective activities (in particular against *Leishmania major*) with a comparably low cytotoxicity against mammalian cells, we have developed a concept for the identification of the pharmacophore of such N,C-coupled arylisoquinolines using structure-activity relationship (SAR) studies. We managed to successfully elaborate a most efficient synthetic strategy giving rise to the generation of more than 250 structurally simplified derivatives showing the broad substrate variability of the pathway. By changing particular structural parameters we could specifically improve the selectivity of the activity against a given parasite (e.g., *Plasmodium falciparum*,

sensing of staphylococci and identified the role of regulatory factors such as Agr and SaeR and verified our results in biofilm experiments. Further work included metabolic and regulatory network modelling with a focus on protein complexes in *S. aureus* under different conditions as well as *Salmonella* metabolism and bioinformatic analyses on *E. coli* and *L. lactis* strains.

Finally, we are also interested in modelling animal-microbial interactions, for instance the Camponotus ant and its interaction with its endosymbiont Blochmannia, as well as Gram-negative pathogenic bacteria. Host pathogen interactions of man and pathogenic fungi are currently studied within the umbrella of several projects in the Collaborative Research Centre TRR124 Pathogenic fungi and their human host: Networks of interaction.

FUTURE DIRECTIONS

The knowledge gained from dynamic modelling of host-pathogen interactions will be instrumental in further projects, for instance to target antibiotics according to metabolic networks (new project ApsMetNet within the EU ERA-net program, Infect-ERA). It will also be used to improve strategies against fungal and bacterial infections. In the future, understanding the physiology of these different organisms and their interactions with the host will remain a key topic of our group.

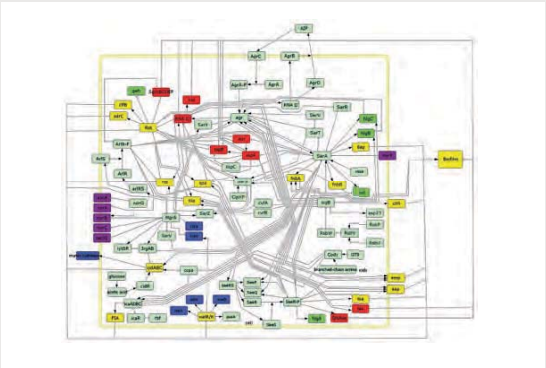


Fig. 1: We combine systems biological modeling of various pathogens and their host interaction as well as bioinformatical analysis of genome and transcriptome. To illustrate this, we present a Boolean model of biofilm production in *S.aureus*. The model was validated by biofilm experiments and transcriptomics data and allows to study the effect of regulators such as SaeR and Agr (Audretsch *et al.*, Mol. Biosystems, 2013). Nodes for different functions are coloured.

SELECTED PUBLICATIONS

Hemer S, Konrad C, Spiliotis M, Koziol U, Schaack D, Förster S, Gelmedin V, Stadelmann B, **Dandekar T**, Hemphill A, Brehm K (2013) *Host insulin stimulates Echinococcus multilocularis insulin signalling pathways and larval development.* **BMC Biology** in press

Winstel V, Liang C, Sanchez-Carballo P, Steglich M, Munar M, Bröker BM, Penadés JR, Nübel U, Holst O, **Dandekar T**, Peschel A, Xia G (2013) *Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens.* **Nature Communications** 4:2345

Karl S, **Dandekar T** (2013) *Jimena: efficient computing and system state identification for genetic regulatory networks.* **BMC Bioinformatics** 14:306

Naseem M, **Dandekar T** (2012) *The role of auxin-cytokinin antagonism in plant pathogen interactions.* **PLoS Pathogens** 8:e1003026

Naseem M, Philippi N, Hussain A, Wangorsch G, Ahmed N, **Dandekar T** (2012) *Integrated systems view on networking by hormones in arabidopsis immunity reveals multiple crosstalk for cytokinin.* **Plant Cell** 24:1793-814

4. ZINF MEMBERS ASSOCIATE WITH OTHER INSTITUTES

4.2. BIOINFORMATICS

Infections are complex multiparametric processes, involving many cell types, molecular networks and different environmental conditions. The group is interested in applying broad bioinformatic approaches to model host-pathogen interactions, from individual molecules to metabolic reconstructions as well as systems biology modeling approaches to regulatory networks and their dynamics.

PROF. THOMAS DANDEKAR

DEPARTMENT OF

BIOINFORMATICS

Lehrstuhl für Bioinformatik
Biozentrum
Am Hubland
D-97074 Würzburg
T +49 (0)931-3184551
M dandekar@biozentrum.uni-wuerzburg.de

INTRODUCTION

Bioinformatics traditionally follows the genetic flow of information from DNA to RNA to proteins. The assembled components have to be interpreted as a complete system with emergent properties, requiring cutting edge methods in systems biology and network modelling. The group's strong interest in infection biology results from the fact that all these aspects are combined in the interaction between host and pathogen during the infection process, two cellular systems in intimate contact involved in an intricate battle for survival. Starting from metabolic models, we also investigate system responses as well as regulatory components. Our approach is generic, we are interested in various infection models that include different hosts such as plants *(Arabidopsis)* and animals (*Camponotus* ants; mouse, man) and different infectious agents from fungi (*Candida albicans, Aspergillus fumigatus*) to bacteria *(Salmonella enterica, Staphylococcus aureus, Listeria)* and viridae (HIV, foamy virus, vaccinia virus). To fulfill our goals, we are developing new algorithms to model regulatory and metabolic networks as well as generating different and specific biological models of metabolism and regulation in different infection processes.

RESEARCH HIGHLIGHTS

Recently, we developed Jimena, a new Java genetic regulatory network simulation framework, to improve the efficiency of dynamic modelling of regulatory networks. The Jimena algorithm turns Boolean models into dynamic models by allowing choices between different interpolation methods. It is fully compatible with, as well as reproduces results from, other methods such as the Squad algorithm. Furthermore, it is able to enumerate all system states in networks, which is a major success compared to earlier efforts and shown in different examples including plant-pathogen interactions.

Our modelling of plant pathogen interactions has focused on plant hormones and the role of cytokinins in the immune response. We have described the delicate balance between immunoprotective hormones in the plant host as well as immune compromising hormones, often triggered by a parasitic pathogen. Cytokinins modulate the immune dynamics in various plant species including *Arabidopsis*, tobacco and rice when challenged with different pathogens (types: biotrophic, necrotrophic and hemibiotrophic). By using dynamic modeling and system analysis, we revealed that they are able to influence the central immune pathways such as jasmonate and salicylate pathways of resistance. Since low and higher cytokinin levels change the immune response further, the result of an infection is also determined by the concentration changes in cytokinins. We have shown that cytokinins have a role beyond galls and green islands, stress responses, plant growth and development as they shape the outcome of infection by different pathogens.

We are also studying staphylococcal pathophysiology. We have participated in a study analysing wall teichoic acid structure in *Staphylococcus aureus* strain PS187 as this changes the evolution of the strain, its metabolism as well as its vulnerability to phage infection. Furthermore, we have modelled in detail the response in quorum

to the attached part of the flagellum, which wraps around the parasite in a characteristic turn, resulting in rotation of the asymmetric cell body. Importantly, we found that trypanosome motion is optimized for navigation in mammalian blood. Microfluidics, micropillars and advanced high-speed microscopy revealed that correct spacing and diameter of blood cells is required for fast forward swimming of the parasites. Unexpectedly, trypanosomes also have a 'reverse gear': when they are trapped, for example in tissues spaces, they effectively reverse the flagellar beat direction. This is unique among eukaryotes and raises the question how such a reversal can work on a molecular and biophysical level. Thus, we can expect more fundamental insights from trypanosomes.

Another highlight of our recent work is a more holistic view of antigenic variation and the role of VSG in trypanosome virulence. We found that VSG structure has been shaped by evolution for maximum crowding and mobility on the surface. Correct VSG density can only be maintained by posttranslational modifications that minimize attractive forces. Furthermore, we have been able to show that the VSG protein itself controls its monoallelic expression. An ectopic genetic system was established that for the first time allows induction of ES silencing. Using this tool, we have revealed that three expression site-associated genes (ESAG) sense the ES activity and – unexpectedly – are involved in cell cycle progression and developmental competence. Thus, our work revises the view on the trypanosome expression site, which in fact can be regarded a global genetic regulator of parasite virulence.

FUTURE DIRECTIONS

We will intensify the genetic and biophysical analyses of VSG coat maintenance. This includes identifying factors that control ES switching and development of the parasite. A set of mutant VSGs has already been generated that reveal highly specific defects in routing to and from the cell surface. We have also invested significantly in technology that enables the observation of the motion of single VSG molecules on the cell surface and within the complex endocytic recycling system. Furthermore, we have established tsetse fly breeding in our laboratory, enabling analyses on the behaviour of trypanosomes in the tsetse fly. Here, our future interest is to genetically manipulate the parasite and insect vector for deciphering developmental transitions.

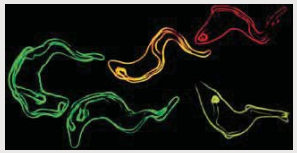


Fig. 1: Like many of the parasites that cause tropical diseases, *Trypanosoma brucei* employs genetic trickery to evade the immune systems of humans and other mammals. This involves changing the variant surface glycoprotein (VSG) coat that surrounds the parasite on a regular basis in order to remain one step ahead of the immune system of its host: while the immune system looks for invaders wearing a particular coat, the parasites are spreading through the host in a completely different coat. This image shows trypanosomes expressing different types of VSG, detected by distinct antibodies (green, yellow, red).

Fig. 2: Blood feeding of tsetse flies in the newly built insectary at the Department of Cell and Developmental Biology.

SELECTED PUBLICATIONS

Heddergott N, Krüger, T, Babu SB, Wei A, Stellamans E, Uppaluri S, Pfohl T, Stark H, **Engstler M** (2012) *Trypanosome motion represents an adaptation to the crowded environment of the vertebrate bloodstream.* **PLoS Pathogens** 8: e1003023

Uppaluri S, Heddergott N, Stellamanns E, Herminghaus S, Zöttl A, Stark H, **Engstler M**, Pfohl T (2012) *Flow Loading Induces Oscillatory Trajectories in a Bloodstream Parasite.* **Biophysical Journal** 103: 1162-1169

Abuillan W, Vorobiev A, Hartel A, Jones NG, **Engstler M**, Tanaka M (2012) *Quantitative determination of the lateral density and intermolecular correlation between proteins anchored on the membrane surfaces using grazing incidence small-angle X-ray scattering and grazing incidence X-ray fluorescence.* **Journal of Chemical Physics** 137: 204907

Weiße S, Heddergott N, Heydt M, Pflästerer D, Maier T, Haraszti T, Grunze M, **Engstler M**, Rosenhahn A (2012) *A Quantitative 3D Motility Analysis of Trypanosoma brucei by Use of Digital In-line Holographic Microscopy.* **PLoS One** 7:e37296

4.3. MOLECULAR AND PHYSICAL PARASITOLOGY

Motion is a hallmark of life. We study motion on very different scales, from molecules to organelles to cells and beyond. Our model system is the African trypanosome, a deadly blood parasite.

PROF. MARKUS ENGSTLER

DEPARTMENT OF CELL AND DEVELOPMENTAL BIOLOGY

Lehrstuhl für Zell- und Entwicklungsbiologie
Biozentrum
Am Hubland
D-97074 Würzburg
T +49 (0)931-3180060
M markus.engstler@biozentrum.uni-wuerzburg.de

INTRODUCTION

The functioning of all biological processes relies on motion, at times directed and energy-dependent and at other times random and driven by thermodynamics. Signalling molecules must reach their destinations, as must vesicles and organelles. Obviously the environment always influences motion, a fact that is surprisingly neglected. Chemical and physical cues such as viscosity, pH, temperature, boundaries or obstacles must be taken into account for a proper understanding of the behaviour of proteins in the cytoplasm or within biological membranes. Likewise, macromolecular and organelle trafficking is not only controlled by biological motors or the cytoskeleton but also by factors such as crowding and confinement.

African trypanosomes are perfect model organisms for the analysis of motion on different scales. The unicellular parasites are constantly motile throughout their complex life cycle. They prosper in the blood of their mammalian hosts, they penetrate into different tissues and they cross the blood-brain barrier. Once ingested with the bite of the transmitting tsetse fly, they must undergo dramatic cell biological changes in order to adapt to varying environments in the insect, a 30-day journey through mostly unknown 'terrain'. At any time, trypanosomes are covered with a dense coat of glycoproteins. Within the mammalian host the parasites are protected by variant surface glycoproteins (VSG), which completely cover the cell surface with some 10 million copies of the same protein. This VSG coat has to maintain its density and fluidity in order to function – a phenomenon that requires very accurate control of membrane and protein trafficking. This makes trypanosomes ideal models for studying the motion of vesicles and organelles.

Trypanosomes have hundreds of VSG genes of which only one is expressed at any given time from one of 15 expression sites (ES). Stochastic switching to the expression of a new VSG forms the basis of antigenic variation, a process discovered in trypanosomes, but present in many pathogens. How antigenic variation works is only partly understood. Likewise it is unclear how VSG coat density is maintained during the cell division cycle. This is of particular interest, as in trypanosomes all biosynthetic organelles are present only in single copies and have to be duplicated and positioned precisely to guarantee transport and recycling of new coat compounds – a process that requires fine-tuned control of molecular and vesicular motion.

RESEARCH HIGHLIGHTS

Much of our current research is based on the finding that trypanosomes exploit hydrodynamic drag forces for immune evasion. We found that fluid flow generated by incessant cell motility can move host antibody-complexed VSGs in the plane of the plasma membrane. As trypanosome motion is directional, the antibody-tagged VSGs are pushed to the posterior part of the cell, where all endocytosis takes place. In this way host antibodies are cleared from the circulation. More recently, in a truly interdisciplinary approach we have used novel methodology to analyse cell motion with unprecedented accuracy. We have found that trypanosomes exhibit a very complex type of motion, which is directed by planar beating of the free part of their single flagellum. The mechanical force is conveyed

4. ZINF MEMBERS ASSOCIATE WITH OTHER INSTITUTES

4.4. MARINE SPONGE-MICROBE INTERACTIONS

My group aims to provide an in-depth understanding of the physiology, metabolism and molecular mechanisms of interactions between marine sponges and their microbial symbiotic communities. Furthermore, we seek to isolate novel anti-infective secondary metabolites from marine sponge-associated actinomycetes.

PROF. UTE HENTSCHEL HUMEIDA
DEPARTMENT OF BOTANY II

Lehrstuhl für Botanik II
Julius-von-Sachs Institut für Biowissenschaften
Julius-von-Sachs Platz 3
T +49 (0)931-3182581
M ute.hentschel@uni-wuerzburg.de

INTRODUCTION

Many species of sponges (phylum Porifera) harbor enormously dense and diverse communities of symbiotic microorganisms in their tissues, which can comprise up to 35% of the total sponge biomass. This remarkable microbial and chemical diversity, coupled with the ancient nature of the sponge-microorganism association, renders sponges important model systems to study metazoan host-microorganism evolution and interactions. Collectively, the animals and their microbial consortia boast an impressive metabolic and chemical repertoire that not only contributes to their nutritional ecology but has also fostered interest from the pharmaceutical industry due to their production of bioactive compounds.

In terms of microbial diversity, as many as 29 bacterial phyla, among them 12 candidate phyla and two archaeal lineages have thus far been identified in sponges. Recent amplicon sequencing studies have indicated the existence of as many as several thousand lineages of symbionts, making sponges one of the most diverse host-microbe associations in the marine environment. Sponges are now considered valuable systems for the study of high-diversity marine host- microorganism associations that resemble the human gut microbiome in several aspects. With the aid of next-generation sequencing technologies and the greater sequencing depth that they afford, a clearer picture of microbial diversity in these hosts is emerging and the factors that influence this diversity are being identified.

In terms of microbial function, we are now beginning to unravel the functions of sponge symbionts. Besides a dedicated role in nitrogen metabolism where they recycle metabolic waste products of the sponge host such as ammonia, some lineages also seem to participate in carbon degradation. Moreover, the microbial symbionts may supplement the animal host with vitamins. However, much remains to be learnt about the various functions of microbial symbionts in the context of the sponge "holobiont".

Sponge-associated microorganisms are also of relevance from a bioprospecting perspective. These sessile animals are a particularly rich source of actinomycetes, which are prolific producers of secondary metabolites with various clinically relevant bioactivities. In this context, large strain collections have been established which are being screened for bioactivity. Bioactivity-guided fractionation in combination with metabolomics and genome sequencing is being used to isolate novel natural products with anti-infective properties. For example, we have reported the novel antioxidant and anti-protease activities of diazepinomicin, which has attracted considerable interest owing to its broad-spectrum anti-tumor activity.

RESEARCH HIGHLIGHTS

One recent research highlight pertains to the candidate phylum Poribacteria, members of which are nearly exclusively found in sponges. We have employed single-cell genomics to obtain comprehensive insights into the metabolic potential of individual poribacterial cells. Detailed analysis of carbohydrate metabolism revealed their ability to degrade diverse carbon sources that likely originate from seawater and the host itself. Furthermore, the presence of specific glycoside hydrolases, uronic acid degradation pathways

as well as several specific sulfatases provides strong support that Poribacteria also degrade glycosaminoglycan (GAG) chains of proteoglycans, which are key components of the sponge host matrix. Therefore, Poribacteria may be viewed as efficient scavengers and recyclers of a particular suite of carbon compounds that are unique to sponges as microbial ecosystems.

The group was also involved in the discovery of another candidate phylum, Tectomicrobia in collaboration with J. Piel (ETH Zürich) and colleagues, members of which are highly enriched in the sponge *Theonella swinhoei.* The combination of metagenomics and single-cell genomics revealed that one phylotype, "Candidatus Entotheonella factor TSY1", produces almost all of the numerous natural products that were previously isolated from the sponge host. Overall, our efforts are directed at providing a deeper understanding of the high-complexity microbial ecosystems within sponges, and at providing research strategies to sustainably use this natural resource.

FUTURE DIRECTIONS

During the next few years, we will aim to combine in-situ work involving experimental manipulation and state-of-the-art physiological measurements with high-throughput -omics technologies to address basic questions in sponge microbiology and symbiosis research. In other words, we will place our omics-generated hypotheses into an ecological context. With regards to novel antiinfective discovery, we will sequence more actinomycete genomes from our collections with the aim of uncovering the hidden genomic potential for secondary metabolism. Genomics-data will be integrated with metabolomics-data to identify elusive metabolites that have been missed by current protocols. We will further explore the power of co-cultivation to elicit metabolites that are not produced in pure culture.

Fig. 1: The Mediterranean sponge *Aplysina aerophoba* as a model for sponge microbiology. Underwater photography: Janine Kamke, University of Würzburg.

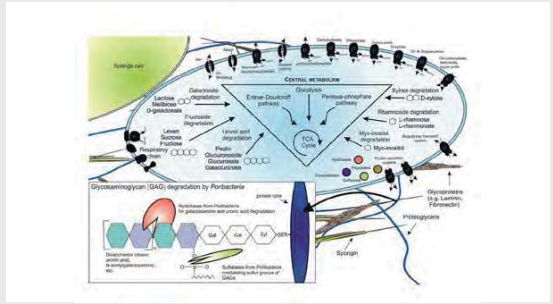


Fig. 2: Schematic overview of a poribacterial cell within the sponge extracellular matrix illustrating pathways of carbohydrate metabolism and glycosaminoglycan degradation by poribacterial enzymes. (Kamke et al. ISME J 2013)

SELECTED PUBLICATIONS

Wilson MC, Mori T, Rückert C, Uria AR, Helf MJ, Takada K, Gernert C, Steffens UA, Heycke N, Schmitt S, Rinke C, Helfrich EJ, Brachmann AO, Gurgui C, Wakimoto T, Kracht M, Crüsemann M, **Hentschel U**, Abe I, Matsunaga S, Kalinowski J, Takeyama H, Piel J (2013) *An environmental bacterial taxon with a large and distinct metabolic repertoire.* **Nature** 506:58-62

Kamke J, Sczyrba A, Ivanova N, Schwientek P, Rinke C, Mavromatis K, Woyke T, **Hentschel U** (2013) *Single-cell genomics reveals complex carbohydrate degradation patterns in poribacterial symbionts of marine sponges.* **ISME Journal** 7: 2287-2300

McFall-Ngai M, Hadfield M, Bosch T, Carey H, Domazet-Loso T, Douglas A, Dubilier N, Eberl G, Fukami T, Gilbert S, **Hentschel U**, King N, Kjelleberg S, Knol, AH, Kremer N, Mazmanian S, Metcalf J, Nealson K, Pierce N, Rawls J, Reid A, Ruby E, Rumpho, M, Sanders J, Tautz, D, Wernegreen (2013) *Animals in a bacterial world: a new imperative for the life sciences.* **PNAS** 110: 3229-3236

Hentschel U, Piel J, Degnan SM, Taylor MW (2012) *Genomic insights into the marine sponge microbiome.* **Nature Reviews Microbiology** 10: 641-654

Abdelmohsen UR, Szesny M, Othman EM, Schirmeister T, Grond S, Stopper H, **Hentschel U** (2012) *Antioxidant and anti-protease activities of diazepinomicin from the sponge-associated Micromonospora strain RV115.* **Marine Drugs** 10: 2208-2221

PRIZES AND AWARDS

2014 Marine Drugs Best Paper Award

have proven to be an excellent starting point for further drug development resulting in preclinical candidates.

While the fluoroquinolones are gyrase inhibitors with high antiinfective activity against Gram-positive and -negative bacteria, they do not have any activity against protozoa. Interestingly, amidation of the carboxylic acid group, which is essential for the bactericidal effect, with benzyl amine groups results in antitrypanosomal activity in the nanomolar range and very low cytotoxicity (high selectivity). Unfortunately, these compounds are not very water soluble. Several rounds of optimization resulted in drugs with improved activity against trypanosoma and increased solubility that can be further improved by a corresponding formulation of the drug (for structure see Fig. 2). In vivo experiments using an appropriate mouse model revealed the quinolonamides are able to cure trypanosoma-infected mice without any sign of toxicity. Initial studies to elucidate the mechanism of action revealed that the amides did not target the gyrase, which is in contrast to the fluoroquiniolones in clinical use. However, the mode of action remains to be elucidated.

FUTURE DIRECTIONS

Since the development of LpMip and BpMip inhibitors resulted in promising lead structures, we will aim to use these to design inhibitors which are active also against Chlamydia, Neisseria, and *Trypansoma cruzi* Mip and develop preclinical candidates with optimized pharmacokinetic properties. With regard to the antitrypanosomal fluoroquinolonamide derivatives we will elucidate the mode of action, this is required before we are able to further optimize the already highly active lead compound to produce preclinical candidates.

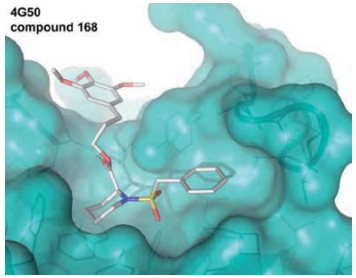
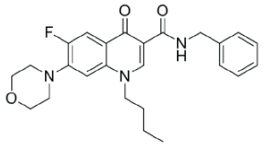


Fig. 1: Compound 168 sitting in the binding pocket of the *Burkholderia* Mip.



IC_{50} (*T.b.brucei*, 72h) 47 nM
IC_{50} (*BSF T.b.b.*, 48h) = 23 nM
IC_{50} (*T.b. rhodesiense*, 72h) 9 nM
IC_{50} (*J774.1* macrophages) 57 µM

Fig. 2: One of the most active antitrypanosomal fluoroquinolonamides.

SELECTED PUBLICATIONS

Stich A, Ponte-Sucre A, **Holzgrabe U** (2013) *Do we need new drugs against Human African Trypanosomiasis?* **Lancet Infectious Diseases** 13: 733-4

Laufer S, **Holzgrabe U**, Steinhilber D (2013) *Drug Discovery: A modern Decathlon.* **Angewandte Chemie** International Edition 52: 4072- 4076

Hiltensperger G, Jones N, Niedermeier S, Stich A, Kaiser M, Jung J, Puhl S, Damme A, Braunschweig H, Meinel L, Engstler M, **Holzgrabe U** (2012) *Synthesis and Structure-Activity Relationships of New Quinolone-type Molecules against Trypanosoma brucei.* **Journal of Medicinal Chemistry** 55: 2538-2548

Tischer M, Pradel G, Ohlsen K, **Holzgrabe U** (2012) *Bisquaternary Ammonium salts and their antimicrobial potential – targets or unspecific interactions?* **ChemMedChem** 7: 22-31

Djukic M, Munz M, Sörgel F, Eiffert H, **Holzgrabe U**, Nau R (2012) Overton`s rule helps to estimate the penetration of antiinfectives into the cerebrospinal fluid in patients. **Antimicrobial Agents and Chemotherapy** 56: 979-988

4. ZINF MEMBERS ASSOCIATE WITH OTHER INSTITUTES

4.5. MEDICINAL CHEMISTRY

New antiinfective drugs against Gram-negative bacteria and protozoa, such as trypanosomes, are urgently needed due to the increasing levels of resistance against current antibiotics. We are employing structure-based drug design to develop and optimize antitrypanosomal compounds and inhibitors of the virulence factor Mip (macrophage infectivity potentiator) protein present in some Gram-negative pathogens.

PROF. ULRIKE HOLZGRABE
INSTITUTE FOR PHARMACY AND FOOD CHEMISTRY

Institut für Pharmazie und Lebensmittelchemie
Am Hubland
D-97074 Würzburg
T +49 (0)931-3185461
M u.holzgrabe@pharmazie.uni-wuerzburg.de

INTRODUCTION

The decline in the design and development of antiinfective drugs by the pharmaceutical industry has resulted in an almost empty drug pipeline. As many pathogens become increasingly resistant to one or more antibiotics we are in danger of losing the arsenal of drugs against infections. This is especially true for Gram-negative bacteria. Recently, the WHO stated that infections by *Pseudomonas, Klebsiella, Chlamydia,* and *Neisseria* are becoming therapeutically problematic. However, for tropical diseases such as malaria, sleeping sickness and leishmanial infections the situation is even worse, because of the lack of effective drugs with no severe adverse effects in addition to resistance problems. Therefore, the Collaborative Research Center SFB630 at the University of Würzburg, has been focusing on the recognition, preparation and functional analysis of agents against infectious diseases.

In order to overcome these problems it is necessary to find novel drugs against new targets, which first require validation of their lethality and also new chemical structures. Until now most of the antibiotics in clinical use either interfere with the replication of bacteria and protozoa or with protein and cell wall biosynthesis. However, blocking the entry of the bacteria and their dissemination in the host, by targeting virulence factors, are also promising strategies to inhibit infections. Many of the Gram-negative bacteria express "macrophage infectivity potentiator" (Mip) proteins which are involved in these processes. Inhibition of Mip in *Legionella* and *Burkholderia* has been shown to inhibit infection.

RESEARCH HIGHLIGHTS

Within the framework of the Collaborative Research Center SFB630 we have developed effective inhibitors of the *Legionella pneumophila* (Lp) Mip by rational drug design. For this we have utilised the available crystal structure of the protein liganded with rapamycin and elucidated the solution structure of prolyl peptidyl isomerase (PPIase) domain of Mip by NMR spectroscopy. Although rapamacin is known to inhibit Mip, it cannot be used due to its immunosuppressive activity. Therefore, we have optimized the PPIase inhibitory activity and reduced the cytotoxicity by making use of the part of the rapamycin molecule that is responsible for the PPIase inhibition. Activity optimization was achieved by systematic variation of the molecule based on the structural information of protein, NMR spectroscopy and bioassays. Moreover, the pipecolic acid derivatives (Fig. 1),which were highly active against LpMip, showed even higher activity against the *Burkholderia pseudomallei* (Bp) Mip and to certain extent also against *Francisella tularensis* and *Yersinia pestis* Mip, all of which are structurally related to the LpMip. The crystal structure of the BpMip liganded with our inhibitors (Fig. 1) revealed a similar binding mode of the pipecolic acids to LpMip even though they function as better BpMip inhibitors. The most efficient inhibitors were tested in macrophages infected with Bp and were found to substantially reduce the cell death (in collaboration with D. Begley and P. Myler, Seattle, USA, I. Norville, Exeter, UK and M. Tyson, Perth, Australia). These compounds will be studied in an appropriate mouse model in the near future. Taken together the pipecolic acid derivatives

synthesis is blocked. Hence, we propose that the FAS is a valid and promising drug target for the development of novel anti-staphylococcal drugs. Based on our structural data, inhibitors targeting *S. aureus* FabI may be further improved raising the hope for novel antibiotics to treat resistant pathogens.

The mycobacterial condensing enzyme KasA is pivotal for the synthesis of very long fatty acids, the precursors of mycolic acids. We have determined the structure of this protein in complex with a mycobacterial phospholipid and with several thiolactomycin derivatives that were designed as substrate analogs. Our structures provide snapshots for each step of the reaction and support an induced fit mechanism in which a wide cavity is established. The analysis of the binding process provides mechanistic insights into the induced fit recognition in this system and serves as a foundation for the development of high affinity KasA inhibitors.

FUTURE DIRECTIONS

Our recent studies on the enzymes involved in fatty acid biosynthesis have provided new insights into catalysis and how this knowledge can be utilized to develop high affinity inhibitors. An additional aim of our structure-based-drug design approach is the analysis of targets that may not be essential for the infectious state of *M. tuberculosis* but for the latent phase, i.e. the chronic stage of infection. We have therefore initiated studies on a mycobacterial target involved in cholesterol metabolism. Furthermore, we will explore the potential of targeting protein-protein interaction sites for drug development. In bacteria, the growing acyl chain is transported by the acyl-carrier protein sequentially from one enzyme to the next in the FAS cycle. The acyl carrier protein forms relatively short-lived complexes with its interaction partners but these interactions are essential for catalysis. We will characterize complexes formed between the acyl-carrier protein and its interaction partners in the FAS system and utilize this knowledge to develop new lead compounds that interfere with these interaction sites.

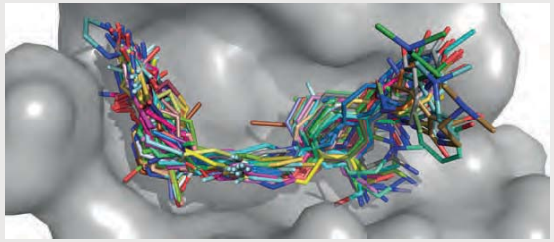


Fig. 1: The active site of S. aureus FabI. Based on our crystal structure we designed new potential inhibitors which are shown in different colors in the *S. aureus* FabI substrate binding pocket (gray).

