RESEARCH REPORT 2008/2009

HELMHOLTZ
ZENTRUM FÜR
INFEKTIONSFORSCHUNG



SCIENTIFIC REPORTS

The Helmholtz Centre for Infection Research

The Helmholtz Centre for Infection Research in Braunschweig lives up to its name: here approximately 250 scientists and 350 technical and administrative staff lay the foundations for new preventative procedures, diagnostic approaches, medicines and drugs with which infectious diseases can be better cured or more effectively prevented. The paths that lead to this goal are as varied as the paths by which the pathogens enter our bodies. Microbiologists investigate how bacteria and viruses manage to enter our bodies, how bacteria communicate with one another and how exactly they make us ill. Geneticists investigate our genetic make-up in a search for reasons why one person falls ill with flu, for example, whilst their neighbour at the same table does not. Immunologists investigate how organisms react to an intruder and resist it. Structural biologists research the molecular structures of key molecules and their interactions. Chemists use this knowledge to investigate and develop new agents that can in turn be employed to combat disease. Vaccine researchers have their sights set on the best way to combat germs using preventative approaches before an illness occurs.

New diagnostic approaches, vaccines and medicines can only be successfully developed when the mechanisms of infectious diseases have been properly understood. A starting point from which researchers at the Helmholtz Centre for Infection Research approach the complex network of "infection" is the cell – both that of the host and that of the pathogen. One example is opportunistic infections which are a problem in hospitals. In a place where patients with weakened immune systems are treated, bacteria transform themselves from unobtrusive companions to dangerous aggressors. In extreme cases they ensconce themselves permanently in our bodies by forming a so-called biofilm, causing chronic illness under some circumstances. In biofilms the bacteria are surrounded by a protective sheath that protects them very effectively against attacks from the immune system or antibiotics. But what has to occur in order for a seemingly harmless germ to become an aggressor? How do bacteria communicate with one another and the host? Only when scientists have understood how the pathogens and their host cells interact and the mechanisms behind these interactions can they disrupt the communication between bacteria in a targeted manner.

Infections are always caused by molecular interactions between the host and pathogens, and between the pathogens themselves. The central elements in these interactions are the proteins involved in the infection. Consequently they play a key role in the understanding of infectious diseases. Microorganisms organise and catalyse their lives with thousands of proteins. The goal of infection researchers is not only to describe these proteins but also to understand their functions. The proteins on the cell surface, in particular, are responsible for contact with the host, and the most pertinent question in this area of research is: how do bacteria manage to infect us? The arsenal of different pathogenity factors, with which bacteria, viruses and other microbial pathogens interfere with processes within human cells is a modest one. However, knowing where these are located and being able to name these factors does not mean that they are understood. Proteins are very large molecules with complex structures and it is precisely in these structures that the secret of their success lies. Scientists at the HZI take a close look at their structure atom by atom. Details of which provide fundamental clues as to how they interact with one another and allows a rationale of their involvement in infection.

Once the structural biologists have familiarised themselves with these critical regions in the molecular structure the chemists can begin to put this knowledge to further use. Their goal is to develop tailored molecules that fit these structures exactly, and subsequently have the opportunity to interrupt an infection. To this end chemists look for natural inhibitors or create new synthetic ones, using these to block the functions of the pathogen proteins in a targeted approach.

The basis for new active agents, which are able to target selected regions of the bacterial proteins, are often natural agents. These often originate from traditional medicinal plants, fungi or bacteria. A special role at the HZI is played by the myxobacteria. These live in the soil and defend themselves against bacterial competitors using a range of novel secondary metabolites, substances that are highly active when carefully isolated and analysed by natural product chemists and then subsequently developed into antiinfectives. Microbial active metabolites for combating infectious diseases are therefore seen as the optimal starting substances for developing new therapies against infections for use in hospitals and elsewhere.

The task of the chemist in infection research is easily described but difficult to implement: chemists search for active components that enable myxobacteria to defend themselves against other microorganisms or are a basic component of a medicinal plant. In these molecules they search for particular structural characteristics and chemical groups in order to recreate these and even improve their effectiveness. For HZI researchers synthesising or modifying natural agents to create potent medicines is a discipline that bridges the gap between pure research and clinical applications.

Vaccine researchers at the HZI also tread a fine line between pure science and the clinic. Vaccines are held to be the most effective and economical method for protecting humans and animals against pathogens. Although scientists obviously search for new vaccines against illnesses such as AIDS or flu, one of their specialities is the optimisation of vaccine application. Adjuvants are the key. These agents have no effect on infectious pathogens themselves, but help the vaccines to develop their full potential.

An understanding of infection requires detailed knowledge of the pathogens, the host organism and environmental factors. As the environmental factors have not proven readily comprehensible thus far, HZI researchers are focusing upon the interaction between pathogen and host. The research field that aims to address this highly-complex question is in its first stages of development and involves system genetics. A whole series of genes determine whether a host responds sensitively or imperviously to an infection. In this context, genes influence one another, with the consequence that a defective gene may be compensated for by another gene or defects in genetic material may reinforce one another.

The system-genetic investigation of such interrelations will lead to new therapeutic approaches. The tools for this are not limited to the petri dish, microscope or mouse, but are above all computers.

Research at the Helmholtz Centre for Infection Research covers a wide field of disciplines ranging from an understanding of the molecular interactions between pathogen and host to the discovery of new active agents, and methods for disease prevention. Co-operation with the Hannover Medical School has resulted in the Twincore in Hanover – a translation centre that is a research meeting point for clinic and pure science. Medical personnel and pure scientists co-operate together under one roof to provide joint solutions to problems encountered in infectious diseases that lead to public health benfits. The involvement in the new research centres enables the Helmholtz Centre for Infection Research to reinforce the health research capabilities of the Braunschweig-Hanover area. Thus furthering progress that is being made towards resolving an old - and yet current - problem: protecting people against infectious diseases.