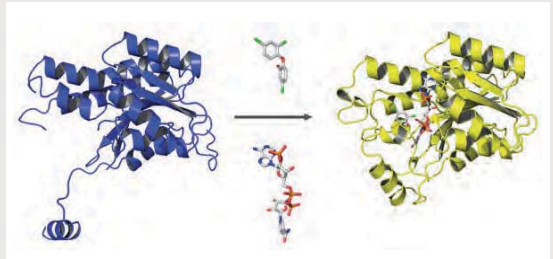


Fig. 2: Active site flexibility of saFabI. The active site region, located in the upper part of the chair-like saFabI structure, is in an open conformation in the unliganded form (blue cartoon) and adopts a closed arrangement (yellow) upon binding of $NADP^+$ and the diphenyl ether inhibitor triclosan (grey stick models).

SELECTED PUBLICATIONS

Schiebel, J, Kapilashrami, K, Fekete, A, Bommineni, GR, Schaefer, CM, Mueller, MJ, Tonge, PJ, **Kisker C** (2013) *Structural basis for the recognition of mycolic acid precursors by KasA, a condensing enzyme and drug target from Mycobacterium tuberculosis* **Journal of Biological Chemistry** 288: 34190-204

Chang, A, Schiebel, J, Yu, W, Bommineni, GR, Pan, P, Baxter, MV, Khanna, A, Sotriffer, CA, **Kisker, C,** Tonge PJ (2013) *Rational optimization of drug-target residence time: insights from inhibitor binding to the Staphylococcus aureus FabI enzyme-product complex* **Biochemistry** 52:4217-28

Schiebel, J, Chang, A, Lu, H, Baxter, MV, Tonge, PJ, **Kisker C** (2012) *Staphylococcus aureus FabI: inhibition, substrate recognition, and potential implications for in vivo essentiality* **Structure** 20:802-13

Hirschbeck, MW, Kuper, J, Lu, H, Liu, N, Neckles, C, Shah, S, Wagner, S, Sotriffer, CA, Tonge, PJ, **Kisker C** (2012) *Structure of the Yersinia pestis FabV enoyl-ACP reductase and its interaction with two 2-pyridoneinhibitors.* **Structure** 20:89-100

PRIZES AND AWARDS

2013 Vice-Chair of the Scientific Committee of the Helmholtz-Center for Infection Research, Braunschweig, Germany

2013 Member of the Scientific Advisory Board of the Bavarian Science Foundation

4. ZINF MEMBERS ASSOCIATE WITH OTHER INSTITUTES

4.6. STRUCTURE BASED DRUG DESIGN

The increasing emergence of pathogenic bacteria resistant to common antibiotics is a worldwide medical concern. My group aims to characterize new targets and to identify new lead compounds for the treatment of specific infectious diseases.

PROF. CAROLINE KISKER
RUDOLF VIRCHOW CENTER FOR EXPERIMENTAL BIOMEDICINE

Rudolf Virchow Zentrum für Experimentelle Biomedizin
Josef-Schneider-Str. 2/D15
D-97080 Würzburg
T +49 (0)931-3180381
M caroline.kisker@virchow.uni-wuerzburg.de

INTRODUCTION

Based on estimates from the World Health Organization approximately one third of the world's population is infected with *Mycobacterium tuberculosis* and about 10% of these individuals will develop an active infection. Critical issues in the treatment and control of the disease include the emergence of multi-, extensively- and, more recently, also totally-drug-resistant strains. Another pathogenic organism, *Staphylococcus aureus*, which is carried intermittently or persistently by 30-50% of the adult population, has rapidly adapted to the presence of drugs that are used to treat staphylococcal infections. Hence, infectious diseases caused by drug resistant bacterial strains are becoming an urgent world-wide problem.

Since phospholipids are an integral part of the essential bacterial cell envelope, fatty acid biosynthesis (FAS) is a promising but yet comparatively unexplored drug target. Bacterial FAS fundamentally differs from its mammalian counterpart and thus may be selectively inhibited. We are therefore focusing our structure-based-drug design efforts on two essential proteins in the bacterial FAS pathway, FabI (InhA in *M. tuberculosis*) and KasA. Based on our structural and functional analyses, inhibitors targeting these enzymes may be further improved raising the hope for novel antibiotics to treat resistant pathogens.

RESEARCH HIGHLIGHTS

Recently, the validity of targeting the FAS for the development of drugs active against Gram-positive bacteria was challenged due to their ability to accept fatty acids from the human blood during infection. Nevertheless, three drug candidates that inhibit the *S. aureus* enoyl-ACP reductase (saFabI) have shown excellent *in vivo* potency and are currently in clinical development.

As an integral member of a structure-based-drug design collaboration for the development of compounds inhibiting saFabI, we have solved several structures of this enzyme. Intriguingly, the region of the protein that forms the active site is extremely flexible in the absence of cofactor and inhibitor. Only upon binding of both ligands does the active site of the enzyme become fully established via an induced-fit mechanism. This process is associated with a dimer-tetramer transition and we have shown that upon treatment of saFabI with $NADP^+$ and diphenyl ether inhibitors, the protein changes its quaternary structure to a tetrameric assembly. During this process, the ordering of the active site region directly contributes to the formation of the dimer-dimer interface.

SaFabI displays several features that are uncommon for typical enoyl-ACP reductases. Notably, the fatty acid profile of *S. aureus* fundamentally differs from other bacterial species. Branched-chain fatty acids (BCFA) are a unique and major fraction of the cell membrane in some Gram-positive bacteria including staphylococci, where they been shown to be required for its *in vivo* fitness. We hypothesized that the additional saFabI flexibility is necessary for the effective reduction of branched-chain substrates and we experimentally revealed that saFabI more readily reduces substrates leading to branched- in contrast to straight-chain fatty acids. Importantly, the human blood only contains a very low level of BCFAs that may not be sufficient for the survival of this pathogen if endogenous fatty acid

Preparation, and Functional Analysis of Agents against Infectious Diseases" (SFB 630). This has led to the testing of synthetic molecules as possible drugs, and the successful identification of strong candidates with favourable therapeutic index.

The department has a strong research and training cooperation with the Catholic University of Health and Allied Sciences in Mwanza, Tanzania. The Bugando Medical Centre was the site of a double-blind randomised controlled clinical trial examining the effect of low dose steroids on the evolution of HIV infections. In cooperation with the Institute of Virology (Würzburg) and University of Stellenbosch, South Africa, we have revealed an alarming rate of primary drug resistance in patients with HIV infections. Both studies have been brought to the attention of WHO and have the potential to influence the treatment naive policy guidelines of HIV in Africa.

Parasitic infections are still highly prevalent in rural, semi-urban and even urban populations in Africa. We are involved in studies on novel approaches in the diagnosis of malaria, the prevalence of intestinal parasites such as *Strongyloides stercoralis* and *Giardia intestinalis* and on the co-morbidity of schistosomiasis and Hepatitis B.

FUTURE DIRECTIONS

During the next years we will intensify our cooperation with African universities in Tanzania, Ghana and South Africa, with HIV infection remaining a strong focus. HIV treatment programmes will be used to help to establish African health systems for the management of chronic non-communicable diseases such as hypertension and diabetes. Recently, the German Leprosy and Tuberculosis Relief Association, the world's largest leprosy relief organisation, which is based in Würzburg, has developed a new research agenda. In close cooperation with this non-governmental organisation, we will intensify our work on tuberculosis, leprosy and Buruli ulcer. In addition Würzburg is the study centre of a new nationwide project to detect and manage patients with Chagas disease in Germany. This work will be supported in collaboration with the *Universidad Católica de las Misiones* in Posadas, Argentina.

Fig. 1: Community in the Congo

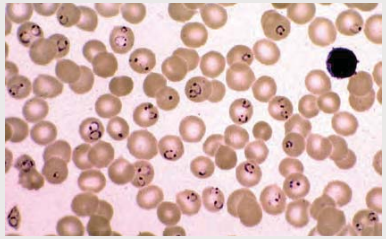


Fig. 2: Plasmodium falciparum in a patient with severe malaria

SELECTED PUBLICATIONS

Tappe D, **Stich A,** Langeheinecke A, von Sonnenburg F, Muntau B, Schafer J, Slesak G Suspected new wave of muscular sarcocystosis in travellers returning from Tioman Island, Malaysia. **Eurosurveillance** *in press*

Strasen J, Williams T, Ertl G, Zoller T, **Stich A,** Ritter O Epidemiology of Chagas disease in Europe: many calculations, little knowledge. **Clinical Research in Cardiolology** *in press*

Tappe D, Ernestus K, Rauthe S, Schoen C, Frosch M, Müller A, **Stich A** (2013) Initial patient cluster and first positive biopsy findings in an outbreak of acute muscular sarcocystislike infection in travelers returning from Tioman island, peninsular Malaysia, in 2011. **Journal of Clinical Microbiology** 51:725-6

Stich A, Ponte-Sucre A, Holzgrabe U (2013) *Do we need new drugs against human African trypanosomiasis.* **Lancet Infectious Diseases** 13:733-4

Tappe D, Nemecek A, Zipp F, Emmerich P, Gabriel M, Günther S, Dobler G, Schmidt-Chanasit J, **Stich A** (2013)Two laboratory-confirmed cases of Japanese encephalitis imported to Germany by travelers returning from Southeast Asia. **Journal of Clinical Virology** 54: 282-5

PRIZES AND AWARDS

Elected Vice President of the German Society of Tropical Medicine and International Health

Elected Speaker of the treatment centers in the Working Group of Highly Contagious Diseases STAKOB at the Robert Koch Institute Belin

2013 Elected Vice President of the German Leprosy and Tb Relief Association

2012 Elected Speaker of the Africa Centre of the Julius- Maximilians University of Würzburg

4. ZINF MEMBERS ASSOCIATE WITH OTHER INSTITUTES

4.7. TROPICAL MEDICINE

Tropical Medicine is a multisectorial field. It comprises travel medicine, which involves providing advice on vaccinations and how to avoid contracting infectious diseases. It also involves the diagnosis and treatment of tropical diseases and exotic infections. A side issue is migrant health, which focuses on improving the health of refugees and asylum seekers in this country. The most challenging aspect is medicine in the tropics, which frequently relates to medical care in resource-limited settings.

PROF. AUGUST STICH
MEDICAL MISSION HOSPITAL

Missionsärztliche Klinik
Tropenmedizinische Abteilung
Salvatorstr. 7
D-97074 Würzburg
T +49 (0)931 7912821
M august.stich@medmissio.de

INTRODUCTION

Usually the "Tropics" are geographically defined as an area of high temperature and humidity where the sun is directly overhead at least once a year. More than 8 million Germans annually travel to tropical and subtropical countries, thus exposing themselves to diseases that are unknown to many medical practitioners in this country. Pre-travel clinics help to reduce the risk by administration of necessary vaccinations and by advising travellers on the best methods of prophylaxis against malaria and other tropical diseases.

Two percent of all returning travellers consult their doctor regarding symptoms connected with exposure to tropical diseases. Fever is the most alarming symptom and malaria must be ruled out in any person returning from endemic countries and with elevated temperature. In Germany, some 600 people are diagnosed with a plasmodial infection each year. Another important febrile disease, which is already more common than malaria, is dengue fever. This viral infection is transmitted by day active mosquitoes, which, due to global climate change, are already establishing stable colonies in southern Germany. Returning patients displaying symptoms such as diarrhoea are often infected by parasites, most commonly by the difficult-to-diagnose *Giardia intestinalis*. Skin conditions such as *Larva migrans cutanea*, staphylococcal pyodermia or erysipelas are also very common in returning travellers.

Another important aspect of tropical medicine is the area of migrant health. Eighteen percent of the population in Germany has a migrant background. Many suffer from diseases that are unknown among medical practitioners in this country, for example, sickle cell disease of familial Mediterranean fever. In some migrant communities, diseases such as chronic hepatitis C or HIV are more prevalent than in the German population. Others, such as extrapulmonary tuberculosis, are difficult to diagnose and require specialized knowledge for correct clinical management. Therefore medical care for migrants requires additional expertise. Würzburg is one of the leading centres in Germany with respect to this issue. A serious problem is the fact that refugees and asylum seekers in Germany have only limited access to our health care system, which places additional social responsibility from personnel involved in the medical care of this vulnerable and underprivileged group.

Tropical medicine also involves devising approaches to improve access to medical systems in many resource-limited settings. Social determinants such as a lack of education, gender inequity, conflicts and war, climatic changes leading to the loss of natural resources and poverty have direct and indirect influences on the health of millions of people. Thus tropical medicine is an access point into the new field of "Global Health".

RESEARCH HIGHLIGHTS

As part of the daily clinical routines, the department has been the first to detect a worldwide outbreak of sarcocystosis originating from Tioman Island in Malaysia, to report on the first European patient with Tana pox disease and to detect the first imported case of Japanese Encephalitis from Bali. There is also ongoing research on *Trypanosoma brucei*, the agent of African sleeping sickness, within the collaborative research centre "Recognition,

gastroenteritis to date. As in the case of *Salmonella*, there are currently no optimal *in vitro* cell culture or animal models that comprehensively mimic the human disease for these two prevalent pathogens. In this project, together with Cynthia Sharma (Würzburg), we have developed new human 3D *in vitro* infection models of the intestine as well as stomach to study host-pathogen interactions and pathogenesis of *Helicobacter* and *Campylobacter*.

We have also developed human lower airway tissue models (see Figure B) to study interactions with respiratory syncytial viruses and bacteria (Prof. Gross, Dr. Krempl, Würzburg). Humans are the only natural hosts of *Bordetella pertussis* and there is a lack of human tissue test systems that reflect the *in vivo* situation of the trachea and bronchi. We have recently generated a 3D tissue-engineered test system that closely resembles natural human airway mucosa based on a clinically implemented biological scaffold. It consists of a polarized respiratory epithelium with ciliated cells, mucus-producing cells and basal cells. Despite vaccination programs whooping cough is reemergining in industrialized populations and there is a high need of such a 3D test system to further improve vaccination strategies and to investigate interactions between this obligate human pathogen and its host.

FUTURE DIRECTIONS

We are aiming to further improve our models by using human primary co-cultures and applying a constant flow of medium in the apical and pulsatile flow in the vascular compartment using a peristaltic pump and a bioreactor system. Cells cultured under dynamic conditions show more *in vivo* like characteristics e.g. in morphology and protein expression levels. The vascular compartment will be supplemented with purified human peripheral blood leukocyte preparations with specific subsets (e.g., monocytes or neutrophils) depleted or enriched to study their contribution to eradication of infection disseminating into the vascular compartment.

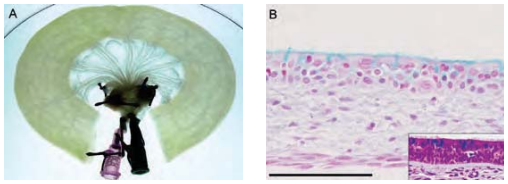
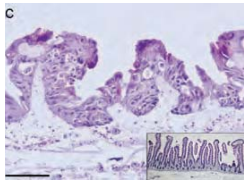


Fig. 1: BioVaSc® (Biological Vascularized Scaffold), a biological collagen matrix produced by decellularization of jejunal gut segments of a juvenile pig, which contains intact vessel structures. (B) Alcian blue staining of a 3D test system of the human airway mucosa. Blue staining indicates mucus production.



(C) H&E staining of human intestinal test system, which contains all major cell types of the gut i.e. enterocytes, goblet and enteroendocrine cells. Insets in B, C show histological staining of respective *in vivo* tissue. Scale bars: 100 µm.

SELECTED PUBLICATIONS

Schenke-Layland K, **Walles H** (2013) *Strategies in tissue engineering and regenerative medicine.* **Biotechnology Journal** 8:278-9

Stratmann AT*, Fecher D*, Wangorsch G*, Göttlich C, Walles T, **Walles H**, Dandekar T, Dandekar G, Nietzer SL (2013) *Establishment of a human 3D lung cancer model based on a biological tissue matrix combined with a Boolean in silico model.* **Molecular Oncology** *8:351-65*

Scheller K, Dally I, Hartmann N, Münst B, Braspenning J, **Walles H** (2013) *UpcyteR micro-vascular endothelial cells repopulate decellularized scaffold.* **Tissue Engineering** Part C Methods 19:57-67

Groeber F, Kahlig A, Loff S, **Walles H**, Hansmann J (2013) *A bioreactor system for interfacial culture and physiological perfusion of vascularized tissue equivalents.* **Biotechnology Journal** *8:308-16*

Steinke M, Gross R, Bauer S, Walles T, **Walles H** (2013) *A human airway mucosa tissue model to investigate whooping cough.* **European Respiratory Journal** *42: 374s*

4. ZINF MEMBERS ASSOCIATE WITH OTHER INSTITUTES

4.8. TISSUE ENGINEERING

Tissue engineering has successfully been applied to create replacement structures for reconstructive surgery. The group is interested in developing and applying complex multicellular three-dimensional (3D) tissue cultures that are able to mimic the microenvironment of human tissues to study important human bacterial or viral infections.

PROF. HEIKE WALLES
DEPARTMENT OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Lehrstuhl Tissue Engineering & Regenerative Medizin
Röntgenring 11
D-97070 Würzburg
T +49 (0)931-3188828
M heike.walles@uni-wuerzburg.de

INTRODUCTION

Tissue Engineering and Regenerative Medicine is an emerging multidisciplinary field involving biology, medicine and engineering. One of the aims of tissue engineering is to recapitulate and maintain the physiological function of cells or tissues *in vitro* for a longer period of time than is possible in simple two-dimensional cultures. This includes the development of novel biomaterials, bioreactors, and co-cultivation techniques. Thus, tissue engineered human tissue models reflecting normal and pathological situations can be designed in order to investigate the underlying cellular and molecular mechanisms involved in distinct infectious diseases. In this respect, of particular interest are models representing human barrier organs such as the gastrointestinal tract, the respiratory tract and the skin, which pose the main contact surface for pathogenic microbes.

RESEARCH HIGHLIGHTS

The group has used primary (stem) cell protocols combined with synthetic or biological-based matrices that specifically mimic the *in vivo* microenvironment of selected tissues. We have previously established a process for manufacturing a collagen matrix with a persisting blood circulation system (BioVaSc® technology, see Figure A). The matrix is based on a modified acellular porcine intestine, with intact blood vessel structures. Based on the BioVaSc® technology, we have established and functionally tested *in vitro* models of the gastrointestinal and respiratory tracts. Furthermore, we have shown that mechanical parameters such as media flow, rotation, tension, extension or pulsation stress are critical for the development of bioartificial tissues. These 3D tissue equivalents can also be engineered to contain specific immune or vascular components and enable us to monitor different stages of infection without the need for testing in animals.

The family of Enterobacteriaceae harbors many causative agents of severe microbial infections of the human host including *Salmonella enterica*. *Salmonella* Typhi causes life-threatening typhoid fever, since it is a primate restricted subspecies, its pathogenesis can only be studied in primate *in vivo* models. An *in vivo* model of *Salmonella* Typhimurium induced enteritis in humans does not exist because the pathogen causes systemic infection and typhoid fever in mice. Therefore, the study of bacterial transmission across in the human intestinal epithelium by these two pathogens requires the generation of new models to study the biologically relevant infection mechanisms. Therefore, we are using our human gastrointestinal barrier models (Figure C), which include a variety of differentiated cell types such as enterocytes, mucus-producing goblet cells and enteroendocrine cells (see Figure C) to study the infection process together with the laboratory of Jörg Vogel (Würzburg). The intestinal architecture more closely mimicks the in vivo situation and will contribute to an improved understanding of the host factors underlying the different progression of bacterial infections in humans.

The same human gastrointestinal barrier models are being used to study Helicobacter pylori and *Campylobacter jejuni* infections. About 50% of the world's population is infected with Helicobacter, the causative agent of gastritis, ulcers, and gastric cancer. The related Epsilonproteobacterium, *Campylobacter*, is the most common cause of bacterial

05

RESEARCH PROGRAMMES AND INFRASTRUCTURE

5. RESEARCH PROGRAMMES AND INFRASTRUCTURE

5.1.
Collaborative Research Centre SFB 630
Recognition, preparation and functional analysis of agents against infectious diseases

The fight against infectious diseases is currently one of the biggest challenges in industrialized, emerging, and developing countries. The latter two are severely affected by tropical diseases, which are of relatively low interest for the development of new drugs by the pharmaceutical industry. In the industrialized countries a serious health problem arises due to infections caused by multi-drug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* strains (MRSA). The CRC/SFB 630 initiative focuses on the development of new drugs against infections caused by *Trypanosomes, Leishmania, Plasmodia,* staphylococci especially MRSA, mycobacteria, *Candida*, *Neisseria* and *Chlamydia*, with a special emphasis on new lead compounds which target molecules in the pathogens that are not targets of currently available antibiotics.

Research groups in project area A of the SFB design and synthesize novel compounds or isolate them from plants and sponges. These compounds are tested in the centralized project Z1 with respect to their anti-infectious properties and toxicity while the detailed mechanisms of action of the most effective compounds are characterized in project area B with state-of-the-art technologies. Finally, project area C provides a detailed understanding at the molecular level via computer-based approaches, which ultimately results in a further optimization of the lead compounds and their pharmacological properties.

Candidate compounds will undergo further pharmaceutical improvements and will be analyzed in animals following a corresponding "galenical formulation". This represents a prerequisite for pre-clinical studies, which will be pursued with the help of non-profit organizations such as the "Drugs for Neglected Diseases Initiative" or the "Medicine for Malaria Venture".

Projects involving ZINF members

Project Area A: Preparation, characterization and optimization of agents

A1 Ulrike Holzgrabe
(Institute for Pharmacy and Food Chemistry)
Small molecules for the treatment of infectious diseases

A2 Gerhard Bringmann
(Dept. of Organic Chemistry)
A new class of active agents against infectious diseases

A5 Ute Hentschel-Humeida
(Dept. of Botany II)
Sponge-associated actinomycetes as sources for novel anti-infectives

Project Area B: Interaction with cellular and molecular systems

B2 Joachim Morschhäuser
(Institute for Molecular Infection Biology)
Inhibition of virulence and resistance mechanisms of *Candida albicans*

B3 Heidrun Moll
(Institute for Molecular Infection Biology)
Mitochondria, endosomes and autophagolysosomes as targets of leishmanicidal agents

B5 Knut Ohlsen
(Institute for Molecular Infection Biology)
Drug-induced gene expression in staphylococci and magnetic resonance-based imaging of infections

B7 Caroline Kisker
(Rudolf-Virchow Center)
Structure-based drug design on essential enzymes from pathogens

B8 Markus Engstler
(Dept. of Cell and Developmental Biology)
VSG as an unexpected drug target for sleeping sickness

B9 Thomas Rudel and Vera Kozjak-Pavlovic
(Dept. of Microbiology)
Active agents against acute and disseminating *Neisseria* infections

Central Project

Z1 Tobias Ölschläger
(Institute for Molecular Infection Biology)
August Stich
(Medical Mission Clinic)
Laboratory for the central evaluation of potential anti-infective agents

5.2.
Collaborative Research Centre SFB 581
Molecular models for diseases of the nervous system

Diseases of the nervous system follow a complex course of primary and secondary pathophysiological processes leading from a causative cellular dysfunction to the disease phenotype. Despite progress in uncovering gene defects during the last two decades, which has been made possible by the genome projects for human, mouse, drosophila and other species, it is often not possible to understand the progression of pathophysiology from the primary cause of these diseases. For example, how a gene defect leading to the specific disease phenotype can be used to develop new therapeutic strategies.

This situation calls for a cell biology oriented neurobiology approach, together in a network with clinical researchers to investigate the cell biological basis of disease development using suitable disease models. Thus the main emphasis of CRC/SFB 581 is to generate mouse and *Drosophila* models, to not only investigate the direct effects of signal transduction on cellular structures and functions in the nervous system, but also the pathophysiological processes involving interactions between different cell types involved in neuroimmunological and neurodegenerative diseases.

The goal of the SFB is to investigate the complex course of primary and secondary pathophysiological processes in diseases of the nervous system. This will be achieved by connecting molecular and cell biology oriented basic research with *in vivo* models to understand of the complex course of disease processes. Since this can only be achieved in an interdisciplinary approach, the CRC/SFB 581 brings together groups working with different methods and model systems for neurodegenerative and neuroimmunological disease processes.

Projects involving ZINF members

A5 Thomas Hünig
(Institute for Virology und Immunobiology)
Induction and therapy of ovalbumin-specific autoimmune encephalomyelitis

A9 Manfred Lutz
(Institute for Virology und Immunobiology)
Presentation of cerebral glycolipids by dendritic cells using CD1d

5.3.
Collaborative Research Centre / Transregio 34
Pathophysiology of staphylococci in the post-genome era

Staphylococcus aureus is a dangerous pathogen, a leading cause of bacterial infection in hospitals and in the community throughout the world. The microorganism is a prominent example of the crisis of antibiotic resistance, one of the major threats to health in the 21st century. At the same time, *S. aureus* is a fascinating model organism to study host-pathogen interactions. We are exposed to the bacteria, often within the first hours of life. The encounters with the versatile microorganism are multi-facetted ranging from symptom-less colonisation and mild skin infections to life threatening disease. Despite extensive efforts there is no effective anti-*S. aureus* vaccine. *S. aureus* is equipped with an impressive assortment of fitness and virulence factors, including a wide variety of immune evasive compounds. Intricate regulation networks enable the bacteria to withstand hostile environmental conditions, such as nutrient limitation, oxidative stress or anaerobic conditions. In recent years, *S. aureus* has been increasingly recognised as a facultative intracellular pathogen. The bacteria can persist inside endothelial and epithelial cells and establish chronic infection. The multiple dimensions of the topic call for an inter-disciplinary approach; its complexity requires new methodology. The CRC/TRR34 brings together bacteriologic and immunologic expertise with that in quantitative biomolecular analytics, structural biology, genomics, bioinformatics, haematology, and imaging. In the postgenomic era, the availability of whole genome sequences of *S. aureus* and its human host has paved the way for comprehensive analysis of transcription profiles, proteins and metabolites. It is now possible to obtain biological fingerprints of bacteria and host at unprecedented detail. This opens avenues for a new quality in the understanding of cell physiology, pathophysiology and infection biology. The CRC/TRR34 will focus on host-pathogen interaction during S. *aureus* colonisation and infection, progressing from cell culture systems of increasing complexity to animal models of infection and studies with human subjects.

Projects involving ZINF members

A2 Knut Ohlsen
(Institute for Molecular Infection Biology)
Phosphoproteomic analysis of *Staphylococcus aureus*: Functional characterization of kinases and identification of their substrates

A8 Thomas. Dandekar
(Dept. of Bioinformatics)
A systems biology perspective of metabolic and regulatory adaptation of *Staphylococcus aureus* to infection-related conditions

B4 Wilma Ziebuhr
(Institute for Molecular Infection Biology)
Regulation of methionine metabolism in staphylococci: Impact on fitness and virulence

C6 Jörg Vogel
(Institute for Molecular Infection Biology)
Post-invasion events in *Staphylococcus aureus* infected host cells – A combined transcriptomics/ proteomics in *in vivo* approach

C11 Thomas Rudel and Martin Fraunholz
(Dept. of Microbiology)
Host cell death induced by *Staphylococcus aureus* and its linkage to phagosomal escape

Z1 Thomas Dandekar
(Dept. of Bioinformatics)
An integrated view of adaptation of *Staphylococcus aureus*

Z3 Knut Ohlsen
(Institute for Molecular Infection Biology)
In vivo imaging of *Staphylococcus aureus* infections

5.4.
Collaborative Research Centre / Transregio 124
Pathogenic fungi and their human host: Networks of interaction

The incidence of invasive mycoses due to opportunistic fungal pathogens has increased significantly over the past two decades. This increase in infections is associated with excessive morbidity and mortality and is directly related to a growing number of patients at risk of developing serious fungal infections. Despite this, the current diagnosis of life-threatening fungal infections remains difficult and and often comes too late. There are only limited options for therapies, which are often ineffective. The yeast *Candida albicans* and the filamentous fungus *Aspergillus fumigatus* are by far the most important causes of life-threatening invasive mycoses in Europe. Both fungi have developed multiple sophisticated, specific and unique pathogenicity mechanisms, many of which are not well understood.

This CRC/Transregio brings together researchers from the Friedrich Schiller University and Hans Knöll Institute, Jena and the Re-

involved in cytoskeletal dynamics. As major membrane components, sphingolipids and their ceramide metabolites play a key role in the dynamics of activated membrane microdomains. These are implicated in steps decisive for the interaction of a host cell with pathogens such as attachment, entry or invasion, intracellular trafficking, compartimentalization and regulation of cell autonomous defense responses. Because immune responses can also be regulated at the level of sphingolipid dynamics, this pathway most likely controls decisive elements in the pathogenesis of infectious diseases where pathogen uptake, spread and dissemination are counteracted by host cell autonomous, innate and adaptive immune responses.

To address the role of sphingolipid dynamics in infection control the research unit brings together relevant groups from the University of Würzburg and the University of Essen. The unit contains expertise in the infection biology of medically important pathogens such as measles virus (MV), *Neisseria meningitidis*, *Neisseria gonorrhoeae and Mycobacterium tuberculosis,* sphingolipid biology in infectious viral and bacterial disease pathogenesis, T cell biology and immunotherapy as well as macrophage biology.

The unit will focus on the regulatory role of sphingolipid dynamics in both the host and pathogen. This includes regulating the adhesion, activation, differentiation and effector functions of T cells at a molecular and cellular level as well as in experimental infection models. In addition the effect of sphingolipid dynamics on pathogen adhesion and invasion, trafficking and modulation of host cell functions that are essential in the control of bacterial pathogens will be analysed.

Projects involving ZINF members

TP1: Sibylle Schneider-Schaulies
(Institute for Virology and Immunobiology)
Sphingomyelinase activation in T cells: Implications for T cell activation and paralysis

TP2: Niklas Beyersdorf and Jürgen Schneider-Schaulies
(Institute for Virology and Immunobiology)
Role of sphingolipids in the regulation of anti-viral T cell responses

TP3: Alexandra Schubert-Unkmeir
(Institute for Hygiene and Microbiology)
Analysis of the functional relevance of sphingomyelinases and ceramide in meningococcal pathogenesis

TP4: T. Rudel *(Dept. of Microbiology)*
Sphingolipids in gonococcal infection

5.7. DFG Priority Program SPP 1258
Sensory and regulatory RNA in prokaryotes

The discovery of many different classes of non-coding RNAs that impact gene expression, questioned the view that proteins are the sole major regulators in the cell. The importance of RNA-mediated regulation is becoming clear based on the large number of important physiological processes under their control. While the number of identified non-coding RNAs has rapidly increased during recent years, in the vast majority of cases their functions remain to be described. These RNAs bind to mRNAs or proteins with high specificity to modulate their activities. This enables them to control entire signalling cascades and corresponding biological processes; in the case of pathogens they can also control virulence. RNAs such as riboswitches can also respond to changes in the physiological state of the cell by binding specific metabolites. Likewise changes in environmental conditions such as temperature upon infection of a host, alter the conformation of RNA-thermometers, subsequently impacting their regulatory role on the levels of virulence factors. The aim of the SPP is to use several different model organisms to understand central questions such as: How many prokaryotic RNAs exist? Which general structural and regulatory features can be identified? How do regulatory RNAs achieve their high binding specificity to their targets? To which extent do RNAs influence cellular metabolism and how important are they for viability under different environmental conditions?

Projects involving ZINF members

Jörg Vogel *(Institute for Molecular Infection Biology)*
A conserved small RNA in the RpoS regulon.

Jörg Vogel *(Institute for Molecular Infection Biology)*
Multiple target regulation by GcvB sRNA.

Jörg Vogel *(Institute for Molecular Infection Biology)*
Central project: Deep sequencing as a tool for prokaryotic RNA research.

5.8. DFG Priority Program SPP 1316
Host-adapted metabolism of bacterial pathogens

Pathogens encounter many different environments during the infection process. To survive and replicate within a host cell pathogens must adapt their metabolism to the available nutrients and physical conditions. During this time they must also coordinate their metabolism with their life-cycle. Therefore, to understand how bacteria adapt to the host environment and cause disease it is not sufficient to understand the function of specific virulence determinants such as toxins or invasins. It is clear that the co-evolution of host and pathogen has also resulted in an adaptation of the metabolism of the pathogen. Researchers within SPP1316 will investigate how bacterial pathogens adapt their metabolism during colonisation of host organisms, how the metabolism of pathogenic bacteria and the host organism is interconnected and which mechanisms of control are active. Projects in this SPP thus aim to identify metabolic pathways that are important for the bacteria during infection and to determine the metabolic fluxes. This will reveal the metabolic reactions of the host organisms and the genetic mechanisms of metabolic adaptation.

search Center for Infectious Diseases in Würzburg to obtain comprehensive insight into the medically important fungi *C. albicans* and *A. fumigatus* and their interactions with the human host. The aims of the CRC/Transregio are to identify pathogenic determinants specific for each fungus and investigate the specific roles of epithelial barriers, the mechanisms of the innate immunity and potential contributions of the adaptive immune system to the pathogenesis of fungal infections. These will form the basis for elucidating the complex mechanisms of fungal infections and identify common principles of fungal pathogenesis. Finally, the insight gained from these studies will be applied to develop new therapeutic approaches. To obtain a comprehensive description and understanding of complex invasive fungal infections, a systems biological approach will be undertaken as a third dimension to the pathobiology of the pathogens and the response of the immune system. Systems biology will help to reveal the structure and dynamics of molecular and cellular cause-effect relations within these pathogenic interactions. The vision of systems biology is the generation of a 'virtual infection model' that enables the prediction of the consequences of changing parameters, such as reduced activity of certain immune effector cells or receptors for the infection.

A detailed knowledge of the infection biology of *A. fumigatus* and *C. albicans* and the immune response mechanisms will provide the basis for better diagnosis and therapy of systemic infections. Due to the involvement of two very active clinical departments a sufficient number of clinical samples will be available for the analyses and greatly contribute to translational medicine (bench to bedside).

Projects involving ZINF members

A2 Hermann Einsele and Jürgen Löffler
(Dept. of Internal Medicine II)
Interaction of *Aspergillus fumigatus* with human natural killer cells, dendritic cells and human alveolar epithelia

A3 Andreas Beilhack *(Dept. of Internal Medicine II)*
In vivo analysis of temporal and spatial disease progression and immune cell recruitment during invasive *Aspergillus fumigatus* and *Candida albicans* infections

A4 Max Topp *(Dept. of Internal Medicine II)*
Impact of regulatory T cells on human infections caused by *Aspergillus fumigatus*

B1 Thomas Dandekar *(Dept. of Bioinformatics)*
Modelling interactions between the host and fungal pathogens by combining metabolic pathway analysis and evolutionary game theory

B2 Thomas Dandekar *(Dept. of Bioinformatics)*
Interaction networks of signalling molecules and path ways between the pathogenic fungi *Aspergillus fumigatus* and *Candida albicans* and their human host

C2 Joachim Morschhäuser
(Institute for Molecular Infection Biology)
Regulation of *Candida albicans* virulence traits by protein kinases

C6 Thomas Hünig amd Niklas Beyersdorf
(Institute for Virology and Immunobiology
Role of secreted *Candida albicans* proteins in immune evasion and pathogenicity

5.5. Research Unit 1680
Unravelling the prokaryotic immune system

The CRISPR-Cas system (CRISPR: clustered regularly interspaced short palindromic repeats, Cas: CRISPR-associated) is an adaptive and heritable resistance mechanism against foreign genetic elements. The CRISPR-Cas system consists of clusters of repetitive chromosomal DNA in which short palindromic DNA repeats are separated by short spacers, the latter being sequences derived from the invader. In addition, a set of proteins, the Cas proteins, is involved. The system is functionally analogous to RNA interference in eukaryotes and it is of great interest to compare the prokaryotic and eukaryotic mechanisms.

CRISPR-Cas ribonucleoprotein complexes target homologous nucleic acids: DNA in case of the bacterial CRISPR interference system and RNA in case of the archaeal CRISPR-RAMP subtype. However, this major difference is only based on observations in a single archaeal species *(Pyrococcus furiosus)* and a few selected bacteria, and a clear link to the respective relevant protein components is so far missing. Furthermore, the identity of the target, and how targeted invading elements are inactivated or even destroyed, remains unknown. Similarly, it is not known how the spacer sequences are acquired and incorporated into the bacterial genome. The CRISPR-Cas system in prokaryotes has some conserved features but seems to be also highly variable. The CRISPR spacer and repeat sequences have different sequences and lengths. The Cas proteins belong to approximately 45 different protein families. For most of these proteins their functional roles are unclear. Moreover, bioinformatic analyses suggest the presence of certain protein components in cyanobacteria and some chloroflexi, which otherwise occur exclusively in archaea. Despite the progress made in understanding CRISPR function, many questions regarding the structure and function of its key components remain to be answered.

The novel approach of this Research Unit is to take seven different bacterial and archaeal organisms to define the common main features of the CRISPR system and to unravel the species-specific unique subsystems using a comparative approach with the help of mass spectrometry, crystallography and bioinformatics.

Project involving ZINF members

B2 Jörg Vogel *(Institute for Molecular Infection Biology)*
A CRISPR/Cas subtype Nmeni/CASS4 system in the human pathogen *Neisseria meningitidis*

5.6. Research Unit 2123
Sphingolipid dynamics in infection control

Lipid ordered membrane microdomains enriched for sphingomyelin and sterols are believed to serve as platforms compartimentalizing membrane associated proteins such as receptors and membrane-proximal signaling components, and regulating processes

Manfred Lutz *(Institute for Virology and Immunobiology)*
Protective and productive inflammatory responses induced by microbial products studied at the level of dendritic cells

Thomas Hünig *(Institute for Virology and Immunobiology)*
The role of CD28 mediated costimulation in the control of secondary immune responses to infectious agents

5.11. German-African Cooperation Projects in Infectiology

The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) funds joint research projects between scientists in Germany and Africa investigating infectious diseases and their social implications. The program focuses on the investigation of neglected infectious diseases in humans and animals but also research on topics of their social and economical impact.

African Sleeping Sickness is a deadly neglected disease. Transmitted by the infamous tsetse fly, the unicellular trypanosomes not only infect humans, but also sheep, goat and cattle. The socio-economic burden in sub-Saharan Africa is enormous. The few drugs available are ancient and highly toxic. Furthermore, diagnosis is very difficult. Although trypanosomes are widely ignored as infectious agents, the parasites have become a model system for molecular cell biologists. The main reason for the attention trypanosomes have received in the past decades is their ability to adapt to very diverse environments, such as the mammalian circulation or the flys midgut. In host blood, trypanosomes evade the immune response through antigenic variation of their cell surface, which consists of a dense layer of variant surface glycoproteins (VSG). A second mechanism that allows trypanosomes to prosper in blood was discovered in the course of the current project: the parasites remove antibodies from the cell surface by exploiting hydrodynamic flow, which acts on the cell surface as the result of incessant, directional motility. In this way, antibody-bound VSGs are dragged against the swimming direction towards the posterior end of the cell, where the flagellar pocket, which harbours the unusually processive endocytosis machinery, is located. The antibodies are internalized and transported to the lysosome for destruction. The aim of our cooperative research project is to unravel the role of antibody removal as a trypanosome virulence factor. The first funding period had a strong capacity building aspect. We equipped our partner laboratory at ICIPE in Nairobi and established reliable and solid logistics. Since experimental animal infections are crucial for the success of our endeavour, we teamed up with KARI-TRC at Muguga, Kenya. During the next funding period we will focus on the role of various trypanosome morphotypes and cell cycle stages found in the course of natural infections. For this, we will not only apply modern techniques such as RNA-seq, electron tomography and dSTORM, but also conduct fieldwork in remote endemic regions in Kenya, Uganda and the Congo.

Projects involving ZINF members

Markus Engstler *(Dept. of Cell and Developmental Biology)*
Antibody clearance as a virulance factor in African sleeping sickness

5.12. BMBF/NGFN (National Genome Research Network) – RNomics of Infectious Diseases

A large portion of eukaryotic and other genomes are transcribed as noncoding RNAs (ncRNAs). It has become apparent that eukaryotes and prokaryotes encode a wide variety of ncRNAs with many being involved in regulating gene expression. The central research objective of the RNomics of Infectious Diseases network is to study the functions of non-coding RNAs (ncRNAs) in pathogens including viruses, bacteria and eukaryotic parasites as well as infection-responsive ncRNAs in host cells. Infections and the fast developing antibiotic resistance in pathogens are still a major threat to human health. Due to the possibility of targeting RNAs through base complementarity, the study of ncRNAs and the use of antagonistic nucleic acid derivatives is a highly promising approach to achieve important advances in biomedical research. Eventually such approaches could be developed to produce effective new treatments augmenting the current repertoire of drugs and to counteract the alarming pace of pathogen resistance.

The key aims of the initiative aims to identify and functionally validate ncRNAs with key regulatory functions in highly relevant viral, bacterial, and eukaryotic parasite infectious diseases: AIDS, salmonellosis, chlamydiosis, meningococcal meningitis, infectious gastritis, malaria, toxoplasmosis, and giardiasis. The three subprojects dealing with viral, bacterial, and eukaryotic parasite infections, respectively, will produce libraries of infected and control cells. The fourth subproject will apply ultra- high-parallel sequencing in combination with bioinformatic analyses to identify ncRNAs that are regulated due to infection. Particular attention will be paid to ncRNAs that are regulated in different infection models. The impact of candidate ncRNAs on infection will be corroborated by manipulating ncRNA in established *in vitro* models. Moreover, the therapeutic potential of such candidate ncRNAs will be tested *in vivo* by using transgenic/knockout technology for established mouse models (bacterial and eukaryotic parasite infection) or by employing non-human primate models (SIV infection).

Projects involving ZINF members

Thomas Rudel *(Dept. of Microbiology)*
Jörg Vogel *(Institute for Molecular Infection Biology)*
RNomics of bacterial infections

5.13. BMBF Medical Infection Genomics

The funding initiative "Medizinische Infektionsgenomik" (Medical Infection Genomics) is a research program financially supported by the Federal Ministry of Education and Research (BMBF). It consists of eleven research clusters that focus on genome research of pathogenic microorganisms. During the funding period from 2011 to 2013 the participating groups of the Medical Infection Genomics network have focused on human pathogenic bacteria that are of high socioeconomic relevance for the public health system in Germany. This is especially the case for those that are widely dis-

Projects involving ZINF members

Thomas Dandekar *(Dept. of Bioinformatics)*
Modeling metabolism in bacterial infections

Thomas Dandekar *(Dept. of Bioinformatics)*
Modeling metabolism in intracellular infections comparing *Salmonella* and *Listeria*

Thomas Dandekar *(Dept. of Bioinformatics)*
Metabolism of intracellular *Salmonella enterica:* One lifestyle in intra-cellular infections

Christoph Schoen *(Institute for Hygiene and Microbiology)*
Gene regulatory mechanisms of metabolic adaptation in *Neisseria meningitidis* in *ex vivo* infection models

Jörg Vogel *(Institute for Molecular Infection Biology)*
A post-transcriptional link between *Salmonella* metabolism and virulence

5.9. DFG Priority Program SPP 1617 Phenotypic heterogeneity and sociobiology of bacterial populations

The DFG-funded Priority Program SPP1617 teams microbiologists from all fields of bacteriology (e.g., infection biology, terrestrial microbiology, biotechnology etc.) with theoreticians from the mathematical and physical sciences. In a combined interdisciplinary effort, SPP1617 aims at a deeper understanding of the complexity of bacterial populations and the theoretical modelling and prediction of their diversity. A focal point of the research is the generation of phenotypic variation in bacterial communities, the evolutionary mechanisms that gave rise to genotypes expressing diverse phenotypes as well as the biological significance of the process. Individual projects cover cell-cell communications and the production of common goods, the division of labour as well as bet-hedging strategies in bacteria of medical, biotechnological and ecological interest.

Projects involving ZINF members

Daniel Lopez *(Research Centre for Infectious Diseases)*
Molecular characterization of the cell types required for the development of *Staphylococcus aureus* biofilms

Wilma Ziebuhr *(Institute for Molecular Infection Biology)*
Heterogeneous gene expression, metabolic variability and differentiation in *Staphylococcus epidermidis* biofilms

5.10. International Research Training Group IRTG 1522 HIV/AIDS and associated infectious diseases in South Africa

The International Research Training Group 1522 (IRTG 1522) on "HIV/AIDS and associated Infectious Diseases in Southern Africa" involves universities in Würzburg and Cape Town. The overall aim is to intensify the scientific exchange and relations between Germany and South Africa. The IRTG is thematically divided in three areas consisting of 12 research projects on infectious diseases. Projects within area 1 address questions on clinical virology, HIV and virus-induced immunosuppression, area 2 comprises studies of HIV-associated infectious agents and projects in area 3 investigate the immunology of infectious agents. A synergistic aspect of the IRTG is that partners from South Africa supply patient samples that cannot be obtained in Germany, which are analysed by methods available in Würzburg but only rarely in South Africa. The main corner stone of this IRTG is a student exchange program between the participating universities that permits students from Würzburg to spend research time in Cape Town and *vice versa*. At the University Würzburg eleven PhD studentships and two MD student stipends are financed by the DFG. Likewise, twelve PhD student stipends are financed by the National Research Foundation (NRF) at the Universities of Stellenbosch and Cape Town.

Projects involving ZINF members

Hartwig Klinker *(Dept. of Internal Medicine)*
August Stich *(Missionsärztliche Klinik)*
The impact of therapeutic drug monitoring on antiretroviral therapy

Axel Rethwilm *(Institute for Virology and Immunobiology)*
Molecular Epidemiology of HIV

Sibylle Schneider-Schaulies *(Institute for Virology and Immunobiology)*
Targets, mechanisms and consequences of regulated T cell pre-mRNA splicing and their relevance as genetic markers of virally induced or general T cell suppression.

Joachim Morschhäuser (*Institute for Molecular Infection Biology)*
Epidemiology, diagnosis, and molecular mechanisms of multidrug resistance in *Candida albicans* and its impact on host-fungus interactions

Klaus Brehm *(Institute for Hygiene and Microbiology)*
Characterization of the influence of excretory/secretory products from *Echinococcus multilocularis* larvae on dendritic cell maturation and the interaction of Echinococcus E/S products with TLR and CTL surface receptors

Heidrun Moll *(Institute for Molecular Infection Biology)*
Characterization of the role of C-type lectins in dendritic cell interactions with *Leishmania* parasites

5.16. ERA-NET

Infectious diseases (ID) cause tens of thousands of deaths each year in Europe. Despite all the measures taken to address ID, different factors have contributed to recent challenges: (i) the threat of emerging ID (16 new and 5 re-emerging infectious diseases were identified in the last 2 decades - NIH), (ii) mass migration, global travelers and growth of congested urban slums, (iii) misuse and overuse of antibiotics (iv) co-infection with at least two pathogens. Hence, continuous global effort and novel avenues of research are required to decipher the role of the new factors in the development of ID. Through this initiative, ERA-NET partners aim to understand all basic aspects of complex human infection biology questions such as co-infection that are not limited to specific pathogens, the cross-talk between host and pathogens, as well as the relationship between microbes' environment and infection. The ERA-NET consortium funds high quality and cutting edge transnational and translational research bringing together basic, applied, technology-driven and clinical research approaches to a broad variety of topics regarding human infectious diseases.

Networks involving ZINF members

PathoGenoMics

Herman Einsele *(Dept. Internal Medicine II)*
Invasive aspergillosis

Invasive aspergillosis (IA) is the most detrimental infection in patients with haematological malignancies. Although, IA may be perceived to be an uncommon disease with an incidence of 10,000 patients annually in Europe, there is increasing evidence that IA is affecting a broader range of patients. In addition, IA is the most expensive opportunistic infection in immunosuppressed patients; the annual cost in Europe is >100 million Euro. A major problem in the management of IA is the poor diagnosis. Therefore, the network aspBIOmics aims to evaluate a battery of in vitro assays for a comprehensive multimodality analysis, combining the detection of Aspergillus fumigatus elements (DNA, RNA, polysaccharides, proteins), host factors and the individual genetic susceptibility of the patients. The advance of this combined approach will be the availability of a panel of biomarkers incorporated into rapid and sensitive *ex vivo* assays. For the first time, a multi-parameter diagnostic strategy is undertaken to target IA. This strategy has the potential to identify patients who are at highest risk of IA before the infection occurs. Consequently, effective tailored prophylaxis can be administered and the success of antifungal therapy can be monitored.

Infect-ERA

Thomas Dandekar *(Dept. of Bioinformatics)*
Systematic identification of antifungal drug targets by a metabolic network approach (AspMetNet)

Fungal infections pose an increasing threat for the immunocompromised. Limitations in antifungal therapy arise from non-specific symptoms of infection, poor diagnostics and comparatively few options for treatment. Currently established antifungal drugs interfere with the fungal cell wall or plasma membrane and are characterized by limited efficacy, severe side effects, or emerging pathogen resistance. Despite their promise to serve as highly specific antifungal targets, fungal metabolic pathways have been widely neglected. Because of the fact that *Aspergillus*, the causative agent of aspergillosis, apparently lacks specific virulence factors, its general characteristics, such as growth and tissue penetration, strongly correlate with the outcome after infection of a susceptible host. These traits strictly rely on nutrient acquisition and metabolic turnover and, therefore, make biosynthetic pathways a prime target in antimycotic therapy. The basic concept of this transnational consortium is to explore the metabolism of the main pathogenic species *A. fumigatus* on a comprehensive scale as essential virulence determinant. Emerging from transcriptome profiling data that are mapped on the annotated genome sequence of *A. fumigatus*, metabolic network reconstruction will serve to identify fungal-specific biosynthetic pathways and key reactions. Predictions for unique enzymes will result in a candidate list of genes, the inactivation of which is likely to result in an auxotrophic phenotype based on conditional essentiality of the biosynthetic reaction. Phenotypic and molecular characterization of these genes will culminate in virulence studies to test infectivity in established animal models of aspergillosis. Based on the resulting data collections, the metabolic network model will be refined in an iterative manner to yield further candidate genes that again will be experimentally validated. In essence, this systematically applied metabolic network approach will yield novel antifungal drug targets based on the metabolism of *A. fumigatus* that will serve as promising candidates for therapeutic intervention to fight fungal infections.

Thomas Rudel *(Dept. of Microbiology)*
Co-infection as a cause of ovarian cancer (CINOCA)

The clinical impact of bacteria-virus co-infections and the subsequent chronic infections are both poorly understood, partly due to the difficulty in drawing conclusive etiological links years after the infection. This transnational network aims to investigate the contribution of chronic co-infections with human herpes viruses (HHVs) and the intracellular bacterium *Chlamydia trachomatis* (Ctr) to the onset of ovarian cancers. Recent epidemiological studies suggest a strong association of ovarian cancers with both agents and –surprisingly – only to a minor extent with human papilloma virus, a known etiologic agent of cervical cancer. An important paradigm shift in recent years now firmly assigns the origin of ovarian cancer to the epithelial lining of the Fallopian tube (FT), a prime meeting site for chronic, often asymptomatic infections by both HHVs and Ctr. Thus, accumulating evidence warrants a careful analysis of the molecular events by which these pathogens synergize in establishing their infectious niche and co-operatively promote malignant transformation. This consortium encompasses leading European laboratories in the areas of HHVs and *Chlamydia* research and two highly committed clinical and SME partners. Together with the clinical partner, who has generated an organoid model of normal human FT cells, suitable for *ex vivo* and *in vitro* studies will be performed. This novel infection model will provide the basis for in-

seminated in hospitals or that pose a particular threat for the public health system due to their high rate of antibiotic resistance or their high virulence potential. Examples of the pathogens studied in the network include, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecali, Salmonella* Typhimurium, *Chlamydia trachomatis Mycobacterium tuberculosis, Legionella pneumophila Helicobacter pylori, Streptococcus pneumonia* and *Neisseria meningitidis.*

The eleven research clusters aim for a comprehensive understanding of the infectious agents and their adaptation to the human host during the infection process. By unraveling the complex interactions between the pathogen and the human host the ultimate goal of the funding initiative is to provide the basis for further improving the prevention, diagnosis and therapy of infectious diseases. The Medical Infection Genomics network is coordinated by Prof. Matthias Frosch, head of the Institute for Hygiene and Microbiology at the University of Würzburg.

Projects involving ZINF members

Matthias Frosch *(Institute for Hygiene and Microbiology)*
Central management of the Medical Infection Genomics consortium

Thomas Rudel *(Dept. of Microbiology)*
Pathogen host interactomes and signaling complexes in bacterial infections

Ulrich Vogel *(Institute for Hygiene and Microbiology)*
Proteomics of meningococci and pneumococci – from *in vitro* biofilms to *in vivo* infection

Jörg Vogel *(Institute for Molecular Infection Biology)*
Next generation transcriptomics for bacterial infections

5.14. BMBF MedVet-Staph – Interdisciplinary Research Network on the Zoonotic Impact of *Staphylococcus aureus*/MRSA

Staphylococcus aureus, including methicillin-resistant *S. aureus* (MRSA) are major human and zoonotic pathogens. Recently, certain defined MRSA clonal lineages were found to spread in industrialized animal husbandry. These livestock-associated (LA)-MRSA were detected in food-producing animals and food samples, but they also occur as colonizers in companion animals and humans. LA-MRSAs have a significant potential to cause disease and they add to the increasing burden of healthcare associated infections in Germany. The MedVetStaph consortium brings together the expertise of microbiologists, infectiologists, epidemiologists and veterinarians to study the epidemiology, molecular biology and ecology of LA-MRSA. The goal of the programme is to provide policy makers and public health authorities with solid scientific data to implement efficient intervention and control measures to contain the further spread of these bacteria.

Projects involving ZINF members

IP 7 Wilma Ziebuhr
(Institute for Molecular Infection Biology)
Influence of antibiotic resistant staphylococcal species on persistence and dissemination of LA-MRSA

5.15. The Bavarian Research Network for Molecular Biosystems (BiosysNet)

In the past, scientists have studied individual genes and gene products, leading to a detailed mechanistic understanding of the functions within living cells in health and disease. Recently, new systemic methods have become available that significantly extend these classical approaches. They have made it possible to sequence entire genomes, to identify most gene products in living cells, and to map interaction networks between components of living systems. This has led to the founding of a new research field called molecular systems biology or molecular systems research. This new research field requires a high level of interdisciplinarity, involving geneticists, molecular biologists, structural biologists, mathematicians and bioinformaticians. It provides a means to answer fundamental and complex biological questions and provide insight into the inner workings of cells and revealing the molecular mechanisms that generate and maintain living systems.

The Bavarian State Ministry of Sciences, Research and the Arts established the Bavarian Research Network for Molecular Biosystems at the end of 2011, as part of its effort to strengthen research, innovation and technology in Bavaria. The objective for the network is to bring together local expertise in systems biology within Bavaria to obtain a holistic view of living cells. The network comprises of 24 groups and supports five new, independent junior researchers in developing their own independent research programs as well as established junior and senior groups with interests in molecular biosystems.

Projects involving ZINF Members

Ana Eulalio *(Institute for Molecular Infection Biology)*
RNA: the missing link in bacterial pathogen-host interactions

Cynthia Sharma *(Research Centre for Infectious Diseases)*
Exploring RNA-binding proteins in *Campylobacter jejuni*

Jörg Vogel *(Institute for Molecular Infection Biology)*
Temporal control of gene expression by small RNAs

5.17. International Network for Strategic Initiatives in Global HIV Trials (INSIGHT)

The mission of the National Institutes of Health (NIH) sponsored INSIGHT network is to develop strategies for the optimization of treatment—antiretroviral therapies (ART), immunomodulatory therapies, and interventions to prevent and treat complications of HIV and ART—in order to prolong disease-free survival in a demographically, geographically, and socio-economically diverse population of individuals infected with HIV. In order to carry out this mission, the research agenda will be pursued through:

- Large randomized trials with morbidity and mortality outcomes, preceded, where appropriate, by vanguard (smaller, pilot) studies to refine design parameters
- Studies relevant to both resource-abundant and resource-constrained countries
- Studies directed at minimizing the adverse effects of long-term treatment, while maximizing treatment benefits
- Substudies conducted as part of larger trials
- Studies designed to allow for co-enrollment, so that multiple major research questions can be addressed in the cohorts under follow-up
- Carefully planned epidemiological analyses, including nested case-control studies that take advantage of a large cross-study database and associated specimen repositories; and
- Linkages with other networks, in order to maximize efficiency and research productivity.

During this seven-year funding cycle, INSIGHT will conduct 7 major clinical trials, three of which are already underway, and 3 vanguard trials at approximately 400 sites in 37 countries. Each of the trials will have carefully planned substudies that add value to the experimental design of the parent protocols. These substudies will investigate mechanistic questions and evaluate the experimental interventions for important secondary outcomes in a cost-effective way. Two of the trials will be preceded by intermediate-size vanguard studies to refine protocols for larger scale investigation, e.g., to estimate parameters for sample size or to more precisely define the study arms.

Projects involving ZINF members

Hartwig Klinker *(Dept. of Internal Medicine II)*
Strategic Timing of AntiRetroviral Treatment (START)

depth genomic and epigenomic analyses that will allow tracing the infection-driven events of host cell transformation on a genomic scale. In concert, this consortium will illuminate the molecular mechanisms by which HHVs and Ctr jointly reprogram human epithelial cells, providing the basis for malignant transformation.

5.18. National Reference Laboratory for Meningococci and *Haemophilus influenzae*

The National Reference Laboratory (NRL) for meningococci and *Haemophilus influenzae* is hosted at the Institute for Hygiene and Microbiology at the University of Würzburg and headed by Prof. Dr. Matthias Frosch and Prof. Dr. Ulrich Vogel. The NRL has been commissioned by the Robert Koch-Institute to conduct representative laboratory surveillance of invasive meningococcal disease and invasive infections caused by *Haemiophilus influenzae* in Germany. Laboratory surveillance for both infectious agents is performed in close collaboration with the Robert Koch-Institute. NRL data are regularly matched with statutory notification data to achieve comprehensive datasets, which are also reported to the European Centre for Disease Prevention and Control (ECDC). The NRL advises laboratories and public health authorities with respect to diagnosis, epidemiology and prevention of meningococcal disease. It collaborates with international networks, e.g. the ECDC IBD labnet and the European Meningococcal Disease Society (EMGM). The NRL annually processes 800 samples from patients with invasive bacterial infections. Key parameters assessed include serogroup or serotype, clonal finetype, and antibiotic resistance. Culture-independent analysis by sensitive PCR assays and DNA sequencing is performed on 100-150 samples per annum. The reference laboratory further conducts serological investigation of vaccine responses. It analyses the strain coverage of meningococcus B vaccines. Annual reports for both infectious agents are available at http://www.nrzmhi.de/. A geographic information system can be accessed at www.episcangis.org.

5.19. The Consulting Laboratory for Echinococcosis

The Robert Koch Institute appoints the consulting laboratory for echinococcosis every second year for consultation, quality management and development of diagnostic procedures. The Institute for Hygiene and Microbiology has hosted the consulting laboratory for echinococcosis since 1997. The consulting laboratory is an assigned set point laboratory for interlaboratory comparison tests. It is also involved in the preparation and updating of quality standards for microbiological diagnostic procedures (MIQ). The consulting laboratory offers the following services:

1. Information regarding the prevention and epidemiology of different types of echinococcosis.
2. Information on the diagnosis, differential diagnosis, and therapy.
3. Detection of antibodies against *Echinococcus multilocularis* and *E. granulosus* in human sera.
4. Microscopy of cyst aspirates, sputa and other liquid samples as well as solid tissue obtained at surgery for echinococcal structures.

5. Parasitological analysis of stained and covered microscopic slides for echinococcal structures and differentiation of the parasite.
6. Detection of echinococcal DNA by PCR (after consultation with the treating physician).

There is a close connection of the consulting laboratory and the research group of Prof. Dr. Klaus Brehm of the Institute for Hygiene and Microbiology, who investigates the host parasite relationship of alveolar echinococcosis. The consulting laboratory is available online at http://www.echinococcus.de.

5.20. IBD-labnet – Coordination of activities for laboratory surveillance of invasive diseases *(N. meningitidis, H. influenzae, S. pneumoniae)* in the EU

The European Centre for Disease Prevention and Control (ECDC) has funded the IBD-labnet since September 2008 within the framework of the program "Laboratory surveillance and external quality assurance of invasive bacterial diseases in EU" and since October 2011 the follow-up program "Coordination of Activities for Laboratory Surveillance of Invasive Bacteria Diseases."

The IBD-labnet is coordinated by Prof. Dr. Matthias Frosch from the Institute for Hygiene and Microbiology and aims to harmonize the laboratory surveillance of invasive bacterial diseases caused by *Neisseria meningitidis*, *Haemophilus influenza* and *Streptococcus pneumoniae* in Europe and EEA/EFTA countries.

The critical importance of valid and accurate typing data for comparability of surveillance data means that a key objective of the laboratory network is the standardization and harmonization of typing methods. The members of this consortium, the National Reference Laboratories for *N. meningitidis*, *H. influenzae* and *S. pneumonia,* are also supported in the strengthening of their laboratory capacity to accurately characterize the invasive isolates. Therefore, the IBD-labnet assists the participating National Reference Laboratories to continuously improve their laboratory performance in the identification and characterization of *N. meningitidis*, *H. influenzae* and *S. pneumonia* as well as the implementation of new techniques for routine work.

The major activities of the IBD-labnet include:

1. The assessment of the laboratory performances by the distribution of external quality assurance exercises.
2. The improvement of the laboratory performances by the organisation of training workshops or exchange programs.
3. The harmonization of methods for antimicrobial susceptibility testing, interpretation and reporting for *N. meningitidis*, *H. influenzae* and *S. pneumoniae*.
4. The standardisation and harmonization of methods for DNA-based serotyping and molecular typing of *S. pneumoniae*.
5. The establishment of a European meningococcal strain collection.

5.21. Interdisciplinary Center for Clinical Research (IZKF)

The IZKF Würzburg organizes the Medical Faculty's internal funding for research. It was founded in 1996 within the federal advancement program "Health Research 2000" of the Federal Ministry of Education and Research. Since 2004, it has been entirely funded by the Free State of Bavaria. Its major goal is the strengthening of clinical research through interdisciplinary cooperation between clinical research and basic research in the biomedical sciences. In 2013, its funding volume was approx. 5 Mio. Euro.

The IZKF supports interdisciplinary clinically relevant research at the university through three main programs:

- Research project grants within defined scientific areas.
- The promotion of young researchers in medicine through the establishment of IZKF Junior Research Groups.
- Infrastructure development through the establishment of core facilities and instrumentation.

The funding decisions are based on peer review methods and transparent fund management, there are three different levels of internal research management.

- The general assembly („Zentrumskonferenz")
- The executive board, which is responsible for decisions on funding requests.
- The External Scientific Advisory Board, which plays an active role in the center's activities and participates in the assessment of each project proposal.

The aim of IZKF-project grants is to facilitate interdisciplinary research between groups at the university and to enable the funding of research into new topics within the focus of the Medical Faculty. Cooperation between clinical researchers and basic researchers in biomedicine is a precondition for a successful grant application. After up to three years of an IZKF-promotion it is expected to transfer the projects into external third-party funding.

The IZKF-Junior Career Program aims to support young independent researchers in merging clinical and biomedical research at the earliest possible stage of their medical career. Together with the direct Junior Career Programs, the IZKF also supports young and motivated scientists of the Medical Faculty with IZKF-research grants.

In order to enhance the local infrastructure, the IZKF has established several Core Facilities. Those with specific relevance to infectious disease research include:

- The core facility "Microarray-Unit" was established in 2001 and broadened the spectrum of services as the "IZKF-Service Unit for Microarray applications and bioinformatic analysis of high throughput methods". In 2013, it was incorporated into the Core Unit "Systems Medicine" which is co-financed by the Medical Faculty. The Core Unit "System Medicine" is led by Dr. Eberhard Krauß. It is a service partner for high throughput techniques for researchers at the University and the University Clinics. The Core Unit currently consists of the

Training activities
The training activities total a minimum of 4-6 hours per week and consist of seminars, journal clubs, program seminars, methods courses and transferable skills workshops as well as retreats and international conferences.

Common Graduation Commission
The participating faculties form a common Graduation Commission within the respective graduate school. The commission is responsible for the conferral of all doctoral degrees within the graduate school. This enforces common standards across disciplines and fosters interdisciplinary cooperation in graduate training.

Section Infection and Immunity Section Speakers:
Thomas Hünig *(Chair of Immunology)*
Joachim Morschhäuser *(Institute for Molecular Infection Biology)*

The topic "Infection and Immunity" represents an internationally recognized major research focus of the University of Würzburg. Strong interdisciplinary bonds between the Faculties of Medicine, Biology, and Chemistry & Pharmacy are hallmarks of this research field in Würzburg. Scientists from the participating faculties cover all the relevant disciplines and methodological approaches in infectious disease research. The network of researchers in the section "Infection and Immunity" however also explores such -seemingly- quite different phenomena as the genesis and control of cancer or aspects of symbiosis in plant biology. The scientific program spans research on host-pathogen interactions, genome research in pathogenic microbes, identification and characterization of novel anti-infectives, molecular processes of immune response in various host organisms including humans, mechanisms of tumorigenic processes induced by microbes, and new concepts in immune therapy. This comprehensive coverage of topics will guarantee the broadest possible training for doctoral researchers, yet provide a focus on common and converging mechanisms.

5.23. Core Unit for Systems Medicine

To obtain a holistic view of a biological system requires high-throughput technologies such as mass-spectrometry for proteomics and metabolomics, next-generation sequencing and high-content screening platforms. The large quantities of data generated by these methods can be integrated and used to model biological entities in a global fashion. While such approaches open doors to new insights they require additional expertise to handle devices and to establish necessary protocols as well as to computationally evaluate the data. Due to these requirements the hurdle to establish one or more high-throughput methods is rather high for a single research group. To lower this barrier the Medical Faculty at the University of Würzburg has decided to create a central institution that can assist such groups by providing access to the necessary instruments and expertise. For this purpose the Core Unit Systems Medicine was founded and has offered several services since the end of 2013. Currently it comprises five subunits:

- The RNAi and Chemical Screening subunit - supervised by Eberhard Krauß – establishes workflows for high-content screening of compounds, which can form the basis for testing large libraries of reagents. For this purpose the subunit strongly relies on lab automation methods and provides different formats to groups that are interested in scaling-up their testing pipelines.

- The Microarray subunit is led by Claus Scholz and funded by the IZKF of the University Hospital Würzburg. It has been integrated into the Core Unit System Medicine as it has a long-standing experience in supporting research groups of the medical campus in their microarray based expression profiling, gene regulation analyses and genetic association screens.

- Three subunits headed by Konrad Förstner offers high-throughput sequencing based service covering all steps starting from the design of the experiment, the library preparation, the sequencing and the bioinformatical analysis. The DNA-Seq subunit assists with genome, exom and amplicon sequencing while the RNA-Seq subunit focuses on the transcriptome analysis and related services such as RNA-protein interaction studies. The Single Cell Analysis subunit (also funded by the IKFZ) supports in the isolation of single cells and the extraction of desired molecules.

As a joint institution the Core Unit Systems Medicine provides its services to research groups of the university and the university clinics. Consulted by a steering committee, it will continue to expand its portfolio of high-throughput methods based on requests of the local research community.

units "Genome and Transcriptome Sequencing" and the "Microarray-Unit" with the affiliated Bioinformatics. In the future it will also consist of "Single Cell Analysis" and "RNAi/Chemical Screening".
- The IZKF extended support for the "Center for Experimental Molecular Medicine (ZEMM)" which allows IZKF members access to the ZEMM's central animal management.
- The IZKF has also recently established the "Service Unit for confocal microscopy and flow cytometry-based cell sorting".

Projects Involving ZINF members

Andreas Beilhack *(IZKF, Dept. of Internal Medicine II)*
Pathogenisis of pneumoviral infection after stem cell transplantation in a natural infection model

Hartwig Klinker (*Dept. of Internal Medicine II*) and
Axel Rethwilm *(Institute for Virology and Immunobiology)*
Therapeutic drug monitoring in the therapy of HIV and HCV infections

Joachim Morschhäuser
(Institute for Molecular Infection Biology)
Molecular studies on oocyte maturation, gamete interactions and their influence on uropathogens.

Christian Perez (IZKF)
Candida-host interactions

Sibylle Schneider-Schaulies
(Institute for Virology and Immunobiology)
HERV and immune reactions during pregnancy

Sibylle Schneider-Schaulies
(Institute for Virology and Immunobiology)
The importance of cellular receptors for measles virus-modulated myelopoiesis and myelostasis in healthy volunteers and patients

Andrea Schubert-Unkmeir
(Institute for Hygiene and Microbiology)
Infection susceptibility and mitochondrial-dependent transplant dysfunction in pulmonary ischemia-reperfusion injury

Cynthia Sharma (ZINF), Heike Walles (*Dept. Tissue Engineering and Regenerative Medicine*), Jürgen Löffler (*Dept. of Internal Medicine II*)
New infection models based on tissue engineering for the human pathogens *Helicobacter pylori* and *Campylobacter jejuni*

5.22. Graduate School of Life Sciences (GSLS)

For many years the Faculties of Medicine and Biology at the University of Würzburg have offered high-level structured graduate training. This culminated in the foundation of the International Graduate School (IGS) by the University Senate in 2003 and the University of Würzburg Graduate Schools (UWGS) in 2006, which ensured common graduation standards and rules for all graduate schools. The Graduate School of Life Sciences (GSLS) was successful in its application to the "Excellence Initiative of the Federal and State Governments" and obtained funds to support fellowships and other activities within the GSLS. In addition to the section Biomedicine and the MD/PhD program three further sections, i.e. Infection and Immunity, Neuroscience and Integrative Biology, were founded.

The Graduate School of Life Sciences (GSLS) is the largest and most strongly integrated graduate school at the University of Würzburg after the successful implementation of programs from the Excellence Initiative application.

The GSLS now houses doctoral researchers of all collaborative research programs – such as the DFG-funded collaborative research centers ("Sonderforschungsbereiche"), research training groups ("Graduiertenkollegs") and clinical research groups ("Klinische Forschergruppen"), as well as other collaborative programs funded by the Federal Ministry of Education and Research (BMBF), the European Union and other sources. The school is currently divided into five separate sections, Biomedicine, Infection and Immunity, Neuroscience, Integrative Biology and Clinical Sciences. Each section usually comprises different programs of about 15 to 25 doctoral researchers. These programs are the scientific as well as social "home" of the doctoral researchers.

A special fellowship program of the GSLS is the core element of funding by the Excellence Initiative. The eighth round of international recruitment is currently underway (2014). To date more than 2000 standardized written applications have been evaluated, and more than 350 candidates have been interviewed. So far 88 fellows from 21 different countries, have been supported by the GSLS. The number of formal members of the GSLS has risen to more than 200 principal investigators from all participating faculties. In 2013 the number of doctoral researchers enrolled in the doctoral study program "Life Sciences" rose to more than 340. In July 2012, the renewal proposal in the framework of the second phase of the Excellence Initiative was approved. Besides establishing an international MSc program and a program for postdoctoral fellows to foster their early independence, the introduction of an excellence program for MD doctoral studies, addressing the top 20% of the medical students, will be implemented.

Key elements of training in the Graduate Schools
- The traditional single supervisor ("Doktorvater") is replaced by a thesis committee of three principal investigators (PIs).
- A panel of training activities is offered, from which an individual program is tailored to each doctoral researcher.
- Doctoral researchers actively participate in the program by offering and organizing courses and symposia.
- A set of requirements has to be met to warrant a common quality standard.

Mentoring System
Each doctoral researcher has an individual thesis committee, which meets with the doctoral researcher at regular intervals to monitor progress and adjust the research and training activities. Additionally, the doctoral researchers report the status of their project within the research groups and programs, to exchange ideas and obtain feedback within their peer-group.

06

APPENDIX

6. APPENDIX

6.1. ALUMNI YOUNG INVESTIGATOR GROUP LEADERS

Since the founding of the Research Center for Infectious Diseases many former Young Investigator group leaders have been appointed to highly competitive positions at various universities and industrial companies.

FORMER YOUNG INVESTIGATOR GROUP LEADERS

HEIDRUN MOLL

CURRENT POSITION
C3-Professorship at the University of Würzburg
Institut für Molekulare Infektionsbiologie

RESEARCH AT THE ZINF
1993 – 1999
Pathogenicity of *Leishmania*

MICHAEL LANZER

CURRENT POSITION
C4-Professorship at the University of Heidelberg
Universitätsklinikum Heidelberg
Hygiene-Institut/ Abt. Parasitologie

RESEARCH AT THE ZINF
1994 – 1999
Pathogenicity of human malarial parasites

JOACHIM MORSCHHÄUSER

CURRENT POSITION
C3-Professorship at the University of Würzburg
Institut für Molekulare Infektionsbiologie

RESEARCH AT THE ZINF
1997 – 2000
Pathogenicity of *Candida*

JOACHIM REIDL

CURRENT POSITION
Professorship at the University of Graz
Karl Franzens Universität Graz

RESEARCH AT THE ZINF
1996 – 2003
Virulence of Gram-negative bacteria

KATJA BECKER

CURRENT POSITION
C4-Professorship at the University of Gießen
IFZ- Biochemie der Ernährung des Menschen

RESEARCH AT THE ZINF
1999 – 2000
Malarial parasites as targets for the development of antiparasitic drugs

KLAUS ERB

CURRENT POSITION
Head of Dept. Allergologie und Immunologie
Department of Pulmonary Research, Boehringer Ingelheim Pharma GmbH & Co. KG

RESEARCH AT THE ZINF
1999-2004
Immunology of intracellular pathogens and allergic disorders

MATTHIAS LEIPPE

CURRENT POSITION
C4-Professorship at the University of Kiel
Zoologisches Institut, Abteilung Zoophysiologie
Christian-Albrechts-Universität

RESEARCH AT THE ZINF
2001-2003
Molecular Parasitology

CHRISTOF HAUCK

CURRENT POSITION
W3-Professorship at the University of Konstanz
Lehrstuhl für Zellbiologie

RESEARCH AT THE ZINF
2001-2006
Pathogen-host communication

SVEN HAMMERSCHMIDT

CURRENT POSITION
W3-Professorship at the University of Greifswald
Interfakultäres Institut für Genetik
und Funktionelle Genomforschung

RESEARCH AT THE ZINF
2003 – 2007
Pathogenicity of *Streptococcus pneumoniae*

UTE HENTSCHEL

CURRENT POSITION
W2-Professorship at the University of Würzburg
Julius-von-Sachs-Institut für Biowissenschaften
Mikrobielle Ökologie

RESEARCH AT THE ZINF
2004 – 2008
Novel Antiinfectives

ANN-KRISTIN MÜLLER

CURRENT POSITION
Group Leader at the Department of Parasitology
Universitätsklinikum Heidelberg
Hygiene-Institut Abt. Parasitologie

RESEARCH AT THE ZINF
2007 – 2008
Biology of rodent malaria parasites

GABRIELE PRADEL

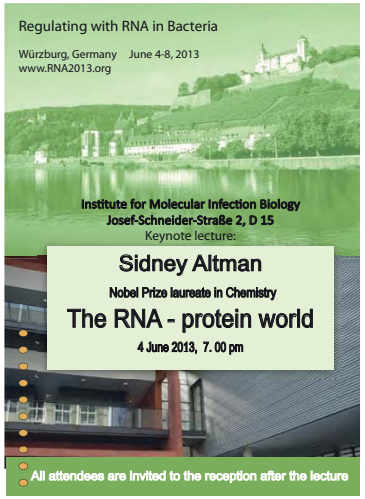
CURRENT POSITION
W2 professorship at the RWTH Aachen
RWTH - Institut für Molekulare Biotechnologie

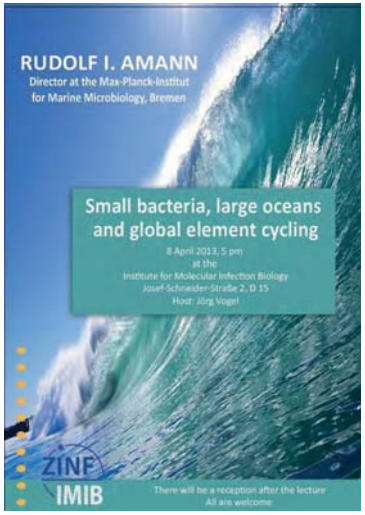
RESEARCH AT THE ZINF
2005 – 2011
Malaria: Transmission blocking strategies

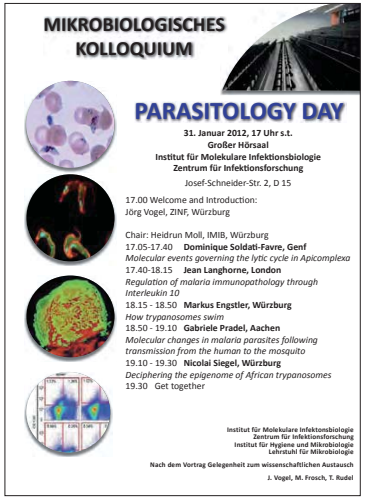
SVEN KRAPPMANN

CURRENT POSITION
W2-Professorship at the University of Erlangen
Friedrich-Alexander-Universität Erlangen-Nürnberg

RESEARCH AT THE ZINF
2005 – 2012
Aspects of *Aspergillus fumigatus* pathogenicity







International Symposium "MMMW 2012 – 2nd Mol Micro Meeting 2012"
Institute for Molecular Infection Biology, Würzburg , 25 – 27 April, 2012

7th Würzburger Infektiologisches Symposium
Clinical Internal Medicine II, Center for Infectiology DGI, Würzburg 14 July, 2012

7th Annual Meeting "Immunology Training Network of Würzburg, Tübingen, Erlangen"
Kloster Schöntal, 15 – 17 July, 2012

7th Joint PhD Students Meeting of the SFB 630 and 766 and FOR 854 "New Trends in Infectious Disease Research"
Würzburg, 14 – 16 November, 2012

6th Würzburger Meningokokken-Workshop „Epidemiologie & Prävention invasiver bakterieller Infektionen"
Würzburg, 22 February, 2013

International Else-Kröner-Forschungs-Symposium for Translational Immunology "From Target to Therapy"
Würzburg, 21 – 22 March, 2013

11th Deutscher Chlamydien Workshop (DCW)
Würzburg, 10 – 12 April, 2013

14th Drug Design & Development Seminar of the German Society for Parasitology (DGP)
in collaboration with SFB 630 and the IRTG 1522, Würzburg, 11 – 13 April, 2013

International Symposium "Regulating with RNA in Bacteria"
Institute for Molecular Infection Biology, Würzburg, 4 – 8 June, 2013

20th Anniversary of the Center for Infectious Diseases (ZINF) Symposium "The New Infection Biology"
Institute for Molecular Infection Biology, Würzburg, 28 June, 2013

8th Annual Meeting Immunology Training Network of Würzburg, Tübingen, Erlangen"
Bildungshaus Obertrubach, 21 – 23 July, 2013

XVIIIth International Pathogenic Neisseria Conference (IPNC)
Würzburg, 9 – 14 September, 2013

2nd International Symposium of the SFB630 "Novel Agents against Infectious Diseases – An Interdisciplinary Approach" of the SFB630
Würzburg, 20 – 22 November, 2013

6. APPENDIX
6.3. SEMINARS AND COLLOQUIA

MICROBIOLOGY COLLOQUIUM

17 Dec 2013
Jan Rehwinkel | University of Oxford
Finding (& fighting) the enemy within: how RIG-I and SAM-HD1 detect and protect against viruses

09 Dec 2013
Muhamed-Kheir Taha | Institut Pasteur, Paris
Use of transgenic animal models to explore meningococcal virulence

26 Nov 2013
Maria Mota | Institute of Molecular Medicine, Lisbon
Host-Plasmodium interactions: understanding to intervene

19 Nov 2013
Stefan Niemann | Leibniz Research Centre, Borstel
Whole genome analysis for understanding transmission and evolution of *M. tuberculosis*

12 Nov 2013
Abdelaziz Heddi | INSA de Lyon
Immunity in Insect Symbiosis

05 Nov 2013
Soeren Molin | Technical University of Denmark, Kongens Lyngby
How microbes adapt to new environments – what we have learned from opportunistic bacterial colonization of human airways

29 Oct 2013
Alexander Elsholz | Harvard University
A novel quorum-sensing mechanism for exopolysaccharide synthesis in bacteria

22 Oct 2013
Matthias Horn | University of Vienna
Chlamydiae in the environment - how the analysis of non-model organisms can challenge long-standing knowledge

15 Oct 2013
Aaron Mitchell | Carnegie Mellon University, Pittsburgh
Two vignettes in *Candida albicans* infection biology

16 July 2013
Xavier Nassif | University Paris Descartes
Meningococcemia more than a Gram negative infection

09 July 2013
Mohamed-Ali-Hakimi | UIF-Grenoble
Toxoplasma gondii secretes an armada of effector proteins to co-opt its host cell - transcriptome and microRNome to promote sustained parasitism

02 July 2013
Guy Tran Van Nhieu | CIRB, Paris
Calcium signals and cytoskeletal remodeling during *Shigella* invasion of epithelial cells

25 June 2013
Vojo Deretic | University of New Mexico
Autophagy in infection, inflammation, and immunity

11 June 2013
Jörgen Johansson | Umea University
Regulatory circuits in *Listeria monocytogenes*

4 June 2013
Conference at the IMIB
Regulating RNA in Bacteria

28 May 2013
Olaf Groß | TU Munich
Inflammasomes – beyond IL-1

21 May 2013
Vladimir Pelicic | Imperial College London
DNA-binding type IV pilins as modulators of horizontal gene exchange during natural transformation

14 May 2013
Ralph Bartenschlager | University Hospital of Heidelberg
On the complexities of interactions between the hepatitis C virus and its host cell

07 May 2013
Klaus Erb | Boehringer Ingelheim
Pathogen derived products for the treatment of allergic disorders: balancing inflammation with immune regulation

30 April 2013
Liam Good | University of London
RNA Silencing in Bacteria and a Novel Method for Cellular Delivery of Biopharmaceuticals

23 April 2013
Richard Ffrench-Constant | University of Exeter
Photorhabdus: shedding light on symbiosis and pathogenesis using a bacterium-nematode-insect model

16 April 2013
Christiane Wolz | University Hospital of Tübingen
Interplay of the stringent control and other regulatory circuits to control virulence and survival of *Staphylococcus aureus*

05 Feb 2013
Inigo Lasa | UPNA-CISC, Navarra, Spain
Lessons from the *Staphylococcus aureus* transcriptome

29 Jan 2013
Dirk Haller | TU Munich
Mechanisms of microbe-host interactions for chronic inflammation in the gut

22 Jan 2013
Mathias Herrmann | Saarland University Hospital, Homburg
Disease mechanisms in *Staphylococcus aureus* endovascular infection

15 Jan 2013
Gunther Hartmann | University Hospital, Bonn
RIG-I: a key immune sensor of RNA

08 Jan 2013
Dr. Kay Johswich | University of Toronto, Canada
Of mice and meningococci: The potential of humanized mouse models

08 Jan 2013
Sten Linnarsson | Karolinska Institute, Stockholm, Sweden
Quantitative analysis of single-cell transcription

18 Dec 2012
Karl Kuchler | Medical University Vienna, Austria
Molecular Mechanisms of Fungal Pathogenesis & Host Immune Response - A Tale of Action, Reaction and Over-Reaction

11 Dec 2012
Jan Rupp | University of Lübeck
The impact of a low oxygen environment on host-pathogen interactions

04 Dec 2012
Raphael Valdivia | Duke University Medical Center, Durham, USA
NextGen approaches to tackle the biology of genetically "intractable" microbial pathogens

27 Nov 2012
Martin Thanbichler | MPI Marburg
Compartmentalization of bacterial cells by protein diffusion barriers

20 Nov 2012
Hubert Hilbi | LMU Munich
Pathogen vacuole formation in phagocytes - the *Legionella* paradigm

13 Nov 2012
Jeremy Mottram | University of Glasgow, UK
Cell death and autophagy in *Leishmania*

06 Nov 2012
Mathias Hornef | MH Hannover
Innate immune recognition and antimicrobial host response by the intestinal epithelium

30 Oct 2012
Konstantin Severinov | Waksman Institute of Microbiology, Rutgers, USA
Small RNA-based adaptive immunity in bacteria

16 Oct 2012
Florian Greten | TU Munich
Cell plasticity, stemness and microflora - novel aspects in the inflammatory microenvironment of colon cancer

17 July 2012
Rasika Harshey | University of Texas, Austin
Moving on a surface: trials, tribulations and triumphs

03 July 2012
Tâm Mignot | CNRS, Marseille
How do complex macromolecular machines evolve? Insights from the newly discovered surface motility machinery in *Myxococcus*

26 June 2012
Matthias Machner | NIH, Bethesda
A master manipulator of host cell membrane traffic

19 June 2012
Christof Hauck | University of Konstanz
CEA-family glycoproteins as facilitators of mucosal colonization by bacteria

12 June 2012
Jose-Juan Lopez Rubio | Institut Pasteur, Paris
Antigenic variation in *P. falciparum:* from epigenetic regulation to the generation of diversity

05 June 2012
Victor Sourjik | ZMBH, Heidelberg
Self-assembly and partitioning of multi protein complexes inbacteria

29 May 2012
Nassos Typas | EMBL, Heidelberg
From systems microbiology to molecular mechanism

22 May 2012
Jean-Marc Ghigo | InstitutPasteur, Paris
Airborne interactions mediated by volatile molecules produced by bacterial communities

15 May 2012
Christine Josenhans | MH Hannover
Lessons from Epsilon proteobacteria: how to sense energy levels in an extracellular host environment

08 May 2012
Kim Lewis | NortheasternUniversity, Boston
Dormancy rules: persister cells and uncultured microorganisms

24 April 2012
José Penadés | CSIC, Spain
Phage dUTPases control transfer of staphylococcal pathogenicity islands by a proto-oncogenic G-protein like mechanism

17 April 2012
Craig Roy | Yale School of Medicine, USA
Subversion of Rab1 function by *Legionella pneumophila*

28 February 2012
Fritz Melchers | MPI for Infection Biology, Berlin
Microenvironmental guidance of B lymphocyte development

07 Feb 2012
Anne Müller | University of Zürich, Switzerland
Pathogenicand immunomodulatory properties of *Helicobacter pylori* govern disease outcome in gastrointestinaland allergicdisease models

24 Jan 2012
Marc Bramkamp | University of Köln, Germany
Chromosome segregation and division in actinobacteria

17 Jan 2012
Dirk Hofreuter | University of Hannover, Germany
What makes *Campylobacter jejuni* thrive: food for a food-borne pathogen

10 Jan 2012
Angelika Grundling | Imperial College London, UK
Lipoteichoic acid synthesis in Gram-positive bacteria and how *S. aureus* manages to survive without it

VIROLOGY AND IMMUNOBIOLOGY SEMINARS

17 Jan 2012
Hanspeter Pircher | Freiburg
Anti-viral immunity analyzed in the LCMV infection model in mice

23 Jan 2012
Matthias Goebeler | Würzburg
Innate immunity and contact hypersensitivity

30 Jan 2012
Thomas Mertens | Ulm
Monitoring of CMV therapy and resistance

06 Feb 2012
Stephan Gadola | SouthamptonUK
Lipid presenting CD1 proteins and the immune system: Jacks of all trades

16 April 2012
Kai-Michael Töllner | Birmingham (UK)
Differentiation of plasma cells and the role of antibodies for affinity maturation

23 April 2012
Jan Münch | Ulm
Natural inhibitors and enhancer of HIV-1 infection

14 May 2012
David Sansom | Birmingham (UK)
The role of CTLA-4 in regulating T cell responses

04 June 2012
Alessandra Cambi | Nijmegen (NL)
Biophysics of virus binding and entry: nanoscale organization and dynamics of DC-SIGN

11 June 2012
David Raulet | Berkley (USA)
Role of the NKG2D immunoreceptor in host defense and inflammation

02 July 2012
Andrew Lever | Cambridge (UK)
Getting intimate with HIV – Structures and functions of lentiviral RNA genomes

09 July 2012
Martin Löchelt | Heidelberg
Foamy viruses: ancient and unconventional retroviruses

29 Oct 2012
Jörg Wischhusen | Würzburg
Interactions between natural killer and breast cancer cells: elimination, escape and enhanced tumorigenicity

05 Nov 2012
Heiner Schaal | Düsseldorf
Regulation of HIV-1 gene expression

12 Nov 2012
Michael Sixt | Klosterneuburg (AT)
Mechanisms of leukocyte chemotaxis Pathways and barriers of hepatitis C virus replication

03 Dec 2012
Immo Prinz | Hannover
What IL-17 producing T cells can do for you

10 Dec 2012
Christian Kurts | Bonn
Role of dendritic cells in kidney disease

07 Jan 2013
Matthias Gunzer | Essen
Infection immunology in real time

15 Jan 2013
Thomas Pietschmann | Hannover
Pathways and barriers of hepatitis C virus replication

21 Jan 2013
Antoine Gessain | Paris
Emerging of New Retroviruses in Humans in Central Africa: HTLV-3/4 and Simian Foamy viruses. Importance of Interspecies Transmission

28 Jan 2013
Max Löhning | Berlin
Lymphocyte differentiation and immunological memory in viral infections

18 March 2013
Christian Kurts | Bonn
Role of dendritic cells in renal disease

22 April 2013
Andrea Kreß | Erlangen
Modulation of the host cell by HTLV-1/Tax

13 May 2013
Gülsah Gabriel | Hamburg
Good and Bad Importins Provide a Driving Force for Interspecies Transmission of Avian Influenza Viruses

29 May 2013
Fabian Leendertz | Berlin
Retroviruses in a multi-species primate association

17 June 2013
Michael Lohoff | Marburg
Interferon-Regulatory Factors and T-cell subset differentiation

24 June 2013
Olivier Schwartz | Paris
The Virology and Immunology of HIV cell-to-cell transmission

01 July 2013
Matthias Gunzer | Essen
Infection immunology in real time

08 July 2013
Max Löhning | DRFZ Berlin
Lymphocyte differentiation and immunological memory in viral infections

14 Oct 2013
Onur Boyman | Zürich
Cytokine-based regulation of immune responses

04 Nov 2013
Marc Schmidt-Supprian | Martinsried
The role of TCR expression for identity, homeostasis and function of mature NKT and regulatory T cells

25 Nov 2013
Ildiko van Rhijn| Utrecht
T cell recognition of mycobacterial lipids

27 Nov 2013
Martin Röcken | Tübingen
Cancer immune control beyond killing: the strict requirement of immune-induced senescence for cancer control

02 Dec 2013
Andreas Pichlmair | München
Recognition of viral RNA by IFIT proteins

6. APPENDIX
6.4. FUNDING

2.1 CYNTHIA SHARMA

DFG (Sh580/1-1): Functional characterization of two acid-regulated small RNAs in *Helicobacter pylori*

IZKF (Interdisciplinary Center for Clinical Research, Würzburg): New 3D-infection models based on tissue-engineering to study pathogenesis of *Helicobacter pylori* and *Campylobacter jejuni* (Co-applicants: Sharma, Walles, Löffler, Melcher)

BioSysNet, Associated Junior Group: Exploring RNA-binding proteins in *Campylobacter jejuni*

Young Investigator fellowship of the Bavarian Academy of Sciences: Functional characterization of small regulatory RNAs in the human pathogen *Helicobacter pylori*

Postdoc/Young Investigator grant of the Daimler-and-Benz-Foundation: Identification and functional characterization of RNA-binding proteins in *Campylobacter jejuni*

2.2 DANIEL LOPEZ

FG SPP1617: Molecular characterization of the distinct cell types required for the development of *Staphylococcus aureus* biofilms (LO-1804/2)

ERC Starting Grant 2013: BacRafts

2.4 ANA EULALIO

BioSysNet, Junior Group: RNA: the missing link in bacterial pathogen-host interactions

FEBS Long-Term Fellowship

2.6 SEBASTIAN GEIBEL

Elitenetzwerk Bayern, Project: Structural biology of mycobacterial secretion machines, N-BM-2013-246

3.1.1 JÖRG VOGEL

BioSysNet, Associated Senior Group: Temporal control of gene expression by small RNAs

DFG FOR1608: Unravelling the prokaryotic immune system. Project: CRISPR/Cas system in *Neisseria meningitides*

DFG SPP1316: Host-Adapted Metabolism of Bacterial Pathogens. Project: a post-transcriptional link between *Salmonella* metabolism and virulence

DFG grant: Cis/trans control of genes by a pH-responsive 5' UTR

BMBF grant: Next-generation transcriptomics of bacterial infections

DFG SFB/TRR34: Pathophysiology of Staphylococci in the Post-Genome-Era. Project: sRNA-mediated gene regulation in staphylococci: Impact on metabolism and biofilm expression

DFG SPP1258: Sensory and regulatory RNA in prokaryotes. Projects: a) RNA deep sequencing & method development; b) Multiple target regulation by GcvB sRNA; c) Conserved sRNA in the RpoS regulon

BMBF grant: RNomics in Infectious Diseases

3.1.2 HEIDRUN MOLL

DFG SFB630: Identification, isolation and functional analysis of anti-infective compounds. Project: B3 (Moll/Schurigt) Identification and characterization of leishmanicidal compounds

DFG IRTG1522: HIV/AIDS and associated infectious diseases in Southern Africa

3.1.3 JOACHIM MORSCHHÄUSER

DFG IRTG 1522: Epidemiology, diagnosis, and molecular mechanisms of multidrug resistance in *Candida albicans* and its impact on host-fungus interactions

DFG MO 846/6: Phenotypic switching and genomic alterations as host adaptation mechanisms of the opportunistic fungal pathogen *Candida albicans*

DFG MO 846/7: Systematic functional analysis of the zinc cluster transcription factor family of the pathogenic yeast *Candida albicans* by artificial activation

DFG SFB 630: Inhibition of virulence and resistance mechanisms of *Candida albicans*

DFG SFB/TR 124: Regulation of *Candida albicans* virulence traits by protein kinases

3.1.4 TOBIAS ÖLSCHLÄGER

DFG SFB630: Identification, isolation and functional analysis of anti-infective compounds. Project: Z1, Central Laboratory

3.1.5 KNUT OHLSEN

DFG SFB/TRR34: Pathophysiology of Staphylococci in the Post-Genome-Era. Project A2: Phosphoproteome studies to characterize serine/threonine protein kinases and phosphatases in *Staphylococcus aureus*

DFG SFB630: Determination of mode of action of novel anti-staphylococcal com-pounds using DNA-microarray technology. Project B5: Drug-induced gene expression in staphylococci

BMBF-Go-Bio3, FKZ 0315565: Immunotherapy against *Staphylococcus aureus*

BMBF-Infection Genomics, FKZ 0315829E: Hostpathogen interaction: effects of secreted proteins of *Staphylococcus aureus* on cells and components of the immune system

EU FP7 241796: Impact of specific antibiotic therapies on the prevalence of human host resistant bacteria (SAT URN)

3.1.6 WILMA ZIEBUHR

DFG SFB/TRR34: Pathophysiology of Staphylococci in the Post-Genomic Era

DFG SPP1617: Phenotypic heterogeneity of bacterial populations

BMBF MedVet Staph Interdisciplinary Research Network on the Zoonotic Impact of *Staphylococcus aureus*/MRSA

3.2.1 MATTHIAS FROSCH

BMBF grant: Medical Infection Genomics. Central Management

BMG grant: National Reference Laboratory for Meningococci

BMG grant: Consiliary Laboratory for *Haemophilus influenzae*

BMG grant: Consiliary Laboratory for Echinococcosis

EU/ECDC grant: Coordination of activities for laboratory surveillance of invasive bacterial diseases (*N. meningitidis*, *H. influenzae* and *S. pneumoniae*) in Member States and EEA/EFTA countries

3.2.2 KLAUS BREHM

DFG grant: Evolutionary and functional relationships of cytokines expressed by the helminth *E. multilocularis* and its mammalian host

DFG-GSLS: Molecular Characterization of Nuclear Hormone and Fibroblast Growth Factor Signalling in *E. multilocularis*

DFG-GSLS: Characterization of totipotent stem cells and regeneration mechanisms in cestode parasites

DFG – GSLS: Utilization of the *Echinococcus* kinome for the development of novel drugs against echinococcosis.

DFG IRTG1522: HIV/AIDS and associated infectious diseases in Southern Africa. Project: Characterization of the influence of excretory/secretory products from *E. multilocularis* larvae on dendritic cell maturation and the interaction of *Echinococcus* E/S products with TLR and CTL surface receptors

3.2.3 CHRISTOPH SCHOEN

DFG SPP1316 Host-Adapted Metabolism of Bacterial Pathogens. Project:"Gene regulatory mechanisms of metabolic adaptation in *Neisseria meningitidis* in ex vivo infection models" (SCHO 1322/1-1)

3.2.4 ANDREA SCHUBERT-UNKMEIR

DFG- SCHU-2394/1-1 and DFG- SCHU-2394/1-2: Integrin-mediated signal transduction in endothelial cells during infection with *N. meningitides*

3.2.5 ULRICH VOGEL

DFG grant: Mechanisms of host adaptation and immune evasion of *Neisseria meningitidis*: the role of biofilms and blebs

BMBF grant: Medical Infection Genomics, Cluster: Proteomics of meningococci and pneumococci. Project Würzburg

BMG grant: National Reference Laboratory for Meningococci within the frame work of the invasive bacterial infection network

BMG grant: Consulting Laboratory for *Haemophilus influenzae* within the framework of the invasive bacterial infection network

Collaborative Research Agreements with Novartis Vaccines

3.3.1 THOMAS HÜNIG

DFG HU 295/9-1 Autoimmunität oder Induktion von Toleranz: Kontrolle CD8 T-Zell-vermittelter Immunreaktionen gegen Oligodendrozyten des ZNS durch lokale Infektion.

DFG HU 295/12-1 Einfluss des CD28-Signals auf die funktionelle Programmierung und Re-Programmierung von Maus- und humanen Gedächtnis- sowie induzierten regulatorischen T-Zellen

DFG TRR 124/1 TP C06 Einfluss sekretierter Proteine von *Candida albicans* bei der Immunevasion und der Pathogenität

DFG IRTG 1522 BayImmuNet: Entwicklung immunologischer Ansätze zur Prävention und Therapie von Patienten mit Pilzinfektion *Aspergillus fumigatus* nach Stammzelltransplantation

SFB Transregio 52, TP A5 Die Rolle von CD28-Signalen bei der Bildung, Homeostase und Funktion regulatorischer T-Zellen

3.3.2 NIKLAS BEYERSDORF

AiCuris GmbH & Co. KG, Wuppertal: Comparative study of the effects of ILM and 2010 Proleukin on mouse and rat lymphocytes *in vitro* and *in vivo* and therapeutic impact on experimental autoimmune encephalomyelitis of the Lewis rat (2008-2010: Prof. Dr. Thomas Hünig, Würzburg)

DFG SFB-TRR124 Pathogenic fungi and their human host: Role of secreted *Candida albicans* proteins in immune evasion and pathogenicity

DFG Research Unit 2123 Sphingolipid dynamics in infection control: Role of sphingolipids in the regulation of anti-viral T cell responses (P02; with Prof. Dr. Jürgen Schneider-Schaulies, Würzburg)

DFG: The role of CD28-mediated signals in programming and reprogramming of mouse and human memory and induced regulatory T cells (BE4080/2-1; with Prof. Dr. Thomas Hünig, Würzburg)

3.3.3 THOMAS HERRMANN

DFG grant: The rat as new model for the analysis of iNKT cell antigen-recognition and function

Wilhlem-Sander-Therapieeinheit Multiples Myelom:gd T Zell- und Antikörper-vermittelte Immuntherapien gegen das Multiple Myelom: Mechanistische Grundlagen für neue Strategien

3.3.4 MANFRED LUTZ

DFG IRTG1522: Protective and productive inflammatory responses induced by microbial products studied at the level of dendritic cells

DFG/TR52: Tolerogenic and Immunogenic T Helper 2 Programming by Differentially Matured Dendritic Cells

DFG grant: Characterization of myeloid-derived suppressor cell subsets and their induction by *Mycobacterium tuberculosis*

IZKF Würzburg with Prof. M. Eyrich: Immunogenicity of native versus in vitro generated dendritic cells from patients with glioblastoma - investigations on the identification and overcoming of tumor escape mechanisms

DFG grant: Control of the homeostatic regulatory T cell pool by RelB expression in steady state migratory dendritic cells

EU-FP7 OPTATIO

SFB-TRR124 Pathogenic fungi and their human host: Networks of interaction

DFG Clinical Research Unit: Molecular Networks in Multiple Myeloma

Deutsche José Carreras Leukämie-Stiftung – Homing of alloreactive T cells

3.6.3 HARTWIG KLINKER

DFG IRTG 1522: HIV associated infectious diseases in South Africa

NIH and BMBF (01 KG 0915): START - Strategic Timing of Antiretroviral Treatment

IZKF Würzburg: Molecular investigations into the pharmacokinetics and drug monitoring of new direct-acting anti-HIV and anti-HCV antivirals (Z-4/106)

3.6.4 JÜRGEN LÖFFLER

CRC SFBTR124 Pathogenic fungi and their human host: Networks of interaction, Project A2: INtercation of *Aspergillus fumigatus* with human natural killer cells, dendritic cells and human alveolar epithelia

ERA Net PathoGenoMics: AspBIOmics - Invasive aspergillosis: Biomarkers for prevention, diagnosis and treatment response

4.1 GERHARD BRINGMANN

Speaker and coordinator of the DFG Collaborative Research Centre (Sonderforschungsbereich, SFB) 630 „Recognition, Preparation, and Functional Analysis of Agents against Infectious Diseases“, Project A2

DFG Clinical Research Unit (Klinische Forschergruppe) KFO 216 „Characterization of the Oncogenic Signaling Network in Multiple Myeloma: Development of Targeted Therapies"

4.2 THOMAS DANDEKAR

DFG TR34/TPA8: Systems biological analysis of the central carbohydrate metabolism and involved protein complexes in *Staphylococcus aureus*

DFG TR34/TPZ1: Integration of bioinformatical tools for an omics databank in a *Staphylococcus aureus* Wiki environment

DFG Da 208/13-1: Metabolism of intracellular *Salmonella enterica:* One life-style in intra-cellular infections

DFG Da 208/10-2: Host adapted metabolism of bacterial infections: Data integration and refined metabolic modelling

DFG GR1243/7-2: Genetic and immunologic basis of pathogenic and mutualistic interactions between bacteria and their ant hosts (joined PI with Roy Gross)

4.3 MARKUS ENGSTLER

DFG SPP1207: Nature inspired fluid mechanics

DFG SFB630: Recognition, Preparation, and Functional Analysis of Agents against Infectious Diseases. Project: VSG as unexpected drug target for sleeping sickness

DFG German-African Cooperation Projects in Infectology

DFG SPP1726: Microswimmers - From Single Particle Motion to Collective Behaviour

4.4 ULRIKE HOLZGRABE

DFG SFB630: Determination of mode of action of novel anti-staphylococcal com-pounds using DNA-microarray technology. Project B5: Drug-induced gene expression in staphylococci

4.5 UTE HENTSCHEL-HUMEIDA

DFG SFB630: Recognition, preparation and functional analysis of agents against infectious diseases. Project: Novel anti-infective substances from marine sponge-associated microbiota

DFG GRK1342: Molecular and functional analysis of lipid-based signal transduction systems. Project: The role of cuticular lipids in plant surface/microbe interactions

EU 7th Framework Programme: 'SeaBiotech: From sea-bed to test-bed: harvesting the potential of marine biodiversity for industrial biotechnology'. Project: Genomic and metagenomic bioprospecting

Unibund Würzburg: Project: Investigations on Mauke-disease and the development of a diagnostic kit

Bavaria California Technology Center (BaCaTeC): Project: Single-cell genomics of marine sponge-associated microbial symbionts

4.6 CAROLINE KISKER

DFG SFB630: Identification, isolation and functional analysis of anti-infective compounds. Project: Structure based Drug Design of essentiell enzymes of M. tuberculosis and other pathogens

Graduate School of Life Sciences, Excellence Initiative by the German federal and state governments, GSC 106

Rudolf Virchow Center for Experimental Biomedicine – DFG Research Center, FZ82

4.7 AUGUST STICH

DFG SFB630: Identification, isolation and functional analysis of anti-infective compounds. Project: Z1, Central Laboratory

4.8 HEIKE WALLES

Bavarian Research Foundation: ForMosA“

IDEAS-European Research Council

BMBF-CIRM: Characterization and bioengineering of cardiovascular stem cell niches

EU 7FrameworkProgramme: VascuBone

Bayern Fit Programme: Regenerative Technologies in Oncology

Wilhelm Sander Foundation: Functional roles of direct and bystander release of IL-12 by dendritic cells for T cell activation and anti-tumor immunity

3.4.1 AXEL RETHWILM

DFG IRTG 1522: HIV and associated infectious diseases in Southern Africa

BMBF: Foamyvirus Network for Genetic Therapy of Fanconi Anemia

DEUTSCHE KREBSHILFE: Lentivirale Reportergenvektoren zur Darstellung und Isolierung von Tumorstammzellen und Charakterisierung stammzellspezifischer Signaltransduktionswege

RKI: Konsiliarlabor für RVS

RKI: Konsiliarlabor für ZNS-Infektionen

RKI: Konsiliarlabor für RSV im Rahmen des Netzwerks Atemwegsinfektionen

3.4.2 SYBILLE SCHNEIDER-SCHAULIES

DFG SCHN405/5-1: Induction of plasma membrane ceramides in T cells and their role in functional paralysis

DFG SCHN405/6-1: Effectors, mechanisms and consequences of sphingomyelinase-dependent regulation of actin dynamics in measles virus induced T cell paralysis

DFG SCHN405/7-1: Regulation of plasma membrane ceramide generation in dendritic cells (DCs): impact on pathogen uptake and sorting, receptor cross-talk and immune activation

DFG SCHN 405/10-1: Sphingomyelinase activation in T cells: role in T cell activation and paralysis (FOR2123)

DFG SCHN 405/11-1: Central project FOR 2123

DFG IRTG1522 TP7: Targets, mechanisms and consequences of regulated T cell pre-mRNA splicing and their relevance as genetic markers of virally induced or general T cell suppression

Deutsche Krebshilfe: Identification of molecular signatures downstream of PI3-kinase interference and their potential to act as suppressors of stimulated or malignant T cell activation and expansion

3.4.3 JÜRGEN SCHNEIDER-SCHAULIES

DFG SCHN320/17-1: Identification of host cell factors for negative-stranded RNA viruses

DFG SCHN320/18-1: Adaptation of canine distemper virus to human host cell receptors

3.5.1 THOMAS RUDEL

ERA Net PathoGenoMics: Functional genomics of host-pathogen interactions using high-throughput screenings: a novel approach towards identifying therapeutic/prophylactic targets. Project: Bacterial factors required for disseminating gonococcal infections

BMBF NGFNplus: RNomics of Infectious Diseases. Project: RNomics of Bacterial Infections

DFG Deutsch-Israelische Projektförderung (DIP): A search for new cancer drug targets: the E4ORF4 network of cancer cell-specific apoptosis

DFG grant: Import and assembly of mitochondrial outer membrane proteins – identification of new factors and import signals

DFG SFB/TRR34: Pathophysiology of Staphylococci in the Post-Genomic Era (C11)

DFG SFB 630: Active Agents against Acute and Disseminating Infections by *Neisseria* (B9)

BMBF grant: Pathogen-Host Interactomes and Signaling Complexes in Bacterial Infections

DFG SPP1580: Simkania negevensis containing vacuoles: formation, trafficking and subversion of host signaling

IZKF: *Chlamydia* and ovarian cancer

3.5.2 MARTIN FRAUNHOLZ

DFG: FR1504/2-1 „Identification of virulence factors mediating phagosomal escape of *Staphylococcus aureus* by transposon insertion site deep sequencing"

3.5.3 ROY GROSS

DFG GR1243/7-2: Genetic and immunologic basis of pathogenic and mutualistic interactions between bacteria and their ant hosts

COST Action FA0701: Arthropod Symbioses: from fundamental studies to pest and disease management

3.5.4 VERY KOZJAK-PAVLOVIC

SFB 630 Identification, isolation and functional analysis of anti-infective compounds. Project B9: Recognition, Preparation and Functional Analysis of Agents against Infectious Diseases

3.6.1 HERMAN EINSELE

EU FP 6 Strep MANASP: Development of novel management strategies for invasive aspergillosis

EU FP 7 NanoII: Nanoscopically-guided induction and expansion of regulatory hematopoietic cells to treat autoimmune and inflammatory processes

ERA Net PathoGenoMics: AspBIOmics - Invasive aspergillosis: Biomarkers for prevention, diagnosis and treatment response

BayImmuNet: Development of an immunotherapy protocol for the prevention and treatment of *A. fumigatus* infection

BMBF: Development, laboratory and clinical evaluation of an innovative diagnostic system for pathogenic fungi

3.6.2 ANDREAS BEILHACK

Deutsche José Carreras Leukämie-Stiftung – Exploring invasive aspergillosis after allogeneic hematpoietic cell transplantation with in vivo imaging

SFB-TR52 - Transcriptional Programming of Individual T Cell Subsets

EU-FP7 program – NanoII

Else-Kröner-Forschungskolleg for Interdisciplinary Translational Immunology (Physician-Scientist program)

IZKF Research Group for Experimental Stem Cell Transplantation

6. APPENDIX
6.5. PUBLICATIONS

2.1 CYNTHIA SHARMA

Dugar G, Herbig A, Förstner KU, Heidrich N, Reinhardt R, Nieselt K, **Sharma CM** (2013) *High-resolution transcriptome maps reveal strain-specific regulatory features of multiple Campylobacter jejuni isolates.* **PLoS Genet** 9: 5 e1003495

Müller S, Windhof I M, Maximov V, Jurkowski T, Jeltsch A, Forstner K U, **Sharma CM**, Graf R, Nellen W (2013) *Target recognition, RNA methylation activity and transcriptional regulation of the Dictyostelium discoideum Dnmt2-homologue (DnmA).* **Nucleic Acids Res** 41: 18 8615-27

Lioliou E, **Sharma CM** , Altuvia Y, Caldelari I, Romilly C, Helfer A C, Margalit H, Romby P (2013) *In vivo mapping of RNA-RNA interactions in Staphylococcus aureus using the endoribonuclease III.* **Methods** 63: 2 135-43

Zhelyazkova P, **Sharma CM** , Forstner K U, Liere K, Vogel J, Borner T (2012) *The primary transcriptome of barley chloroplasts: numerous noncoding RNAs and the dominating role of the plastid-encoded RNA polymerase.* **Plant Cell** 24: 1 123-36

Wilms I, Overlöper A, Nowrousian M, **Sharma CM** , Narberhaus F (2012) *Deep sequencing uncovers numerous small RNAs on all four replicons of the plant pathogen Agrobacterium tumefaciens.* **RNA Biol** 9: 4 446-57

Sharma CM, Heidrich N (2012) *Small RNAs and virulence in bacterial pathogens.* **RNA Biol** 9: 4 361-3

Schmidtke C, Findeiss S, **Sharma CM** , Kuhfuss J, Hoffmann S, Vogel J, Stadler P F, Bonas U (2012) *Genome-wide transcriptome analysis of the plant pathogen Xanthomonas identifies sRNAs with putative virulence functions.* **Nucleic Acids Res** 40: 5 2020-31

Roscetto E, Angrisano T, Costa V, Casalino M, Forstner K U, **Sharma CM** , Di Nocera P P, De Gregorio E (2012) *Functional characterization of the RNA chaperone Hfq in the opportunistic human pathogen Stenotrophomonas maltophilia.* **J Bacteriol** 194: 21 5864-74

Rieder R, Reinhardt R, **Sharma CM**, Vogel J (2012) *Experimental tools to identify RNA-protein interactions in Helicobacter pylori.* **RNA Biol** 9: 4 520-31

Ramachandran V K, Shearer N, Jacob J J, **Sharma CM** , Thompson A (2012) *The architecture and ppGpp-dependent expression of the primary transcriptome of Salmonella Typhimurium during invasion gene expression.* **BMC Genomics** 13: 25

Pernitzsch S R, **Sharma CM** (2012) *Transcriptome complexity and riboregulation in the human pathogen Helicobacter pylori* **Front Cell Infect Microbiol** 2: 14

Madhugiri R, Pessi G, Voss B, Hahn J, **Sharma CM**, Reinhardt R, Vogel J, Hess WR, Fischer HM, Evguenieva-Hackenberg E (2012) *Small RNAs of the Bradyrhizobium/Rhodopseudomonas lineage and their analysis.* **RNA Biol** 9: 1 47-58

Lioliou E, **Sharma CM**, Caldelari I, Helfer AC, Fechter P, Vandenesch F, Vogel J, Romby P (2012) *Global regulatory functions of the Staphylococcus aureus endoribonuclease III in gene expression.* **PLoS Genet** 8: 6 e1002782

Jager D, Pernitzsch SR, Richter AS, Backofen R, **Sharma CM**, Schmitz RA (2012) *An archaeal sRNA targeting cis- and trans-encoded mRNAs via two distinct domains* **Nucleic Acids Res** 40: 21 10964-79

Chao Y, Papenfort K, Reinhardt R, **Sharma CM**, Vogel J (2012) *An atlas of Hfq-bound transcripts reveals 3' UTRs as a genomic reservoir of regulatory small RNAs* **EMBO J** 31: 20 4005-19

2.2 DANIEL LOPEZ

Mielich-Suss B, Schneider J, **Lopez D** (2013) *Overproduction of flotillin influences cell differentiation and shape in Bacillus subtilis.* **MBio** 4: 6 e00719-13

Audretsch C, **Lopez D**, Srivastava M, Wolz C, Dandekar T (2013) *A semi-quantitative model of Quorum-Sensing in Staphylococcus aureus, approved by microarray meta-analyses and tested by mutation studies.* **Mol Biosyst** 9: 11 2665-80

Yepes A, Schneider J, Mielich B, Koch G, Garcia-Betancur JC, Ramamurthi KS, Vlamakis H, **Lopez D** (2012) *The biofilm formation defect of a Bacillus subtilis flotillin-defective mutant involves the protease FtsH.* **Mol Microbiol** 86: 2 457-71

Schneider J, Yepes A, Garcia-Betancur JC, Westedt I, Mielich B, **Lopez D** (2012) *Streptomycin-induced expression in Bacillus subtilis of YtnP, a lactonase-homologous protein that inhibits development and streptomycin production in Streptomyces griseus.* **Appl Environ Microbiol** 78: 2 599-603

Garcia-Betancur JC, Yepes A, Schneider J, **Lopez D** (2012) Single-cell analysis of Bacillus subtilis biofilms using fluorescence microscopy and flow cytometry. **J Vis Exp** 60

2.3 NICOLAI SIEGEL

Nguyen TN, Muller LS, Park SH, **Siegel TN**, Gunzl A (2014) *Promoter occupancy of the basal class I transcription factor A differs strongly between active and silent VSG expression sites in Trypanosoma brucei.* **Nucleic Acids Res** 42: 5 3164-76

Lopez-Rubio JJ, **Siegel TN**, Scherf A (2013) *Genome-wide chromatin immunoprecipitation-sequencing in Plasmodium.* **Methods Mol Biol** 923: 321-33

Chene A, Vembar SS, Riviere L, Lopez-Rubio JJ, Claes A, **Siegel TN**, Sakamoto H, Scheidig-Benatar C, Hernandez-Rivas R, Scherf A (2012) *PfAlbas constitute a new eukaryotic DNA/RNA-binding protein family in malaria parasites.* **Nucleic Acids Res** 40: 7 3066-77

2.4 ANA EULALIO

Huntzinger E, Kuzuoglu-Öztürk D, Braun JE, **Eulalio A**, Wohlbold L, Izaurralde E (2013) *The interactions of GW182 proteins with PABP and deadenylases are required for both translational repression and degradation of miRNA targets.* **Nucleic Acids Res** 41(2):978-94

Eulálio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M (2012) *Functional screening identifies miRNAs inducing cardiac regeneration.* **Nature** 20;492(7429):376-81

Lovric J, Mano M, Zentilin L, **Eulálio A**, Zacchigna S, Giacca M (2012) *Terminal differentiation of cardiac and skeletal myocytes induces permissivity to AAV transduction by relieving inhibition imposed by DNA damage response proteins.* **Mol Ther** 20(11):2087-97

Eulálio A, Schulte L, Vogel J (2012) *The mammalian microRNA response to bacterial infections.* **RNA Biol** 9(6):742-750

2.5 CHRISTIAN PEREZ

Perez JC, Johnson AD (2013) *Regulatory circuits that enable proliferation of the fungus Candida albicans in a mammalian host.* **PLoS Pathog** 9: 12 e1003780

Perez JC, Kumamoto CA, Johnson AD (2013) *Candida albicans commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit.* **PLoS Biol** 11: 3 e1001510

Fraser HB, Levy S, Chavan A, Shah HB, **Perez JC**, Zhou Y, Siegal ML, Sinha H (2012) *Polygenic cis-regulatory adaptation in the evolution of yeast pathogenicity.* **Genome Res** 22: 10 1930-9

Zwir I, Latifi T, **Perez JC**, Huang H, Groisman EA (2012) *The promoter architectural landscape of the Salmonella PhoP regulon.* **Mol Microbiol** 84: 3 463-85

2.6 SEBASTIAN GEIBEL

Geibel S, Waksman G (2013) *The molecular dissection of the chaperone-usher pathway.* **Biochim Biophys Acta** Oct 16. pii: S0167-4889

Geibel S, Procko E, Hultgren SJ, Baker D, Waksman G (2013) *Structural and energetic basis of folded-protein transport by the FimD usher.* **Nature** 496: 243-246

Rego AT, Johnson JG, **Geibel S**, Enguita FJ, Clegg S, Waksman G (2012) *Crystal structure of the MrkD1P receptor binding domain of Klebsiella pneumoniae and identification of the human collagen V binding interface.* **Mol Microbiol** 86: 882-893

3.1.1 JÖRG VOGEL

Papenfort K, Sun Y, Miyakoshi M, Vanderpool CK, **Vogel J** (2013) *Small RNA-mediated activation of sugar phosphatase mRNA regulates glucose homeostasis.* **Cell** 153:426–437

Fröhlich KS, Papenfort K, Fekete A, **Vogel J** (2013) *A small RNA activates CFA synthase by isoform-specific mRNA stabilization.* **EMBO J** 13;32(22):2963-79

Heidrich N, **Vogel J** (2013) *Same same but different: new structural insight into CRISPR-Cas complexes.* **Mol Cell** 10;52(1):4-7

Zhang Y, Heidrich N, Ampattu BJ, Gunderson CW, Seifert HS, Schoen C, **Vogel J**, Sontheimer EJ (2013) *Processing-Independent CRISPR RNAs Limit Natural Transformation in Neisseria meningitides.* **Mol Cell** 23;50(4):488-503

Holmqvist E, **Vogel J** (2013) *A small RNA serving both the Hfq and CsrA regulons.* **Genes & Development** 27:1073-1078

Göpel Y, Papenfort K, Reichenbach B, **Vogel J**, Görke B (2013) *Targeted decay of a regulatory small RNA by an adaptor protein for RNase E and counteraction by an anti-adaptor RNA.* **Genes Dev** 1;27(5):552-64

Wright PR, Richter AS, Papenfort K, Mann M, **Vogel J**, Hess WR, Backofen R, Georg J (2013) *Comparative genomics boosts target prediction for bacterial small RNAs.* **PNAS** 110(37):E3487-96

Heidrich N, **Vogel J** (2013) *CRISPRs extending their reach: Prokaryotic RNAi protein Cas9 recruited for gene regulation.* **EMBO J** 3;32(13):1802-4

Gutierrez A, Laureti L, Crussard S, Abida H, Rodríguez-Rojas A, Blázquez J, Baharoglu Z, Mazel D, Darfeuille F, **Vogel J**, Matic I (2013) *Beta-lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity.* **Nat Commun** 19;4:1610

Schulte LN, Westerman AJ, **Vogel J** (2013) *Differential activiation and functional specialisation of miR-146 and miR-155 in innate immune sensing.* **Nucleic Acids Res** 41(1):542-53

Vogel J, Bassler BL (2013) *Bacterial Regulatory Mechanisms: The gene and beyond.* **Current Opinion in Microbiology** 16(2):109-11

Caldelari I, Chao Y, Romby P, **Vogel J** (2013) *RNA-mediated regulation in pathogenic bacteria.*
In: **Bacterial Pathogens and Pathogenesis** (eds. Cossart P, Maloy S), CSH Press, Sep 1;3(9). doi:pii: a010298

Papenfort K, Corcoran CP, Gupta SK, Miyakoshi M, Heidrich N, Chao Y, Fröhlich KS, Sharma CM, Ziebuhr W, Böhm A, **Vogel J** (2013) *Regulatory Mechanisms of Special Significance: The role of sRNAs in Virulence.* In: **Regulation of Bacterial Virulence** (eds. Darwin AJ, Vasil ML), ASM Press, pp493-527

Podkaminski D, Bouvier M, **Vogel J** (2013) *Identification and characterization of small noncoding RNAs in bacteria.* In: **Handbook of RNA Biochemistry** (eds. Hartmann RK, Bindereif A, Schön A, Westhof E), Wiley-VCH, in press

Chao Y, Papenfort K, Reinhardt R, Sharma CM, **Vogel J** (2012) *An atlas of Hfq-bound transcripts reveals 3' UTRs as a genomic reservoir of regulatory small RNAs.* **EMBO J** 31(29):4005-19

Westermann AJ, Gorski SA, **Vogel J** (2012) *Dual RNA-seq of pathogen and host.* **Nat Rev Microbiol** 14;10(9):618-30

Flowers SA, Barker KS, Berkow EL, Toner G, Chadwick SG, Gygax SE, **Morschhäuser J**, Rogers PD (2012) *Gain-of-function mutations in UPC2 are a frequent cause of ERG11 upregulation in azole-resistant clinical isolates of Candida albicans*. **Eukaryot Cell** 11:1289-1299

Sasse C, Dunkel N, Schäfer T, Schneider S, Dierolf F, Ohlsen K, **Morschhäuser J** (2012) *The stepwise acquisition of fluconazole resistance mutations causes a gradual loss of fitness in Candida albicans.* **Mol Microbiol** 86:539-556

3.1.4 TOBIAS ÖLSCHLÄGER

Becker S, **Oelschlaeger TA**, Wullaert A, Vlantis K, Pasparakis M, Wehkamp J, Stange EF, Gersemann M (2013) *Bacteria regulate intestinal epithelial cell differentiation factors both in vitro and in vivo*. **PLoS One** 8(2)

Bringmann G, Zhang G, **Oelschlaeger T**, Stich A, Wu J, Chatterjee M, Brun R (2013) *Highly selective antiplasmodial naphthylisoquinoline alkaloids from Ancistrocladus tectorius* . **Phytochemistry** 91:220-8

Memmel E, Homann A, **Oelschlaeger TA**, Seibel J (2013) *Metabolic glycoengineering of Staphylococcus aureus reduces its adherence to human T24 bladder carcinoma cells.* **Chem Commun** (Camb) 25;49(66):7301-3

Rund SA, Rohde H, Sonnenborn U, **Oelschlaeger TA** (2013) *Antagonistic effects of probiotic Escherichia coli Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7.* **Int J Med Microbiol** 303(1):1-8

Troge A, Scheppach W, Schroeder BO, Rund SA, Heuner K, Wehkamp J, Stange EF, **Oelschlaeger TA** (2012) *More than a marine propeller - the flagellum of the probiotic Escherichia coli strain Nissle 1917 is the major adhesin mediating binding to human mucus.* **Int J Med Microbiol** 302(7-8):304-14

Seo EJ, Weibel S, Wehkamp J, **Oelschlaeger TA** (2012) *Construction of recombinant E. coli Nissle 1917 (EcN) strains for the expression and secretion of defensins.* **Int J Med Microbiol** 302(6):276-87

Fünfstück R, WagenlehnerFME, Ölschläger T, Naber KG (2012) *Harnwegsinfektionen: Zystitis, Pyelonephritis, Urosepsis* **Dtsch Med Wochenschr** 137: 198-201

3.1.5 KNUT OHLSEN

Bäsell K, Otto A, Junker S, Zühlke D, Rappen GM, Schmidt S, Hentschker C, Macek B, **Ohlsen K**, Hecker M, Becher D (2013) *The phosphoproteome and its physiological dynamics in Staphylococcus aureus.* **Int J Med Microbiol** S1438-4221(13)193-8

Yu W, Leibig M, Schäfer T, Bertram R, **Ohlsen K**, Götz F (2013) *Mevalonate auxotrophic mutant of Staphylococcus aureus can adapt to mevalonate depletion.* **Antimicrob Agents Chemother** Aug 19. [Epub ahead of print]

Hertlein, T, Sturm V, Jakob P, **Ohlsen K** (2013) *19F Magnetic Resonance Imaging of perfluorocarbons for the evaluation of response to antibiotic therapy in a Staphylococcus aureus infection model.* **PLoS One** 8(5):e64440

Dunkel N, Hertlein T, Franz R, Reuß O, Sasse C, Schäfer T, **Ohlsen K**, Morschhäuser J (2013) *Roles of different peptide transporters in nutrient acquisition in Candida albicans.* **Eukaryot Cell** 12(4):520-8

Feng J, Michalik S, Varming AN, Andersen JH, Albrecht D, Jelsbak L, Krieger S, **Ohlsen K**, Hecker M, Gerth U, Ingmer H, Frees D (2013) *Trapping and Proteomic Identification of Cellular Substrates of the ClpP Protease in Staphylococcus aureus* . **J Proteome Res** 1;12(2):547-58

Sasse C, Dunkel N, Schäfer T, Schneider S, Dierolf F, **Ohlsen K**, Morschhäuser J (2012) *The stepwise acquisition of fluconazole resistance mutations causes a gradual loss of fitness in Candida albicans.* **Mol Microbiol** 86(3):539-56

Marincola G, Schäfer T, Behler J, Bernhardt J, **Ohlsen K**, Goerke C, Wolz C (2012) *RNase Y of Staphylococcus aureus and its role in the activation of virulence genes.* **Mol Microbiol** 85:817-832

Rakette S, Donat S, **Ohlsen K**, Stehle T (2012) *Structural Analysis of Staphylococcus aureus Serine/Threonine Kinase PknB*. **PLoS One** 7(6):e39136

Tischer M, Pradel G, **Ohlsen K**, Holzgrabe U (2012) *Quaternary ammonium salts and their antimicrobial potential: targets or nonspecific interactions?* **ChemMedChem** 2;7(1):22-31

Depke M, Burian M, Schäfer T, Bröker BM, **Ohlsen K**, Völker U (2012) *The alternative sigma factor B modulates virulence gene expression in a murine Staphylococcus aureus infection model but does not influence kidney gene expression pattern of the host.* **Int J Med Microbiol** 302(1):33-9

Donat S, Hasenberg M, Schäfer T, **Ohlsen K**, Gunzer M, Einsele H, Löffler J, Beilhack A, Krappmann S (2012) *Surface display of Gaussia princeps luciferase allows sensitive fungal pathogen detection during cutaneous aspergillosis.* **Virulence** 3(1) [Epub ahead of print]

3.1.6 WILMA ZIEBUHR

Schoenfelder SMK, Marincola G, Geiger T, Goerke C, Wolz C, **Ziebuhr W** (2013) *Methionine Biosynthesis in Staphylococcus aureus is Tightly Controlled by a Hierarchical Network Involving an Initiator tRNA-specific T-box Riboswitch.* **PloS Pathog** 9(9):e003606

Makgotlho PE, Marincola G, Schäfer D, Liu Q, Bae T, Geiger T, Wasserman E, Wolz C, **Ziebuhr W,** Sinha B (2013) *SDS Interferes with SaeS Signaling of Staphylococcus aureus Independently of SaePQ*. **PLoS One** 20;8(8):e71644. doi: 10.1371

Hilmi D, Parcina M, Bode K, Ostrop J, Schuett S, Heeg K, **Ziebuhr W**, Sommerburg O, Bekeredjian-Ding I (2013) *Functional variation reflects intra-strain diversity of Staphylococcus aureus small colony variants in the host-pathogen interaction.* **Int J Med Microbiol** 303(2):61-9

Papenfort K, Corcoran CP, Gupta SK, Miyakoshi M, Heidrich N, Chao Y, Fröhlich KS, Sharma CM, **Ziebuhr W,** Böhm A, Vogel J (2013)

Regulatory Mechanisms of Special Significance: The role of sRNAs in Virulence In: Regulation of Bacterial Virulence (eds. Darwin AJ, Vasil ML), ASM Press, pp493-527

Bandyra K, Said N, Pfeiffer V, Górna MW, **Vogel J#**, Luisi BF# (2012) *The seed region of a small RNA drives the controlled destruction of target mRNA by the endoribonuclease RNase E.* **Mol Cell** 28;47(6):943-53

Lioliou E, Sharma CM, Caldelari I, Helfer AC, Fechter P, Vandenesch F, **Vogel J#**, Romby P# (2012) *Global regulatory functions of the of Staphylococcus aureus endoribonuclease III in gene expression.* **PLoS Genetics** 8(6):e1002782

Eulalio A, Schulte L, **Vogel J** (2012) *The mammalian microRNA response to bacterial infections.* **RNA Biol** 9(6):742-750

Corcoran CP, Rieder R, Podkaminski D, Hofmann B, **Vogel J (2012)** *Use of Aptamer Tagging to Identify In Vivo Protein Binding Partners of Small Regulatory RNAs.* **Methods Mol Biol** 2012;905:177-200

Papenfort K, Podkaminski D, Hinton JC, **Vogel J** (2012) *The ancestral SgrS RNA discriminates horizontally acquired Salmonella mRNAs through a single G-U wobble pair*. **PNAS** 109(13):E757-64

Argaman L, Elgrably-Weiss M, Hershko T, **Vogel J**, Altuvia S (2012) *RelA protein stimulates the activity of RyhB small RNA by acting on RNA-binding protein Hfq.* **PNAS** 109(12):4621-6

Kröger C, Dillon SC, Cameron AD, Papenfort K, Sivasankaran SK, Hokamp K, Chao Y, Sittka A, Hébrard M, Händler K, Colgan A, Leekitcharoenphon P, Langridge GC, Lohan AJ, Loftus B, Lucchini S, Ussery DW, Dorman CJ, Thomson NR, **Vogel J**, Hinton JC (2012) *The transcriptional landscape and small RNAs of Salmonella enterica serovar Typhimurium*. **PNAS** 109(20):E1277-86

Corcoran CP, Podkaminski D, Papenfort K, Urban JH, Hinton JC, **Vogel J** (2012) *Superfolder GFP reporters validate diverse new mRNA targets of the classic porin regulator, MicF RNA.* **Mol Micro** 84(3):428-45

Boehm A, **Vogel J** (2012) *The csgD mRNA as a hub for signal integration via multiplesmall RNAs.* **Molecular Microbiology** 84(1):1-5

Fröhlich KS, Papenfort K, Berger AA, **Vogel J** (2012) *A conserved RpoS-dependent small RNA controls the synthesis of major porin OmpD.* **Nucleic Acids Res** 40(8):3623-40

Rieder R, Reinhardt R, Sharma C, **Vogel J** (2012) *Experimental tools to identify RNA-protein interactions in Helicobacter pylori.* **RNA Biol** 1;9(4)

Schmidtke C, Findeiss S, Sharma CM, Kuhfuss J, Hoffmann S, **Vogel J**, Stadler PF, Bonas U (2012) *Genome-wide transcriptome analysis of the plant pathogen Xanthomonas identifies sRNAs with putative virulence functions*. **Nucleic Acids Res** 40(5):2020-31

Zhelyazkova P, Sharma CM, Förstner KU, Liere K, **Vogel J**, Börner T (2012) *The primary transcriptome of barley chloroplasts: numerous noncoding RNAs and the dominating role of the plastid-encoded RNA polymerase*. **Plant Cell** 24(1):123-36

Madhugiri R, Pessi G, Voss B, Hahn J, Sharma CM, Reinhardt R, **Vogel J**, Hess WR, Fischer HM, Evguenieva-Hackenberg E (2012) *Small RNAs of the Bradyrhizobium/Rhodopseudomonas lineage and their analysis*. RNA Biol 9(1)**:47-58**

3.1.2 HEIdrun Moll

Bringmann G, Thomale K, Bischof S, Schneider C, Schultheis M, Sc**hwarz T**, Moll H, Schurigt *U (2013) A novel Leishmania major amastigote assay in 96-well format for rapid drug screening and its use for the discovery and evaluation of a new class of leishmanicidal quinolini***um salts. Antimicrob Agents Chemother** 57: 3003-3011

Schwarz T, Remer KA, Nahrendorf W, Masic A, Siewe L, Müller W, Roers A, **Moll H** (2013) *T cell-derived IL-10 determines leishmaniasis disease outcome and is suppressed by a dendritic cell-based vaccine* . **PLoS Pathog** 9: e1003476

Masic A, Hurdayal R, Nieuwenhuizen N E, Brombacher F, **Moll H** (2012) *Dendritic cell-mediated vaccination relies on Interleukin-4 Receptor signaling to avoid tissue damage after Leishmania major infection of BALB/c mice.* **PLoS Negl Trop Dis** 6(7): e1721

3.1.3 JOACHIM MORSCHHÄUSER

Sasse C, Hasenberg M, Weyler M, Gunzer M, **Morschhäuser J** (2013) *White-opaque switching of Candida albicans allows immune evasion in an environment-dependent fashion*. **Eukaryot. Cell** 12:50-58.

Dunkel N, Hertlein T, Franz R, Reuß O, Sasse C, Schäfer T, Ohlsen T, **Morschhäuser J** (2013) *Roles of different peptide transporters in nutrient acquisition in Candida albicans.* **Eukaryot. Cell** 12:520-528.

Hennicke F, Grumbt M, Lermann U, Ueberschaar N, Palige K, Böttcher B, Jacobsen ID, Staib C, **Morschhäuser J**, Monod M, Hube B, Hertweck C, Staib P (2013) *Factors supporting cysteine tolerance and sulfite production in Candida albicans.* **Eukaryot Cell** 12:604-613

Palige K, Linde J, Martin R, Böttcher B, Citiulo F, Sullivan DJ, Weber J, Staib C, Rupp S, Hube B, **Morschhäuser J**, Staib P (2013) *Global transcriptome sequencing identifies chlamydospore specific markers in* Candida albicans *and* Candida dubliniensis. **PLoS One** 8:e61940

Schillig R, **Morschhäuser J** (2013) *Analysis of a fungus-specific transcription factor family, the Candida albicans zinc cluster proteins, by artificial activation.* **Mol Microbiol** 89:1003-1017

Ramírez-Zavala B, Weyler M, Gildor T, Schmauch C, Kornitzer D, Arkowitz R, **Morschhäuser J** (2013) *Activation of the Cph1-dependent MAP kinase signaling pathway induces white-opaque switching in Candida albicans.* **PLoS Pathog** 9:e1003696

Sasse C, **Morschhäuser J** (2012) *Gene deletion in Candida albicans wild-type strains using the SAT1-flipping strategy.* **Methods Mol Biol** 845:3-17

Weyler M, **Morschhäuser J** (2012) *Tetracycline-inducible gene expression in Candida albicans.* **Methods Mol Biol** 845:201-210

Sasse C, Schillig R, Reimund A, Merk J, **Morschhäuser J** (2012) *Inducible and constitutive activation of two polymorphic promoter alleles of the Candida albicans multidrug efflux pump MDR1.* **Antimicrob Agents Chemother** 56:4490-4494

Ziebuhr W, Flückiger U (2012) *Coagulase-negative Staphylococci*. In: **Hospital Epidemiology and Infection Control**, Lippincott Williams & Wilkins, Philadelphia, PA p443-462

3.2.1 MATTHIAS FROSCH

Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, Caugant DA, Kriz P, Abad R, Bambini S, Carannante A, Deghmane AE, Fazio C, **Frosch M**, Frosi G, Gilchrist S, Giuliani MM, Hong E, Ledroit M, Lovaglio PG, Lucidarme J, Musilek M, Muzzi A, Oksnes J, Rigat F, Orlandi L, Stella M, Thompson D, Pizza M, Rappuoli R, Serruto D, Comanducci M, Boccadifuoco G, Donnelly JJ, Medini D, Borrow R (2013) *Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment.* **Lancet Infect Dis** 13: 5 416-25

Tappe D, Ernestus K, Rauthe S, Schoen C, **Frosch M**, Muller A, Stich A (2013) *Initial patient cluster and first positive biopsy findings in an outbreak of acute muscular Sarcocystis-like infection in travelers returning from Tioman island, Peninsular Malaysia, in 2011* **J Clin Microbiol** 51: 2 725-6

Hoiseth SK, Murphy E, Andrew L, Vogel U, **Frosch M**, Hellenbrand W, Abad R, Vazquez JA, Borrow R, Findlow J, Taha MK, Deghmane AE, Caugant DA, Kriz P, Musilek M, Mayer LW, Wang X, Macneil JR, York L, Tan CY, Jansen KU, Anderson AS (2013) *A multi-country evaluation of Neisseria meningitidis serogroup B factor H-binding proteins and implications for vaccine coverage in different age groups.* **Pediatr Infect Dis J** 32: 10 1096-101

Hellenbrand W, Elias J, Wichmann O, Dehnert M, **Frosch M**, Vogel U (2013) *Epidemiology of invasive meningococcal disease in Germany, 2002-2010, and impact of vaccination with meningococcal C conjugate vaccine.* **J Infect** 66: 1 48-56

Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, Corton C, Care R, Poolman JT, Zollinger WD, Frasch CE, Stephens DS, Feavers I, **Frosch M**, Parkhill J, Vogel U, Quail MA, Bentley SD, Maiden MC (2013) *Description and nomenclature of Neisseria meningitidis capsule locus.* **Emerg Infect Dis** 19: 4 566-73

Elias J, Heuschmann PU, Schmitt C, Eckhardt F, Boehm H, Maier S, Kolb-Maurer A, Riedmiller H, Mullges W, Weisser C, Wunder C, **Frosch M**, Vogel U (2013) *Prevalence dependent calibration of a predictive model for nasal carriage of methicillin-resistant Staphylococcus aureus.* **BMC Infect Dis** 13: 111

Elias J, Findlow J, Borrow R, Tremmel A, **Frosch M**, Vogel U (2013) *Persistence of antibodies in laboratory staff immunized with quadrivalent meningococcal polysaccharide vaccine.* **J Occup Med Toxicol** 8: 1 4

Atanasov G, Benckert C, Thelen A, Tappe D, **Frosch M**, Teichmann D, Barth TF, Wittekind C, Schubert S, Jonas S (2013) *Alveolar echinococcosis-spreading disease challenging clinicians: a case report and literature review.* **World J Gastroenterol** 19: 26 4257-61

Zlamy M, Hofer J, Elias J, Vogel U, **Frosch M**, Jungraithmayr T, Zimmerhackl LB, Prelog M (2012) *Immunogenicity of meningococcus C vaccination in a patient with atypical hemolytic uremic syndrome (aHUS) on eculizumab therapy.* **Pediatr Transplant** 16: 6 E246-50

Schork S, Schluter A, Blom J, Schneiker-Bekel S, Puhler A, Goesmann A, **Frosch M**, Schoen C (2012) *Genome sequence of a Neisseria meningitidis capsule null locus strain from the clonal complex of sequence type 198.* **J Bacteriol** 194: 18 5144-5

Maiden MC, **Frosch M** (2012) *Can we, should we, eradicate the meningococcus?* **Vaccine** 30 Suppl 2: B52-6

Joseph B, **Frosch M**, Schoen C, Schubert-Unkmeir A (2012) *Transcriptome analyses in the interaction of Neisseria meningitidis with mammalian host cells.* **Methods Mol Biol** 799: 267-93

3.2.2 KLAUS BREHM

Geyer KK, Chalmers IW, MacKintosh N, Hirst JE, Geoghegan R, Badets M, Brophy PM, **Brehm K**, Hoffmann KF (2013) *Cytosine methylation is a conserved epigenetic feature found throughout the phylum Platyhelminthes.* **BMC Genomics** 14: 462

Tsai IJ, Zarowiecki M, Holroyd N, Garciarrubio A, Sanchez-Flores A, Brooks KL, Tracey A, Bobes RJ, Fragoso G, Sciutto E, Aslett M, Beasley H, Bennett HM, Cai J, Camicia F, Clark R, Cucher M, De Silva N, Day TA, Deplazes P, Estrada K, Fernandez C, Holland PW, Hou J, Hu S, Huckvale T, Hung SS, Kamenetzky L, Keane JA, Kiss F, Koziol U, Lambert O, Liu K, Luo X, Luo Y, Macchiaroli N, Nichol S, Paps J, Parkinson J, Pouchkina-Stantcheva N, Riddiford N, Rosenzvit M, Salinas G, Wasmuth JD, Zamanian M, Zheng Y, Cai X, Soberon X, Olson PD, Laclette JP, **Brehm K**, Berriman M (2013) *The genomes of four tapeworm species reveal adaptations to parasitis.* **Nature** 496: 7443 57-63

Koziol U, Krohne G, **Brehm K** (2013) *Anatomy and development of the larval nervous system in Echinococcus multilocularis.* **Front Zool** 10: 1 24

Hsu TL, Lin G, Koizumi A, **Brehm K**, Hada N, Chuang PK, Wong CH, Hsieh SL, Diaz A (2013) *The surface carbohydrates of the Echinococcus granulosus larva interact selectively with the rodent Kupffer cell receptor.* **Mol Biochem Parasitol** 192: 1-2 55-9

Camicia F, Herz M, Prada LC, Kamenetzky L, Simonetta SH, Cucher MA, Bianchi JI, Fernandez C, **Brehm K**, Rosenzvit MC (2013) *The nervous and prenervous roles of serotonin in Echinococcus spp.* **Int J Parasitol** 43: 8 647-59

Olson PD, Zarowiecki M, Kiss F, **Brehm K** (2012) *Cestode genomics - progress and prospects for advancing basic and applied aspects of flatworm biology* **Parasite Immunol** 34: 2-3 130-50

Nono JK, Pletinckx K, Lutz MB, **Brehm K** (2012) *Excretory/secretory-products of Echinococcus multilocularis larvae induce apoptosis and tolerogenic properties in dendritic cells in vitro.* **PLoS Negl Trop Dis** 6: 2 e1516

Hemer S, **Brehm K** (2012) *In vitro efficacy of the anticancer drug imatinib on Echinococcus multilocularis larvae* **Int J Antimicrob Agents** 40: 5 458-62

Brehm K, Carlton JM, Hoffmann KF (2012) *Parasite genomics and post-genomic activities: 21st century resources for the parasite immunologist.* **Parasite Immunol** 34: 2-3 47-9

3.2.3 CHRISTOPH SCHOEN

Zhang Y, Heidrich N, Ampattu BJ, Gunderson CW, Seifert HS, **Schoen C**, Vogel J, Sontheimer EJ (2013) *Processing-independent CRISPR RNAs limit natural transformation in Neisseria meningitidis.* **Mol Cell** 50: 4 488-503

Tappe D, Ernestus K, Rauthe S, **Schoen C,** Frosch M, Muller A, Stich A (2013) *Initial patient cluster and first positive biopsy findings in an outbreak of acute muscular Sarcocystis-like infection in travelers returning from Tioman island, Peninsular Malaysia, in 2011.* **J Clin Microbiol** 51: 2 725-6

Tappe D, Schulze MH, Oesterlein A, Abele-Horn M, Baron S, Durcholz D, Langen HJ, Jany B, **Schoen C** (2012) *Molecular detection of Campylobacter jejuni as a cause of culture-negative spondylodiscitis.* **J Clin Microbiol** 50: 4 1499-500

Schork S, Schluter A, Blom J, Schneiker-Bekel S, Puhler A, Goesmann A, Frosch M, **Schoen C** (2012) *Genome sequence of a Neisseria meningitidis capsule null locus strain from the clonal complex of sequence type 198.* **J Bacteriol** 194: 18 5144-5

Pawlik MC, Hubert K, Joseph B, Claus H, **Schoen C**, Vogel U (2012) *The zinc-responsive regulon of Neisseria meningitidis comprises 17 genes under control of a Zur element.* **J Bacteriol** 194: 23 6594-603

Joseph B, Frosch M, **Schoen C,** Schubert-Unkmeir A (2012) *Transcriptome analyses in the interaction of Neisseria meningitidis with mammalian host cells.* **Methods Mol Biol** 799: 267-93

3.2.4 ANDREA SCHUBERT-UNKMEIR

Slanina H, Mundlein S, Hebling S, **Schubert-Unkmeir A** (2014) *Role of epidermal growth factor receptor signaling in the interaction of Neisseria meningitidis with endothelial cells.* **Infect Immun** 82: 3 1243-55

Schubert-Unkmeir A, Christodoulides M (2013) *Genome-based bacterial vaccines: current state and future outlook.* **BioDrugs** 27: 5 419-30

Slanina H, Schmutzler M, Christodoulides M, Kim KS, **Schubert-Unkmeir A** (2012) *Effective plasmid DNA and small interfering RNA delivery to diseased human brain microvascular endothelial cells.* **J Mol Microbiol Biotechnol** 22: 4 245-57

Slanina H, Hebling S, Hauck CR, **Schubert-Unkmeir A** (2012) *Cell invasion by Neisseria meningitidis requires a functional interplay between the focal adhesion kinase, Src and cortactin.* **PLoS One** 7: 6 e39613

Muller I, Freitag MH, Poggensee G, Scharnetzky E, Straube E, Schoerner C, Hlobil H, Hagedorn HJ, Stanek G, **Schubert-Unkmeir A,** Norris DE, Gensichen J, Hunfeld KP (2012) *Evaluating frequency, diagnostic quality, and cost of Lyme borreliosis testing in Germany: a retrospective model analysis.* **Clin Dev Immunol** 2012: 595427

Joseph B, Frosch M, Schoen C, **Schubert-Unkmeir A** (2012) *Transcriptome analyses in the interaction of Neisseria meningitidis with mammalian host cells.* **Methods Mol Biol** 799: 267-93

3.2.5 ULRICH VOGEL

Fiebig T, Berti F, Freiberger F, Pinto V, Claus H, Romano MR, Proietti D, Brogioni B, Stummeyer K, Berger M, **Vogel U**, Costantino P, Gerardy-Schahn R (2014) *Functional expression of the capsule polymerase of Neisseria meningitidis serogroup X: a new perspective for vaccine development.* **Glycobiology** 24: 2 150-8

Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, Caugant DA, Kriz P, Abad R, Bambini S, Carannante A, Deghmane AE, Fazio C, Frosch M, Frosi G, Gilchrist S, Giuliani MM, Hong E, Ledroit M, Lovaglio PG, Lucidarme J, Musilek M, Muzzi A, Oksnes J, Rigat F, Orlandi L, Stella M, Thompson D, Pizza M, Rappuoli R, Serruto D, Comanducci M, Boccadifuoco G, Donnelly JJ, Medini D, Borrow R (2013) *Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment.* **Lancet Infect Dis** 13: 5 416-25

Romanow A, Haselhorst T, Stummeyer K, Claus H, Bethe A, Muhlenhoff M, **Vogel U**, von Itzstein M, Gerardy-Schahn R (2013) *Biochemical and biophysical characterization of the sialyl-/hexosyltransferase synthesizing the meningococcal serogroup W135 heteropolysaccharide capsule.* **J Biol Chem** 288: 17 11718-30

Marcus U, **Vogel U**, Schubert A, Claus H, Baetzing-Feigenbaum J, Hellenbrand W, Wichmann O (2013) *A cluster of invasive meningococcal disease in young men who have sex with men in Berlin, October 2012 to May 2013.* **Euro Surveill** 18: 28

Lappann M, Otto A, Becher D, **Vogel U** (2013) *Comparative proteome analysis of spontaneous outer membrane vesicles and purified outer membranes of Neisseria meningitidis.* **J Bacteriol** 195: 19 4425-35

Lappann M, Danhof S, Guenther F, Olivares-Florez S, Mordhorst IL, **Vogel U** (2013) *In vitro resistance mechanisms of Neisseria meningitidis against neutrophil extracellular traps.* **Mol Microbiol** 89: 3 433-49

Hubert K, Devos N, Mordhorst I, Tans C, Baudoux G, Feron C, Goraj K, Tommassen J, **Vogel U**, Poolman JT, Weynants V (2013) *ZnuD, a potential candidate for a simple and universal Neisseria meningitidis vaccine.* **Infect Immun** 81: 6 1915-27

Hoiseth SK, Murphy E, Andrew L, **Vogel U**, Frosch M, Hellenbrand W, Abad R, Vazquez JA, Borrow R, Findlow J, Taha MK, Deghmane AE, Caugant DA, Kriz P, Musilek M, Mayer LW, Wang X, Macneil JR, York L, Tan CY, Jansen KU, Anderson AS (2013) *A multi-country evaluation of Neisseria meningitidis serogroup B factor H-binding proteins and implications for vaccine coverage in different age groups.* **Pediatr Infect Dis J** 32: 10 1096-101

Hellenbrand W, Elias J, Wichmann O, Dehnert M, Frosch M, **Vogel U** (2013) *Epidemiology of invasive meningococcal disease in Germany, 2002-2010, and impact of vaccination with meningococcal C conjugate vaccine.* **J Infect** 66: 1 48-56

Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, Corton C, Care R, Poolman JT, Zollinger WD, Frasch CE, Stephens DS, Feavers I, Frosch M, Parkhill J, **Vogel U**, Quail MA, Bentley SD, Maiden MC (2013) *Description and nomenclature of Neisseria meningitidis capsule locus.* **Emerg Infect Dis** 19: 4 566-73

Tuberculosis and after Recent Mycobacterium tuberculosis Infection Suppresses T-Cell Function. **Am J Respir Crit Care Med** 188: 724

Lutz MB (2013) *How quantitative differences in dendritic cell maturation can direct T1/T2-cell polarization.* **Oncoimmunology** 2: 2 e22796

Heuer M, Behlich AS, Lee JS, Ribechini E, Jo EK, **Lutz MB** (2013) *The 30-kDa and 38-kDa antigens from Mycobacterium tuberculosis induce partial maturation of human dendritic cells shifting CD4(+) T cell responses towards IL-4 production.* **BMC Immunol** 14: 48

Nono JK, Pletinckx K, **Lutz MB**, Brehm K (2012) *Excretory/secretory-products of Echinococcus multilocularis larvae induce apoptosis and tolerogenic properties in dendritic cells in vitro.* **PLoS Negl Trop Dis** 6: 2 e1516

Langenhorst D, Gogishvili T, Ribechini E, Kneitz S, McPherson K, Lutz MB, Hunig T (2012) *Sequential induction of effector function, tissue migration and cell death during polyclonal activation of mouse regulatory T-cells.* **PLoS One** 7: 11 e50080

3.4.1 AXEL RETHWILM

Rethwilm A, Bodem J(2013) *Evolution of Foamy viruses: The most ancient of all retroviruses.* **Viruses** 5:2349-2374

Rethwilm A, Lindemann, D (2013) *Foamy Viruses.* In **Fields Virology** Edited by Knipe DM, Howley PM; vol 6th ed.]

Weber C, Armbruster N, Scheller C, Kreppel F, Kochanek S, **Rethwilm A**, Steinert AF (2013) *Foamy virus-adenovirus hybrid vectors for gene therapy of the arthritides.* **J Gene Med** 15:155-167

Plochmann K, Horn A, Gschmack E, Armbruster N, Krieg J, Wiktorowicz T, Weber C, Stirnnagel K, Lindemann D, **Rethwilm A**, et al. (2012) *Heparan sulfate is an attachment factor for foamy virus entry.* **J Virol** 86:10028-10035

Spannaus R, Hartl MJ, Wöhrl BM, **Rethwilm A,** Bodem J (2012) The prototype foamy virus protease is active independently of the integrase domain. **Retrovirology** 2012, 9:41

Jacobs GB, Bock S, Schuch A, Moschall R, Schrom EM, Zahn J, Reuter C, Preiser W, **Rethwilm A**, Engelbrecht S, et al (2012) *Construction of a high titer infectious HIV-1 subtype C proviral clone from South Africa.* **Viruses** 4:1830-1843

Kasang C, Ulmer A, Donhauser N, Schmidt B, Stich A, Klinker H, Kalluvya S, Koutsilieri E, **Rethwilm A**, Scheller C (2012) *HIV patients treated with low-dose prednisolone exhibit lower immune activation than untreated patients.* **BMC Infect Dis** 12:14

3.4.2 JÜRGEN SCHNEIDER-SCHAULIES

Avila M, Alves L, Khosravi M, Ader-Ebert N, Origgi F, **Schneider-Schaulies J**, Zurbriggen A, Plemper RK, Plattet P (2014) *Molecular determinants defining the triggering range of prefusion F complexes of canine distemper virus.* **J Virol** 88: 5 2951-66

Schneider-Schaulies J, Schneider-Schaulies S (2013) *Viral infections and sphingolipids.* **Handb Exp Pharmacol** 216 321-40

Rasbach A, Abel T, Munch RC, Boller K, **Schneider-Schaulies J**, Buchholz CJ (2013) *The receptor attachment function of measles virus hemagglutinin can be replaced with an autonomous protein that binds Her2/neu while maintaining its fusion-helper function.* **J Virol** 87: 11 6246-56

Bieringer M, Han JW, Kendl S, Khosravi M, Plattet P, **Schneider-Schaulies J** (2013) *Experimental adaptation of wild-type canine distemper virus (CDV) to the human entry receptor CD150.* **PLoS One** 8: 3 e57488

Ader N, Brindley M, Avila M, Orvell C, Horvat B, Hiltensperger G, **Schneider-Schaulies J**, Vandevelde M, Zurbriggen A, Plemper RK, Plattet P (2013) *Mechanism for active membrane fusion triggering by morbillivirus attachment protein.* **J Virol** 87: 1 314-26

Reuter D, Sparwasser T, Hunig T, **Schneider-Schaulies J** (2012) *Foxp3+ regulatory T cells control persistence of viral CNS infection.* **PLoS One** 7: 3 e33989

Kneissl S, Abel T, Rasbach A, Brynza J, **Schneider-Schaulies J**, Buchholz CJ (2012) *Measles virus glycoprotein-based lentiviral targeting vectors that avoid neutralizing antibodies.* **PLoS One** 7: 10 e46667

Fehrholz M, Kendl S, Prifert C, Weissbrich B, Lemon K, Rennick L, Duprex PW, Rima BK, Koning FA, Holmes RK, Malim MH, **Schneider-Schaulies J** (2012) *The innate antiviral factor APOBEC3G targets replication of measles, mumps and respiratory syncytial viruses.* **J Gen Virol** 93: Pt 3 565-76

3.4.3 SYBILLE SCHNEIDER-SCHAULIES

Schneider-Schaulies J, **Schneider-Schaulies S** (2013) *Viral infections and sphingolipids.* **Handb Exp Pharmacol** 216 321-40

Riedel A, Mofolo B, Avota E, **Schneider-Schaulies S**, Meintjes A, Mulder N, Kneitz S (2013) *Accumulation of splice variants and transcripts in response to PI3K inhibition in T cells.* **PLoS One** 8: 2 e50695

Avota E, Koethe S, **Schneider-Schaulies S** (2013) *Membrane dynamics and interactions in measles virus dendritic cell infections.* **Cell Microbiol** 15: 2 161-9

Koethe S, Avota E, **Schneider-Schaulies S** (2012) *Measles virus transmission from dendritic cells to T cells: formation of synapse-like interfaces concentrating viral and cellular components.* **J Virol** 86: 18 9773-81

3.5.1 THOMAS RUDEL

Sibilski C, Mueller T, Kollipara L, Zahedi RP, Rapp UR, **Rudel T**, Balju-ls A (2013) *Tyr728 in the kinase domain of the murine kinase suppressor of RAS 1 regulates binding and activation of the mitogen-activated protein kinase kinase.* **J Biol Chem** 288: 49 35237-52

Zeth K, Kozjak-Pavlovic V, Faulstich M, Fraunholz M, Hurwitz R, Kepp O, **Rudel T** (2013) *Structure and function of the PorB porin from disseminating Neisseria gonorrhoeae.* **Biochem J** 449: 3 631-42

Prusty BK, Siegl C, Hauck P, Hain J, Korhonen SJ, Hiltunen-Back E, Puolakkainen M, **Rudel T** (2013) *Chlamydia trachomatis infection induces replication of latent HHV-6.* **PLoS One** 8: 4 e61400

Elias J, Heuschmann PU, Schmitt C, Eckhardt F, Boehm H, Maier S, Kolb-Maurer A, Riedmiller H, Mullges W, Weisser C, Wunder C, Frosch M, **Vogel U** (2013) *Prevalence dependent calibration of a predictive model for nasal carriage of methicillin-resistant Staphylococcus aureus.* **BMC Infect Dis** 13: 111

Elias J, Findlow J, Borrow R, Tremmel A, Frosch M, **Vogel U** (2013) *Persistence of antibodies in laboratory staff immunized with quadrivalent meningococcal polysaccharide vaccine.* **J Occup Med Toxicol** 8: 1 4

Zlamy M, Hofer J, Elias J, **Vogel U**, Frosch M, Jungraithmayr T, Zimmerhackl LB, Prelog M (2012) *Immunogenicity of meningococcus C vaccination in a patient with atypical hemolytic uremic syndrome (aHUS) on eculizumab therapy.* **Pediatr Transplant** 16: 6 E246-50

Vogel U, Szczepanowski R, Claus H, Junemann S, Prior K, Harmsen D (2012) *Ion torrent personal genome machine sequencing for genomic typing of Neisseria meningitidis for rapid determination of multiple layers of typing information.* **J Clin Microbiol** 50: 6 1889-94

Vogel U, Stefanelli P, Vazquez J, Taha MK, Claus H, Donnelly J (2012) *The use of vaccine antigen characterization, for example by MATS, to guide the introduction of meningococcus B vaccines.* **Vaccine** 30 Suppl 2: B73-7

Schwerk C, Papandreou T, Schuhmann D, Nickol L, Borkowski J, Steinmann U, Quednau N, Stump C, Weiss C, Berger J, Wolburg H, Claus H, **Vogel U**, Ishikawa H, Tenenbaum T, Schroten H (2012) *Polar invasion and translocation of Neisseria meningitidis and Streptococcus suis in a novel human model of the blood-cerebrospinal fluid barrier.* **PLoS One** 7: 1 e30069

Plikaytis BD, Stella M, Boccadifuoco G, DeTora LM, Agnusdei M, Santini L, Brunelli B, Orlandi L, Simmini I, Giuliani M, Ledroit M, Hong E, Taha MK, Ellie K, Rajam G, Carlone GM, Claus H, **Vogel U**, Borrow R, Findlow J, Gilchrist S, Stefanelli P, Fazio C, Carannante A, Oksnes J, Fritzsonn E, Klem AM, Caugant DA, Abad R, Vazquez JA, Rappuoli R, Pizza M, Donnelly JJ, Medini D (2012) *Interlaboratory standardization of the sandwich enzyme-linked immunosorbent assay designed for MATS, a rapid, reproducible method for estimating the strain coverage of investigational vaccines.* **Clin Vaccine Immunol** 19: 10 1609-17

Pawlik MC, Hubert K, Joseph B, Claus H, Schoen C, **Vogel U** (2012) *The zinc-responsive regulon of Neisseria meningitidis comprises 17 genes under control of a Zur element.* **J Bacteriol** 194: 23 6594-603

Moremi N, Mshana SE, Kamugisha E, Kataraihya J, Tappe D, **Vogel U**, Lyamuya EF, Claus H (2012) *Predominance of methicillin resistant Staphylococcus aureus -ST88 and new ST1797 causing wound infection and abscesses.* **J Infect Dev Ctries** 6: 8 620-5

Lam TT, Claus H, Elias J, Hellenbrand W, Imohl M, Prelog M, Sing A, van der Linden M, **Vogel U** (2012) *[Infections with pneumococci, menigococci, H. influenzae and diphtheria in Germany: the RKI Reference Network for Invasive Bacterial Infections (IBI) at the 5th Wurzburg Workshop on Epidemiology, Prevention and Therapy for Invasive Meningococcal Diseases 2010 (meeting report)].* **Gesundheitswesen** 74: 11 747-53

Hubert K, Pawlik MC, Claus H, Jarva H, Meri S, **Vogel U** (2012) *Opc expression, LPS immunotype switch and pilin conversion contribute to serum resistance of unencapsulated meningococci.* **PLoS One** 7: 9 e45132

Claus H, Jordens MS, Kriz P, Musilek M, Jarva H, Pawlik MC, Meri S, **Vogel U** (2012) *Capsule null locus meningococci: typing of antigens used in an investigational multicomponent meningococcus serogroup B vaccine.* **Vaccine** 30: 2 155-60

3.3.1 THOMAS HÜNIG

Kuhlhorn F, Rath M, Schmoeckel K, Cziupka K, Nguyen HH, Hildebrandt P, **Hunig T**, Sparwasser T, Huehn J, Potschke C, Broker BM (2013) *Foxp3+ regulatory T cells are required for recovery from severe sepsis.* **PLoS One** 8: 5 e65109

Reuter D, Sparwasser T, **Hunig T**, Schneider-Schaulies J (2012) *Foxp3+ regulatory T cells control persistence of viral CNS infection.* **PLoS One** 7: 3 e33989

Na SY, Hermann A, Sanchez-Ruiz M, Storch A, Deckert M, **Hunig T** (2012) *Oligodendrocytes enforce immune tolerance of the uninfected brain by purging the peripheral repertoire of autoreactive CD8+ T cells.* **Immunity** 37: 1 134-46

Hunig T (2012) *The storm has cleared: lessons from the CD28 superagonist TGN1412 trial.* **Nat Rev Immunol** 12: 5 317-8

3.3.2 NIKLAS BEYERSDORF

Camara M, **Beyersdorf N**, Fischer HJ, Herold MJ, Ip CW, van den Brandt J, Toyka KV, Taurog JD, Hunig T, Herrmann T, Reichardt HM, Weishaupt A, Kerkau T (2013) *CD8(+) T cell help is required for efficient induction of EAE in Lewis rats.* **J Neuroimmunol** 260: 1-2 17-27

Hofmann U, **Beyersdorf N**, Weirather J, Podolskaya A, Bauersachs J, Ertl G, Kerkau T, Frantz S (2012) *Activation of CD4+ T lymphocytes improves wound healing and survival after experimental myocardial infarction in mice.* **Circulation** 125: 13 1652-63

Kerstan A, **Beyersdorf N**, Stoevesandt J, Trautmann A (2012) *Wasp venom immunotherapy expands a subpopulation of CD4(+)CD25+ forkhead box protein 3-positive regulatory T cells expressing the T-cell receptor Vbeta2 and Vbeta5.1 chains.* **J Allergy Clin Immunol** 130: 4 994-6 e3

3.3.3 THOMAS HERRMANN

Looringh van Beeck FA, Leegwater PA, **Herrmann T,** Broere F, Rutten VP, Willemse T, Van Rhijn I (2013) *Tandem repeats modify the structure of the canine CD1D gene.* **Anim Genet** 44: 3 352-5

Monzon-Casanova E, Paletta D, Starick L, Muller I, Sant'Angelo DB, Pyz E, **Herrmann T** (2013) *Direct identification of rat iNKT cells reveals remarkable similarities to human iNKT cells and a profound deficiency in LEW rats.* **Eur J Immunol** 43: 2 404-15

3.3.4 MANFRED LUTZ

du Plessis N, Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, van Helden PD, **Lutz MB**, and Walzl G(2013) *Increased Frequency of Myeloid-derived Suppressor Cells during Active*

Springer J, Morton CO, Perry M, Heinz WJ, Paholcsek M, Alzheimer M, Rogers TR, Barnes RA, **Einsele H**, Loeffler J, White PL (2013) *Multicenter comparison of serum and whole-blood specimens for detection of Aspergillus DNA in high-risk hematological patients.* **J Clin Microbiol** 51: 5 1445-50

Rasche L, Duell J, Morgner C, Chatterjee M, Hensel F, Rosenwald A, **Einsele H**, Topp MS, Brandlein S (2013) *The natural human IgM antibody PAT-SM6 induces apoptosis in primary human multiple myeloma cells by targeting heat shock protein GRP78.* **PLoS One** 8: 5 e63414

Nerreter T, Distler E, Kochel C, **Einsele H**, Herr W, Seggewiss-Bernhardt R (2013) *Combining dasatinib with dexamethasone long-term leads to maintenance of antiviral and antileukemia specific cytotoxic T cell responses in vitro.* **Exp Hematol** 41: 7 604-614 e4

Mueller-Loebnitz C, Ostermann H, Franzke A, Loeffler J, Uharek L, Topp M, **Einsele H** (2013) *Immunological aspects of Candida and Aspergillus systemic fungal infections.* **Interdiscip Perspect Infect Dis** 2013: 102934

Hirsch HH, Martino R, Ward KN, Boeckh M, **Einsele H**, Ljungman P (2013) *Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus.* **Clin Infect Dis** 56: 2 258-66

Hicheri Y, **Einsele H**, Martino R, Cesaro S, Ljungman P, Cordonnier C (2013) *Environmental prevention of infection in stem cell transplant recipients: a survey of the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation.* **Transpl Infect Dis** 15: 3 251-8

Grube M, Loeffler J, Mezger M, Kruger B, Echtenacher B, Hoffmann P, Edinger M, **Einsele H**, Andreesen R, Holler E (2013) *TLR5 stop codon polymorphism is associated with invasive aspergillosis after allogeneic stem cell transplantation.* **Med Mycol** 51: 8 818-25

Furst D, Muller C, Vucinic V, Bunjes D, Herr W, Gramatzki M, Schwerdtfeger R, Arnold R, **Einsele H**, Wulf G, Pfreundschuh M, Glass B, Schrezenmeier H, Schwarz K, Mytilineos J (2013) *High-resolution HLA matching in hematopoietic stem cell transplantation: a retrospective collaborative analysis.* **Blood** 122: 18 3220-9

Fuji S, Kapp M, **Einsele H** (2013) *Monitoring of Pathogen-Specific T-Cell Immune Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation.* **Front Immunol** 4: 276

Fuji S, Kapp M, **Einsele H** (2013) *Alloreactivity of Virus-Specific T Cells: Possible Implication of Graft-Versus-Host Disease and Graft-Versus-Leukemia Effects.* **Front Immunol** 4: 330

Doppler K, Knop S, **Einsele H**, Sommer C, Wessig C (2013) *Sporadic late onset nemaline myopathy and immunoglobulin deposition disease.* **Muscle Nerve** 48: 6 983-8

Bauerlein CA, Riedel SS, Baker J, Brede C, Garrote AL, Chopra M, Ritz M, Beilhack GF, Schulz S, Zeiser R, Schlegel PG, **Einsele H**, Negrin RS, Beilhack A (2013) *A diagnostic window for the treatment of acute graft-versus-host disease prior to visible clinical symptoms in a murine model.* **BMC Med** 11: 134

Zirkel J, Klinker H, Kuhn A, Abele-Horn M, Tappe D, Turnwald D, **Einsele H**, Heinz WJ (2012) *Epidemiology of Candida blood stream infections in patients with hematological malignancies or solid tumors.* **Med Mycol** 50: 1 50-5

Topp MS, Gokbuget N, Zugmaier G, Degenhard E, Goebeler ME, Klinger M, Neumann SA, Horst HA, Raff T, Viardot A, Stelljes M, Schaich M, Kohne-Volland R, Bruggemann M, Ottmann OG, Burmeister T, Baeuerle PA, Nagorsen D, Schmidt M, **Einsele H**, Riethmuller G, Kneba M, Hoelzer D, Kufer P, Bargou RC (2012) *Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL.* **Blood** 120: 26 5185-7

Springer J, Schlossnagel H, Heinz W, Doedt T, Soeller R, **Einsele H**, Loeffler J (2012) *A novel extraction method combining plasma with a whole-blood fraction shows excellent sensitivity and reproducibility for patients at high risk for invasive aspergillosis.* **J Clin Microbiol** 50: 8 2585-91

Springer J, **Einsele H**, Loeffler J (2012) *Molecular techniques in the diagnosis of deep and systemic mycosis.* **Clin Dermatol** 30: 6 651-6

Ruhnke M, Bohme A, Buchheidt D, Cornely O, Donhuijsen K, **Einsele H**, Enzensberger R, Hebart H, Heussel CP, Horger M, Hof H, Karthaus M, Kruger W, Maschmeyer G, Penack O, Ritter J, Schwartz S (2012) *Diagnosis of invasive fungal infections in hematology and oncology--guidelines from the Infectious Diseases Working Party in Haematology and Oncology of the German Society for Haematology and Oncology (AGIHO).* **Ann Oncol** 23: 4 823-33

Rasche L, Bernard C, Topp MS, Kapp M, Duell J, Wesemeier C, Haralambieva E, Maeder U, **Einsele H**, Knop S (2012) *Features of extramedullary myeloma relapse: high proliferation, minimal marrow involvement, adverse cytogenetics: a retrospective single-center study of 24 cases.* **Ann Hematol** 91: 7 1031-7

Ljungman P, Locasciulli A, de Soria VG, Bekassy AN, Brinch L, Espigado I, Ferrant A, Franklin IM, O'Riordan J, Rovira M, Shaw P, **Einsele H** (2012) *Long-term follow-up of HCV-infected hematopoietic SCT patients and effects of antiviral therapy.* **Bone Marrow Transplant** 47: 9 1217-21

Heinz WJ, Grau A, Ulrich A, Helle-Beyersdorf A, Zirkel J, Schirmer D, Lenker U, **Einsele H**, Klinker H (2012) *Impact of benzodiazepines on posaconazole serum concentrations. A population-based pharmacokinetic study on drug interaction.* **Curr Med Res Opin** 28: 4 551-7

Donat S, Hasenberg M, Schafer T, Ohlsen K, Gunzer M, **Einsele H**, Loffler J, Beilhack A, Krappmann S (2012) *Surface display of Gaussia princeps luciferase allows sensitive fungal pathogen detection during cutaneous aspergillosis.* **Virulence** 3: 1 51-61

Brede C, Friedrich M, Jordan-Garrote AL, Riedel SS, Bauerlein CA, Heinze KG, Bopp T, Schulz S, Mottok A, Kiesel C, Mattenheimer K, Ritz M, von Krosigk V, Rosenwald A, **Einsele H**, Negrin RS, Harms GS, Beilhack A (2012) *Mapping immune processes in intact tissues at cellular resolution.* **J Clin Invest** 122: 12 4439-46

Bouzani M, **Einsele H**, Loeffler J (2012) *Functional analysis is a paramount prerequisite for understanding the in vitro interaction of human natural killer cells with Aspergillus fumigatus.* **J Infect Dis** 205: 6 1025-6; author reply 1026-7

Prusty BK, Krohne G, **Rudel T** (2013) *Reactivation of chromosomally integrated human herpesvirus-6 by telomeric circle formation.* **PLoS Genet** 9: 12 e1004033

Ott C, Utech M, Goetz M, **Rudel T**, Kozjak-Pavlovic V (2013) *Requirements for the import of neisserial Omp85 into the outer membrane of human mitochondria.* **Biosci Rep** 33: 2 e00028

Mehlitz A, **Rudel T** (2013) *Modulation of host signaling and cellular responses by Chlamydia.* **Cell Commun Signal** 11: 90

Faulstich M, Bottcher JP, Meyer TF, Fraunholz M, **Rudel T** (2013) *Pilus phase variation switches gonococcal adherence to invasion by caveolin-1-dependent host cell signaling.* **PLoS Pathog** 9: 5 e1003373

Eisenreich W, Heesemann J, **Rudel T**, Goebel W (2013) *Metabolic host responses to infection by intracellular bacterial pathogens.* **Front Cell Infect Microbiol** 3: 24

Castro IC, Breiling A, Luetkenhaus K, Ceteci F, Hausmann S, Kress S, Lyko F, **Rudel T**, Rapp UR (2013) *MYC-induced epigenetic activation of GATA4 in lung adenocarcinoma.* **Mol Cancer Res** 11: 2 161-72

Rudel T (2012) *To die or not to die: Shigella has an answer.* **Cell Host Microbe** 11: 3 219-21

Prusty BK, Bohme L, Bergmann B, Siegl C, Krause E, Mehlitz A, **Rudel T** (2012) *Imbalanced oxidative stress causes chlamydial persistence during non-productive human herpes virus co-infection.* **PLoS One** 7: 10 e47427

Ott C, Ross K, Straub S, Thiede B, Gotz M, Goosmann C, Krischke M, Mueller MJ, Krohne G, **Rudel T**, Kozjak-Pavlovic V (2012) *Sam50 functions in mitochondrial intermembrane space bridging and biogenesis of respiratory complexes.* **Mol Cell Biol** 32: 6 1173-88

Baljuls A, Beck M, Oenel A, Robubi A, Kroschewski R, Hekman M, **Rudel T**, Rapp UR (2012) *The tumor suppressor DiRas3 forms a complex with H-Ras and C-RAF proteins and regulates localization, dimerization, and kinase activity of C-RAF.* **J Biol Chem** 287: 27 23128-40

Rajalingam K, **Rudel T** (2012) *"Prohibitin"g CRAF/MAPK activation with rocaglamides.* **Chem Biol** 19: 9 1077-78

3.5.2 MARTIN FRAUNHOLZ

Grosz M, Kolter J, Paprotka K, Winkler AC, Schafer D, Chatterjee SS, Geiger T, Wolz C, Ohlsen K, Otto M, Rudel T, Sinha B, **Fraunholz M** (2014) *Cytoplasmic replication of Staphylococcus aureus upon phagosomal escape triggered by phenol-soluble modulin alpha.* **Cell Microbiol** 16: 4 451-65

Fraunholz M, Bernhardt J, Schuldes J, Daniel R, Hecker M, Sinha B (2013) *Complete Genome Sequence of Staphylococcus aureus 6850, a Highly Cytotoxic and Clinically Virulent Methicillin-Sensitive Strain with Distant Relatedness to Prototype Strains.* **Genome Announc** 1: 5

Geiger T, Francois P, Liebeke M, **Fraunholz M**, Goerke C, Krismer B, Schrenzel J, Lalk M, Wolz C (2012) *The stringent response of Staphylococcus aureus and its impact on survival after phagocytosis through the induction of intracellular PSMs expression.* **PLoS Pathog** 8: 11 e1003016

Fraunholz M, Sinha B (2012) *Intracellular Staphylococcus aureus: live-in and let die.* **Front Cell Infect Microbiol** 2: 43

3.5.3 ROY GROSS

Ratzka C, **Gross R**, Feldhaar H (2013) *Gene expression analysis of the endosymbiont-bearing midgut tissue during ontogeny of the carpenter ant Camponotus floridanus.* **J Insect Physiol** 59: 6 611-623

Ratzka C, **Gross R**, Feldhaar H (2013) *Systemic gene knockdown in Camponotus floridanus workers by feeding of dsRNA* **Insectes Soc** 60: 475-484

Yevsa T, Ebensen T, Zygmunt B, Libanova R, Fuchs B, **Gross R**, Schulze K, Guzman CA (2013) *Development and characterization of attenuated metabolic mutants of Bordetella bronchiseptica for applications in vaccinology* **Environ Microbiol** 15: 64-76

Ratzka C, **Gross R,** Feldhaar H (2012) *Endosymbiont tolerance and control within insect hosts* **Insects** 3: 553-572

Gross R, Beier D (Editors) (2012) *Two-component systems in bacteria.* Caister Academic Press, United Kingdom. ISBN: 978-1-908230-08-9

Krueger B, Friedrich T, Forster F, Bernhardt J, **Gross R**, Dandekar T (2012) *Different evolutionary modifications as a guide to rewire two-component systems.* **Bioinform Biol Insights** 6: 97-128

Ratzka C, Forster F, Liang C, Kupper M, Dandekar T, Feldhaar H, **Gross R** (2012) *Molecular characterization of antimicrobial peptide genes of the carpenter ant Camponotus floridanus.* **PLoS One** 7: 8 e43036

3.5.4 VERA KOZJAK-PAVLOVIC

Zeth K, **Kozjak-Pavlovic V**, Faulstich M, Fraunholz M, Hurwitz R, Kepp O, Rudel T (2013) *Structure and function of the PorB porin from disseminating Neisseria gonorrhoeae.* **Biochem J** 449: 3 631-42

Ott C., Utech M., Goetz M., Rudel T., **Kozjak-Pavlovic V** (2013) *The requirements for the import of neisserial Omp85 into the outer membrane of human mitochondria* **Biosci Rep** 33:e00028.

3.6.1 HERMAN EINSELE

Tan SM, Kapp M, Flechsig C, Kapp K, Rachor JE, Eyrich M, Loeffler J, **Einsele H**, Grigoleit GU (2013) *Stimulating surface molecules, Th1-polarizing cytokines, proven trafficking--a new protocol for the generation of clinical-grade dendritic cells.* **Cytotherapy** 15: 4 492-506

Styczynski J, Gil L, Tridello G, Ljungman P, Donnelly JP, van der Velden W, Omar H, Martino R, Halkes C, Faraci M, Theunissen K, Kalwak K, Hubacek P, Sica S, Nozzoli C, Fagioli F, Matthes S, Diaz MA, Migliavacca M, Balduzzi A, Tomaszewska A, Camara Rde L, van Biezen A, Hoek J, Iacobelli S, **Einsele H**, Cesaro S (2013) *Response to rituximab-based therapy and risk factor analysis in Epstein Barr Virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation.* **Clin Infect Dis** 57: 6 794-802

3.6.4 JÜRGEN LÖFFLER

Voigt J, Hunniger K, Bouzani M, Jacobsen ID, Barz D, Hube B, **Loeffler J**, Kurzai O (2014) *Human natural killer cells acting as phagocytes against Candida albicans and mounting an inflammatory response that modulates neutrophil antifungal activity.* **J Infect Dis** 209: 4 616-26

Bouzani M, Einsele H, **Loeffler J** (2013) *Functional analysis is a paramount prerequisite for understanding the in vitro interaction of human Natural Killer cells with Aspergillus fumigatus.* **J Infect Dis** 205:1025-1026

Springer J, Morton O, Perry M, Heinz W, Paholcsek M, Alzheimer M, Rogers T, Barnes R, Einsele H, **Loeffler J,** White L (2013) *Comparison of serum and whole blood specimens for the detection of Aspergillus DNA in high-risk haematological patients: A multicenter evaluation.* **J Clin Microbiol** 51:1445-1450

Rogers T, Morton O, Springer J, Conneally E, Heinz W, Kenny C, Frost S, Einsele H, **Loeffler J** (2013) *Combined real-time PCR and galactomannan surveillance improves diagnosis of invasive aspergillosis in high risk patients with haematological malignancies.* **Brit J Haematol** 161:517-524

Cunha C, Aversa F, Lacerda J, Busca A, Kurzai O, Grube M, **Löffler J**, Maertens J, Bell A, Inforzato A, Barbati E, Almeida B, Santos e Sousa P, Barbui A, Potenza L, Caira M, Rodrigues F, Salvatori G, Pagano L, Luppi M, Mantovani A, Velardi A, Romani L, Carvalho A (2014) *Genetic PTX3 deficiency and aspergillosis in stem cell transplantation*. **N Engl J Med** 370: 421-432

3.6.5 ANDREW ULLMANN

Leroux S, **Ullmann AJ** (2013) *Management and diagnostic guidelines for fungal diseases in infectious diseases and clinical microbiology: critical appraisal.* **Clin Microbiol Infect** 19: 1115-1121

Pagano L, Cornely OA, Busca A, Caira M, Cesaro S, Gasbarrino C, Girmenia C, Heinz WJ, Herbrecht R, Lass-Florl C, Nosari A, Potenza L, Racil Z, Rickerts V, Sheppard DC, Simon A, **Ullmann AJ**, Valentini CG, Vehreschild JJ, Candoni A, Vehreschild MJ (2013) *Combined antifungal approach for the treatment of invasive mucormycosis in patients with hematologic diseases: a report from the SEIFEM and FUNGISCOPE registries.* **Haematologica** 98: e127-130

Ullmann AJ, Dolan MC, Sackal CA, Fikrig E, Piesman J, Zeidner NS (2013) *Immunization with adenoviral-vectored tick salivary gland proteins (SALPs) in a murine model of Lyme borreliosis.* **Ticks Tick Borne** Dis 4: 160-163

Ullmann AJ, Johnson A (2013) *A multidisciplinary approach to managing invasive fungal disease. Preface.* **J Antimicrob Chemother** 68 Suppl 3: i1

Weissinger F, Auner HW, Bertz H, Buchheidt D, Cornely OA, Egerer G, Heinz W, Karthaus M, Kiehl M, Kruger W, Penack O, Reuter S, Ruhnke M, Sandherr M, Salwender HJ, **Ullmann AJ,** Waldschmidt DT, Wolf HH (2012) *Antimicrobial therapy of febrile complications after high-dose chemotherapy and autologous hematopoietic stem cell transplantation--guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO).* **Ann Hematol** 91: 1161-1174

4.1 GERHARD BRINGMANN

Baunach M, Ding L, Bruhn T, **Bringmann G**, Hertweck C (2013) *Regiodivergent N-C and N-N aryl coupling reactions of indoloterpenes and cycloether formation mediated by a single bacterial flavoenzyme.* **Angew Chem Int Ed Engl** 52: 9040-9043

Bringmann G, Lombe BK, Steinert C, Ioset KN, Brun R, Turini F, Heubl G, Mudogo V (2013) *Mbandakamines A and B, unsymmetrically coupled dimeric naphthylisoquinoline alkaloids, from a Congolese Ancistrocladus species.* **Org Lett** 15: 2590-2593

Bringmann G, Thomale K, Bischof S, Schneider C, Schultheis M, Schwarz T, Moll H, Schurigt U (2013) *A novel Leishmania major amastigote assay in 96-well format for rapid drug screening and its use for discovery and evaluation of a new class of leishmanicidal quinolinium salts.* **Antimicrob Agents Chemother** 57: 3003-3011

Bringmann G, Zhang G, Buttner T, Bauckmann G, Kupfer T, Braunschweig H, Brun R, Mudogo V (2013) *Jozimine A2: the first dimeric Dioncophyllaceae-type naphthylisoquinoline alkaloid, with three chiral axes and high antiplasmodial activity.* **Chemistry** 19: 916-923

Bringmann G, Zhang G, Olschlager T, Stich A, Wu J, Chatterjee M, Brun R (2013) *Highly selective antiplasmodial naphthylisoquinoline alkaloids from Ancistrocladus tectorius.* **Phytochemistry** 91: 220-228

Jansen N, Ohlendorf B, Erhard A, Bruhn T, **Bringmann G**, Imhoff JF (2013) *Helicusin E, isochromophilone X and isochromophilone XI: new chloroazaphilones produced by the fungus Bartalinia robillardoides strain LF550.* **Mar Drugs** 11: 800-816

Jetter P, Steinert C, Knauer M, Zhang G, Bruhn T, Wiese J, Imhoff JF, Fiedler HP, **Bringmann G** (2013) *New bhimamycins from Streptomyces sp. AK 671.* **J Antibiot (**Tokyo) 66: 719-726

Li J, Li MY, Bruhn T, Katele FZ, Xiao Q, Pedpradab P, Wu J, **Bringmann G** (2013) *Thaixylomolins A-C: limonoids featuring two new motifs from the Thai Xylocarpus moluccensis.* **Org Lett** 15: 3682-3685

Lodige M, Lewis MD, Paulsen ES, Esch HL, Pradel G, Lehmann L, Brun R, **Bringmann G**, Mueller AK (2013) *A primaquine-chloroquine hybrid with dual activity against Plasmodium liver and blood stages.* **Int J Med Microbiol** 303: 539-547

Tatsis EC, Schaumloffel A, Warskulat AC, Massiot G, Schneider B, **Bringmann G** (2013) *Nudicaulins, yellow flower pigments of Papaver nudicaule: revised constitution and assignment of absolute configuration.* **Org Lett** 15: 156-159

Almeida C, Hemberger Y, Schmitt SM, Bouhired S, Natesan L, Kehraus S, Dimas K, Gutschow M, **Bringmann G**, Konig GM (2012) *Marilines A-C: novel phthalimidines from the sponge-derived fungus Stachylidium sp.* **Chemistry** 18: 8827-8834

Goel A, Kumar A, Hemberger Y, Raghuvanshi A, Jeet R, Tiwari G, Knauer M, Kureel J, Singh AK, Gautam A, Trivedi R, Singh D, **Bring-**

Bauer R, Mezger M, Blockhaus C, Schmitt AL, Kurzai O, **Einsele H,** Loeffler J (2012) *40-O -[2-Hydroxyethyl]rapamycin modulates human dendritic cell function during exposure to Aspergillus fumigatus.* **J Basic Microbiol** 52: 3 269-76

3.6.2 ANDREAS BEILHACK

Wolfl M, Schwinn S, Yoo YE, Ress ML, Braun M, Chopra M, Schreiber SC, Ayala VI, Ohlen C, Eyrich M, **Beilhack A**, Schlegel PG (2013) *Src-kinase inhibitors sensitize human cells of myeloid origin to Toll-like-receptor-induced interleukin 12 synthesis.* **Blood** 122: 7 1203-13

Schliesser U, Chopra M, **Beilhack A**, Appelt C, Vogel S, Schumann J, Panov I, Vogt K, Schlickeiser S, Olek S, Wood K, Brandt C, Volk HD, Sawitzki B (2013) *Generation of highly effective and stable murine alloreactive Treg cells by combined anti-CD4 mAb, TGF-beta, and RA treatment.* **Eur J Immunol** 43: 12 3291-305

Ophir E, Or-Geva N, Gurevich I, Tal O, Eidelstein Y, Shezen E, Margalit R, Lask A, Shakhar G, Hagin D, Bachar-Lustig E, Reich-Zeliger S, **Beilhack A**, Negrin R, Reisner Y (2013) *Murine anti-third-party central-memory CD8(+) T cells promote hematopoietic chimerism under mild conditioning: lymph-node sequestration and deletion of anti-donor T cells.* **Blood** 121: 7 1220-8

Bauerlein CA, Riedel SS, Baker J, Brede C, Garrote AL, Chopra M, Ritz M, Beilhack GF, Schulz S, Zeiser R, Schlegel PG, Einsele H, Negrin RS, **Beilhack A** (2013) *A diagnostic window for the treatment of acute graft-versus-host disease prior to visible clinical symptoms in a murine model.* **BMC Med** 11: 134

Donat S, Hasenberg M, Schafer T, Ohlsen K, Gunzer M, Einsele H, Loffler J, **Beilhack A**, Krappmann S (2012) *Surface display of Gaussia princeps luciferase allows sensitive fungal pathogen detection during cutaneous aspergillosis.* **Virulence** 3: 1 51-61

Brede C, Friedrich M, Jordan-Garrote AL, Riedel SS, Bauerlein CA, Heinze KG, Bopp T, Schulz S, Mottok A, Kiesel C, Mattenheimer K, Ritz M, von Krosigk V, Rosenwald A, Einsele H, Negrin RS, Harms GS, **Beilhack A** (2012) *Mapping immune processes in intact tissues at cellular resolution.* **J Clin Invest** 122: 12 4439-46

3.6.3 HARTWIG KLINKER

Bibra M, Rosenkranz B, Pretorius E, Rabie H, Edson C, Lenker U, Cotton M, **Klinker H** (2014) *Are lopinavir and efavirenz serum concentrations in HIV-infected children in the therapeutic range in clinical practice?* **Paediatr Int Child Health** 34: 2 138-41

Burger D, Back D, Buggisch P, Buti M, Craxi A, Foster G, **Klinker H**, Larrey D, Nikitin I, Pol S, Puoti M, Romero-Gomez M, Wedemeyer H, Zeuzem S (2013) *Clinical management of drug-drug interactions in HCV therapy: challenges and solutions.* **J Hepatol** 58: 4 792-800

Dawood FZ, Khan F, Roediger MP, Zhang ZM, Swaminathan S, **Klinker H**, Hoy J, Lundgren JD, Neaton JD, Soliman EZ (2013) *Electrocardiographic spatial QRS-T angle and incident cardiovascular disease in HIV-infected patients (from the Strategies for the Management of Antiretroviral Therapy [SMART] study).* **Am J Cardiol** 111: 1 118-24

Gunda DW, Kasang C, Kidenya BR, Kabangila R, Mshana SE, Kidola J, Kalluvya SE, Kongola GW, **Klinker H** (2013) *Plasma concentrations of efavirenz and nevirapine among HIV-infected patients with immunological failure attending a tertiary hospital in North-western Tanzania.* **PLoS One** 8: 9 e75118

Innes S, Schulte-Kemna E, Cotton MF, Zollner EW, Haubrich R, **Klinker H**, Sun X, Jain S, Edson C, van Niekerk M, Innes ER, Rabie H, Browne SH (2013) *Biceps skin-fold thickness may detect and predict early lipoatrophy in HIV-infected children.* **Pediatr Infect Dis** J 32: 6 e254-62

Kasang C, Ulmer A, Donhauser N, Schmidt B, Stich A, Klinker H, Kalluvya S, Koutsilieri E, Rethwilm A, Scheller C (2012) *HIV patients treated with low-dose prednisolone exhibit lower immune activation than untreated patients.* **BMC Infect Dis** 12: 14

Knop V, Teuber G, **Klinker H**, Moller B, Rasenack J, Hinrichsen H, Gerlach T, Spengler U, Buggisch P, Neumann K, Sarrazin C, Zeuzem S, Berg T (2013) *Prediction of minimal residual viremia in HCV type 1 infected patients receiving interferon-based therapy.* **Ann Hepatol** 12: 2 190-8

Mauss S, **Klinker H** (2013) *Drug-drug interactions in the treatment of HCV among people who inject drugs.* **Clin Infect Dis** 57 Suppl 2: S125-8

Sarrazin C, Berg T, Cornberg M, Dollinger M, Ferenci P, Hinrichsen H, **Klinker H,** Kraus M, Manns M, Mauss S, Peck-Radosavljevic M, Schmidt H, Spengler U, Wedemeyer H, Wirth S, Zeuzem S (2012) *Expert opinion on boceprevir- and telaprevir-based triple therapies of chronic hepatitis C.* **Z Gastroenterol** 50: 1 57-72

Zeuzem S, Buggisch P, Agarwal K, Marcellin P, Sereni D, **Klinker H**, Moreno C, Zarski JP, Horsmans Y, Mo H, Arterburn S, Knox S, Oldach D, McHutchison JG, Manns MP, Foster GR (2012) *The protease inhibitor, GS-9256, and non-nucleoside polymerase inhibitor tegobuvir alone, with ribavirin, or pegylated interferon plus ribavirin in hepatitis C.* **Hepatology** 55: 3 749-58

Zirkel J, **Klinker H,** Kuhn A, Abele-Horn M, Tappe D, Turnwald D, Einsele H, Heinz WJ (2012) *Epidemiology of Candida blood stream infections in patients with hematological malignancies or solid tumors.* **Med Mycol** 50: 1 50-5

Heinz WJ, Einsele H, Helle-Beyersdorf A, Zirkel J, Grau A, Schirmer D, Lenker U, **Klinker H** (2013) *Posaconazole concentrations after allogeneic hematopoietic stem cell transplantation.* **Transpl Infect Dis** 15: 449-56

Heinz WJ, Grau A, Ulrich A, Helle-Beyersdorf A, Zirkel J, Schirmer D, Lenker U, Einsele H, **Klinker H** (2012) *Impact of benzodiazepines on posaconazole serum concentrations. A population-based pharmacokinetic study on drug interaction.* **Curr Med Res Opin** 28:551-7

Helle-Beyersdorf A, Zirkel J, Heidemeier A, **Klinker H**, Heinz WJ (2012) *Tuberculosis as a rare cause for an abdominal mass: report on three cases and review of the literature*. **Z Gastroenterol** 50:288-92

targeting bacterial immunophilins. **Antimicrob Agents Chemother** 58: 3 1458-67

Stich A, Ponte-Sucre A, **Holzgrabe U** (2013) *Do we need new drugs against human African trypanosomiasis?* **Lancet Infect Dis** 13: 9 733-4

Topf CM, Schiebel J, Kisker C, **Holzgrabe U** (2012) *Old and new anti-mycobacterial drugs. Fatty acid synthesis as a target.* **Pharm Unserer Zeit** 41: 1 64-70

Tischer M, Pradel G, Ohlsen K, **Holzgrabe U** (2012) *Quaternary ammonium salts and their antimicrobial potential: targets or nonspecific interactions?* **ChemMedChem** 7: 1 22-31

Hiltensperger G, Jones NG, Niedermeier S, Stich A, Kaiser M, Jung J, Puhl S, Damme A, Braunschweig H, Meinel L, Engstler M, **Holzgrabe U** (2012) *Synthesis and structure-activity relationships of new quinolone-type molecules against Trypanosoma brucei.* **J Med Chem** 55: 6 2538-48

Djukic M, Munz M, Sorgel F, **Holzgrabe U**, Eiffert H, Nau R (2012) *Overton's rule helps to estimate the penetration of anti-infectives into patients' cerebrospinal fluid.* **Antimicrob Agents Chemother** 56: 2 979-88Djukic M, Munz M, Sorgel F, Holzgrabe U, Eiffert H, Nau R (2012)

4.5 UTE HENTSCHEL-HUMEIDA

Kamke J, Sczyrba A, Ivanova N, Schwientek P, Rinke C, Mavromatis K, Woyke T, **Hentschel U** (2013) *Single-cell genomics reveals complex carbohydrate degradation patterns in poribacterial symbionts of marine sponges.* **ISME J** 7(12): 2287-2300

McFall-Ngai M, Hadfield M, Bosch T, Carey H, Domazet-Loso T, Douglas A, Dubilier N, Eberl G, Fukami T, Gilbert S, **Hentschel U**, King N, Kjelleberg S, Knol, AH, Kremer N, Mazmanian S, Metcalf J, Nealson K, Pierce N, Rawls J, Reid A, Ruby E, Rumpho, M, Sanders J, Tautz, D, Wernegreen J (2013) *Animals in a bacterial world: a new imperative for the life sciences.* **Proc Natl Acad Sci** 110(9): 3229-3236

Ngwa CJ, Gloeckner V, Abdelmohsen UR, Scheuermayer M, Fischer R, **Hentschel U**, Pradel G (2013) *16S rRNA gene-based identification of Elizabethkingia meningoseptica as a dominant midgut bacterium of the Asian malaria vector Anopheles stephensi with antimicrobial activities.* **J Med Entomol** 50(2): 404-414

Abdelmohsen UR, Szesny M, Othman EM, Schirmeister T, Grond S, Stopper H and **Hentschel U** (2012) *Antioxidant and anti-protease activities of diazepinomicin from the sponge-associated Micromonospora strain RV115.* **Mar Drugs** 10: 2208-2221

Bayer K, **Hentschel U** (2012) *Einzelzell-Genomik: das Bakterium als Individuum.* **BIOspektrum** 18(6): 596-598

Kamke J, Bayer K, Woyke T, **Hentschel U** (2012) *Exploring symbioses by single-cell genomics.* **Biol Bull** 223 (1): 30-43

Hentschel U, Piel J, Degnan SM, Taylor MW (2012) *Genomic insights into the marine sponge microbiome.* **Nat Rev Microbiol** 10(9): 641-654

Pimentel-Elardo SM, Grozdanov L, Proksch S, **Hentschel U** (2012) *Diversity of nonribosomal peptide synthetase genes in the microbial metagenomes of marine sponges.* **Mar Drugs** 10(6):1192-1202

Bayer K, Scheuermayer M, Fieseler L, **Hentschel U** (2012) *Genomic mining for novel FADH$_2$-dependent halogenases in marine sponge-associated microbial consortia.* **Mar Biotechnol** DOI: 10.1007/s10126-012-9455-2

Angermeier H, Gloeckner V, Pawlik JR, Lindquist NL, **Hentschel U** (2012) *Sponge white patch disease affecting the Caribbean sponge Amphimedon compressa.* **Dis Aquat Organ** 99: 95-102

Abdelmohsen U, Zhang G, Philippe A, Schmitz W, Pimentel-Elardo SM, Hertlein-Amslinger B, **Hentschel U**, Bringmann G (2012) *Cyclodysidins A-D, cyclic lipopeptides from the marine sponge-derived Streptomyces strain RV15.* **Tetrahedron Lett** 53 23-29

Tabares P, Degel B, Schaschke N, **Hentschel U**, Schirmeister T (2012) *Identification of the protease inhibitor miraziridine A in the Red Sea sponge Theonella swinhoei.* **Pharmacognosy Res** 4(1) 63-66

4.6 CAROLINE KISKER

Schiebel J, Kapilashrami K, Fekete A, Bommineni GR, Schaefer CM, Mueller MJ, Tonge PJ, **Kisker C** (2013) *Structural basis for the recognition of mycolic acid precursors by KasA, a condensing enzyme and drug target from Mycobacterium tuberculosis* **J Biol Chem** 288: 47 34190-204

Chang A, Schiebel J, Yu W, Bommineni GR, Pan P, Baxter MV, Khanna A, Sotriffer CA, **Kisker C**, Tonge PJ (2013) *Rational optimization of drug-target residence time: insights from inhibitor binding to the Staphylococcus aureus FabI enzyme-product complex* **Biochemistry** 52: 24 4217-28

Topf CM, Schiebel J, **Kisker C**, Holzgrabe U (2012) *Old and new anti-mycobacterial drugs. Fatty acid synthesis as a target.* **Pharm Unserer Zeit** 41: 1 64-70

Schiebel J, Chang A, Lu H, Baxter MV, Tonge PJ, **Kisker C** (2012) *Staphylococcus aureus FabI: inhibition, substrate recognition, and potential implications for in vivo essentiality* **Structure** 20: 5 802-13

Hirschbeck MW, Kuper J, Lu H, Liu N, Neckles C, Shah S, Wagner S, Sotriffer CA, Tonge PJ, **Kisker C** (2012) *Structure of the Yersinia pestis FabV enoyl-ACP reductase and its interaction with two 2-pyridone inhibitors.* **Structure** 20: 1 89-100

Patsalo V, Yondala MA, Luan B, Shoshani I, **Kisker C**, Green DF, Raleigh DP, Hearing P (2012) *"Biophysical and Functional Analyses Suggest that the Adenovirus E4-ORF3 Protein Requires Higher-Order Multimerization to Function Against PML Nuclear Bodies".* **J Biol Chem** 287: 22573-22583

4.7 AUGUST STICH

Bringmann G, Zhang G, Olschlager T, **Stich A**, Wu J, Chatterjee M, Brun R (2013) *Highly selective antiplasmodial naphthylisoquinoline alkaloids from Ancistrocladus tectorius.* **Phytochemistry** 91: 220-228

mann G (2012) *Synthesis, optical resolution, absolute configuration, and osteogenic activity of cis-pterocarpans.* **Organic & biomolecular chemistry** 10: 9583-9592

Groh M, Meidlinger D, **Bringmann G**, Speicher A (2012) *Atroposelective Heck macrocyclization: enantioselective synthesis of bis(bibenzylic) natural products.* **Organic letters** 14: 4548-4551

Kullmann M, Hipke A, Nuernberger P, Bruhn T, Gotz DC, Sekita M, Guldi DM, **Bringmann G**, Brixner T (2012) *Ultrafast exciton dynamics after Soret- or Q-band excitation of a directly beta,beta'-linked bisporphyrin.* **Physical chemistry chemical physics** 14: 8038-8050

Li J, Li MY, Bruhn T, Gotz DC, Xiao Q, Satyanandamurty T, Wu J, **Bringmann G** (2012) *Andhraxylocarpins A-E: structurally intriguing limonoids from the true mangroves Xylocarpus granatum and Xylocarpus moluccensis.* **Chemistry** 18: 14342-14351

Messi BB, Ndjoko-Ioset K, Hertlein-Amslinger B, Lannang AM, Nkengfack AE, Wolfender JL, Hostettmann K, **Bringmann G** (2012) *Preussianone, a new flavanone-chromone biflavonoid from Garcinia preussii Engl.* **Molecules** 17: 6114-6125

4.2 THOMAS DANDEKAR

Kunz M, Ahmed N, **Dandekar T**, Naseem M (2013) *Hormone Signaling Networks Open Multiple Routes for Immunity and Disease in Plants.* **Biohelikon: Immunity and Diseases** 1:2

Winstel V, Liang C, Sanchez-Carballo P, Steglich M, Munar M, Broker BM, Penades JR, Nubel U, Holst O, **Dandekar T**, Peschel A, Xia G (2013) *Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens.* **Nat Commun** 4: 2345

Naseem M, Kunz M, Ahmed N, **Dandekar T** (2013) *Integration of boolean models on hormonal interactions and prospects of cytokinin-auxin crosstalk in plant immunity.* **Plant Signal Behav** 8: 4 e23890

Naseem M, Kaltdorf M, Hussain A, **Dandekar T** (2013) *The impact of cytokinin on jasmonate-salicylate antagonism in Arabidopsis immunity against infection with Pst DC3000.* **Plant Signal Behav** 8: 10 doi: 10 4161/psb 26791

Audretsch C, Lopez D, Srivastava M, Wolz C, **Dandekar T** (2013) *A semi-quantitative model of Quorum-Sensing in Staphylococcus aureus, approved by microarray meta-analyses and tested by mutation studies.* **Mol Biosyst** 9: 11 2665-80

Ratzka C, Forster F, Liang C, Kupper M, **Dandekar T**, Feldhaar H, Gross R (2012) *Molecular characterization of antimicrobial peptide genes of the carpenter ant Camponotus floridanus.* **PLoS One** 7: 8 e43036

Naseem M, **Dandekar T** (2012) *The role of auxin-cytokinin antagonism in plant-pathogen interactions.* **PLoS Pathog** 8: 11 e1003026

Krueger B, Friedrich T, Forster F, Bernhardt J, Gross R, **Dandekar T** (2012) *Different evolutionary modifications as a guide to rewire two-component systems.* **Bioinform Biol Insights** 6: 97-128

Fuchs TM, Eisenreich W, Kern T, **Dandekar T** (2012) *Toward a Systemic Understanding of Listeria monocytogenes Metabolism during Infection.* **Front Microbiol** 3: 23

Dandekar T, Astrid F, Jasmin P, Hensel M (2012) *Salmonella enterica: a surprisingly well-adapted intracellular lifestyle.* **Front Microbiol** 3: 164

Zirkel J, Cecil A, Schäfer F, Rahlfs S, Ouedraogo A, Xiao K, Sawadogo S, Coulibaly B, Becker K, **Dandekar T** (2012) *Analyzing Thiol-Dependent Redox Networks in the Presence of Methylene Blue and Other Antimalarial Agents with RT-PCR-Supported in silico Modeling.* **Bioinform Biol Insights** 6: 287-302

4.3 MARKUS ENGSTLER

Abuillan W, Vorobiev A, Hartel A, Jones NG, **Engstler M**, Tanaka M (2012) *Quantitative determination of the lateral density and intermolecular correlation between proteins anchored on the membrane surfaces using grazing incidence small-angle X-ray scattering and grazing incidence X-ray fluorescence.* **J Chem Phys** 137: 20 204907

Harrington JM, Scelsi C, Hartel A, Jones NG, **Engstler M**, Capewell P, MacLeod A, Hajduk S (2012) *Novel African trypanocidal agents: membrane rigidifying peptides.* **PLoS One** 7: 9 e44384

Heddergott N, Kruger T, Babu SB, Wei A, Stellamanns E, Uppaluri S, Pfohl T, Stark H, **Engstler M** (2012) *Trypanosome motion represents an adaptation to the crowded environment of the vertebrate bloodstream.* **PLoS Pathog** 8: 11 e1003023

Hiltensperger G, Jones NG, Niedermeier S, Stich A, Kaiser M, Jung J, Puhl S, Damme A, Braunschweig H, Meinel L, **Engstler M**, Holzgrabe U (2012) *Synthesis and structure-activity relationships of new quinolone-type molecules against Trypanosoma brucei.* **J Med Chem** 55: 6 2538-48

Uppaluri S, Heddergott N, Stellamanns E, Herminghaus S, Zottl A, Stark H, **Engstler M**, Pfohl T (2012) *Flow loading induces oscillatory trajectories in a bloodstream parasite.* **Biophys J** 103: 6 1162-9

Weisse S, Heddergott N, Heydt M, Pflasterer D, Maier T, Haraszti T, Grunze M, **Engstler M**, Rosenhahn A (2012) *A quantitative 3D motility analysis of Trypanosoma brucei by use of digital in-line holographic microscopy* **PLoS One** 7: 5 e37296

Engstler M, Heddergott N, Krüger T, Stellamanns E, Uppaluri S, Pfohl T (2012) *African Trypanosomes as Model System for Functional Analyses of Microbial Motility.* In C. Tropea and H. Bleckmann (Eds.): Nature-Inspired Fluid Mechanics, **NNFM** 119, pp. 43-61

4.4 ULRIKE HOLZGRABE

Laufer S, **Holzgrabe U**, Steinhilber D (2013) *Drug Discovery: A modern Decathlon.* **Angewandte Chemie** International Edition 52: 4072-4076

Begley DW, Fox D, 3rd, Jenner D, Juli C, Pierce PG, Abendroth J, Muruthi M, Safford K, Anderson V, Atkins K, Barnes SR, Moen SO, Raymond AC, Stacy R, Myler PJ, Staker BL, Harmer NJ, Norville IH, **Holzgrabe U**, Sarkar-Tyson M, Edwards TE, Lorimer DD (2014) *A structural biology approach enables the development of antimicrobials*

Fitzner S, Wohlfart J, Muller A, **Stich A**, Weiss T (2013) *Fever, leg swelling, coughing, and left upper quadrant pain in a 19-year-old woman.* **Internist** (Berl) 54: 619-623

Tappe D, Ernestus K, Rauthe S, Schoen C, Frosch M, Muller A, **Stich A** (2013) *Initial patient cluster and first positive biopsy findings in an outbreak of acute muscular Sarcocystis-like infection in travelers returning from Tioman island, Peninsular Malaysia, in 2011.* **J Clin Microbiol** 51: 725-726

Tappe D, Mueller A, Weissbrich B, Schubert J, Schargus M, **Stich A** (2013) *Severe conjunctivitis due to multidrug-resistant Neisseria gonorrhoeae and adenovirus 53 coinfection in a traveler returning from Thailand.* **J Travel Med** 20: 403-406

Stich A, Muller A, Tintelnot K (2013) [Coccidioidomycosis with manifestation on the tongue]. **Pathologe** 34: 548-551

Stich A, Ponte-Sucre A, Holzgrabe U (2013) *Do we need new drugs against human African trypanosomiasis?* **Lancet Infect Dis** 13: 733-734

Wichmann D, Poppert S, Von Thien H, Clerinx J, Dieckmann S, Jensenius M, Parola P, Richter J, Schunk M, **Stich A**, Zanger P, Burchard GD, Tannich E (2013) *Prospective European-wide multicentre study on a blood based real-time PCR for the diagnosis of acute schistosomiasis.* **BMC Infect Dis** 13: 55

Hiltensperger G, Jones NG, Niedermeier S, **Stich A**, Kaiser M, Jung J, Puhl S, Damme A, Braunschweig H, Meinel L, Engstler M, Holzgrabe U (2012) *Synthesis and structure-activity relationships of new quinolone-type molecules against Trypanosoma brucei.* **J Med Chem** 55: 2538-2548

Kasang C, Ulmer A, Donhauser N, Schmidt B, **Stich A**, Klinker H, Kalluvya S, Koutsilieri E, Rethwilm A, Scheller C (2012) *HIV patients treated with low-dose prednisolone exhibit lower immune activation than untreated patients.* **BMC Infect Dis** 12: 14

Tappe D, Nemecek A, Zipp F, Emmerich P, Gabriel M, Gunther S, Dobler G, Schmidt-Chanasit J, **Stich A** (2012) *Two laboratory-confirmed cases of Japanese encephalitis imported to Germany by travelers returning from Southeast Asia.* **J Clin Virol 54** 282-285

4.8 HEIKE WALLES

Groeber F, Kahlig A, Loff S, **Walles H,** Hansmann J (2013) *A bioreactor system for interfacial culture and physiological perfusion of vascularized tissue equivalents.* **Biotechnol J** 8: 308-316

Hansmann J, Groeber F, Kahlig A, Kleinhans C, **Walles H** (2013) *Bioreactors in tissue engineering - principles, applications and commercial constraints.* **Biotechnol J** 8: 298-307

Jakob F, Ebert R, Ignatius A, Matsushita T, Watanabe Y, Groll J, **Walles H** (2013) *Bone tissue engineering in osteoporosis.* **Maturitas** 75: 118-124

Scheller K, Dally I, Hartmann N, Munst B, Braspenning J, **Walles H** (2013) *Upcyte(R) microvascular endothelial cells repopulate decellularized scaffold.* **Tissue Eng Part C Methods** 19: 57-67

Schenke-Layland K, **Walles H** (2013) *Strategies in tissue engineering and regenerative medicine.* **Biotechnol J** 8: 278-279

Schimek K, Busek M, Brincker S, Groth B, Hoffmann S, Lauster R, Lindner G, Lorenz A, Menzel U, Sonntag F, **Walles H**, Marx U, Horland R (2013) *Integrating biological vasculature into a multi-organ-chip microsystem.* **Lab Chip** 13: 3588-3598

Jakob F, Ebert R, Rudert M, Noth U, **Walles H**, Docheva D, Schieker M, Meinel L, Groll J (2012) *In situ guided tissue regeneration in musculoskeletal diseases and aging : Implementing pathology into tailored tissue engineering strategies.* **Cell Tissue Res** 347: 725-735

Kleinhans C, Barz J, Wurster S, Willig M, Oehr C, Muller M, **Walles H**, Hirth T, Kluger PJ (2013) *Ammonia plasma treatment of polystyrene surfaces enhances proliferation of primary human mesenchymal stem cells and human endothelial cells.* **Biotechnol J** 8: 327-337

Marx U, **Walles H**, Hoffmann S, Lindner G, Horland R, Sonntag F, Klotzbach U, Sakharov D, Tonevitsky A, Lauster R (2012) *Human-on-a-chip' developments: a translational cutting-edge alternative to systemic safety assessment and efficiency evaluation of substances in laboratory animals and man?* **Altern Lab Anim** 40: 235-257

Moll C, Reboredo J, Schwarz T, Appelt A, Schurlein S, **Walles H,** Nietzer S (2013) *Tissue engineering of a human 3D in vitro tumor test system.* **J Vis Exp** 6;(78) doi: 10.3791/50460

Vorsmann H, Groeber F, **Walles H**, Busch S, Beissert S, Walczak H, Kulms D (2013) *Development of a human three-dimensional organotypic skin-melanoma spheroid model for in vitro drug testing.* **Cell Death Dis** 4: e719

Gryanik A, Wittenberg T, **Walles H**, Steckhan D, Thude S, Kaufmann M, Munzenmayer C (2012) *Automatic Image Quantification for Structural Analysis of in vitro Dermal Samples.* **Biomed Tech** (Berl) doi: 10.1515/bmt-**2012**-4241

Rackwitz L, Eden L, Reppenhagen S, Reichert JC, Jakob F, **Walles H**, Pullig O, Tuan RS, Rudert M, Noth U (2012) *Stem cell- and growth factor-based regenerative therapies for avascular necrosis of the femoral head.* **Stem Cell Res Ther** 3: 7

Schleicher M, Hansmann J, Elkin B, Kluger PJ, Liebscher S, Huber AJ, Fritze O, Schille C, Muller M, Schenke-Layland K, Seifert M, **Walles H**, Wendel HP, Stock UA (2012) *Oligonucleotide and Parylene Surface Coating of Polystyrene and ePTFE for Improved Endothelial Cell Attachment and Hemocompatibility.* **Int J Biomater** 397813

Votteler M, Carvajal Berrio DA, Pudlas M, **Walles H**, Schenke-Layland K (2012) *Non-contact, label-free monitoring of cells and extracellular matrix using Raman spectroscopy.* **J Vis Exp** 29;(63). pii: 3977. doi: 10.3791/3977

Votteler M, Carvajal Berrio DA, Pudlas M, **Walles H**, Stock UA, Schenke-Layland K (2012) *Raman spectroscopy for the non-contact and non-destructive monitoring of collagen damage within tissues.* **J Biophotonics** 5 47-56

IMPRESSUM

Herausgeber
Zentrum für Infektionsforschung
Josef-Schneider-Straße 2/D15
97080 Würzburg
www.zinf.uni-wuerzburg.de

Redaktion
Jörg Vogel
Stan Gorski
Hilde Merkert

Layout
Designbüro Clemens Mahler
mqdesign

Print
G-Medien GmbH
Lohr a. Main

Portraits
Schmelz Fotodesign

Cover photo
False-colored scanning electron microscopy image of Campylobacter jejuni, Image Gaurav Dugar, ZINF