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All Photos: HZI, Gramann

Foreword

Prof. Dr. Rudi Balling | Scientific Director

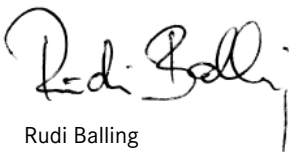
2008 was an exciting year for the Helmholtz Centre for Infection Research (HZI). Following the scientific realignment of the institute, the first appraisal within the scope of the programme-oriented funding was due. During the preparations for this we scientists asked ourselves a number of times whether we had done everything right. Did we set the right focus? Would the assessors share our opinion that we had achieved really good results? “Yes”, the assessors confirmed, evaluating the scientific programme of the HZI as very good. We consequently received the go ahead for further development on the path towards an improved top performance. The objective, to provide a basis for the creation of new antiinfectives and new vaccines.

Now we are moving forward, we need to maintain our performance and expand upon it. We want to prove that our research is in keeping with the Helmholtz mission, namely to solve specific problems in our society. One highlight from the past illustrates that a centre like the HZI is certainly very capable of achieving this. During 2007 the US Food and Drug Administration approved the cancer medication Ixempra. This is based on the natural substance epothilone that was discovered and researched at the HZI – at that time still the GBF.

The conditions are ideal to continue achieving such application-oriented scientific breakthroughs in the future. We have received funding approval for further joint ventures in the region to extend and consolidate our research pipeline, from the laboratory to the patient:

- The Technical University of Braunschweig and the HZI are collaborating on the establishment of the Braunschweig Integrated Centre for Systems Biology (BRICS). Here biologists, mathematicians, IT experts and engineers will be able to work together to learn how to understand complex biological processes in their entirety, as well as modelling and simulating them. New sequencing technologies will aid these tasks. The resulting flow of data and the fact that infectious diseases are extremely complex processes mean that in future we will only be able to manage these with methods such as those established at BRICS.
- A joint drug discovery centre combines the expertise in chemical biology and medical chemistry at the Leibniz University of Hannover and the HZI. With this we aim to develop the potential of the HZI natural product collection for the development of new antiinfectives.
- In future, the transfer of results achieved in basic research to preclinical applications in humans will be made possible by the Hannover Centre for Translational Medicine, which is being established by the Fraunhofer Institute of Toxicology and Experimental Medicine in collaboration with the Medical School Hannover and the HZI. Early clinical studies (phase I/IIa) will be implemented here.

All of this serves to secure the scientific productivity of Niedersachsen and, subsequently, also the future of the HZI. Investments in minds are, however, at least as vital as the investments in buildings. We are working hard to make the HZI an even better employer, so that we can continue to attract the best staff for excellent research.



Rudi Balling

Photos from left to right: The new mousehouse oft he HZi will be inaugurated at the end of August 2009 | The new leading scientists at Twincore: Profs. Ulrich Kalinke, Michael Ott, Tim Greten, Tim Sparwasser, Susanne Häußler, Thomas Pietschmann (from left to right) | Dr. Kathrin Westphal during her work in the lab. She was awarded the prize of the "Arbeitskreis Zellbiologie und Biomedizinische Forschung e.V." for her excellent PhD work *Photos: HZI, Krämer (le) | HZI, Gramann (ce) | HZI, Dornbach (ri)*

Highlights 2007-2009

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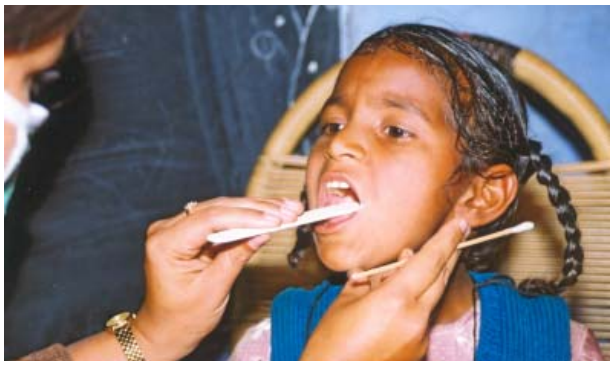
Inhoffen prize awarded to ETH researcher François Diederich The Inhoffen prize winner for 2007 was Prof. François Diederich from the ETH Swiss Federal Institute of Technology in Zürich. He was awarded the prize by the Friends of the HZI association for his scientific work on biological receptors, i.e. molecules on the cell surfaces which accurately detect specific substances. Cells use them to identify messengers, chemical signals and the presence of dangerous pathogens. Diederich investigated exactly how these receptors operate. He artificially produced simplified versions of these receptors in the lab and studied their behaviour under controlled conditions. The research yielded various important discoveries for many biological and medical processes and helped further understanding of diseases.

Prof. François Diederich from the ETH Zürich, winner of the Inhoffen Prize 2007. *Photo: HZI, Hübner*

ASSIST: help for heart disease in children Since March 2007, division manager of the HZI, Singh Chhatwal, has been responsible for coordinating the German-Indian ASSIST cooperative project: over the next few years, the ASSIST researchers are aiming to gather information about the streptococcus bacteria which are very widespread in India. A quick test will then be developed to ensure that the particularly dangerous strains of streptococcus are treated quickly with a suitable antibiotic, thereby halting the development of the infection. The reason: a streptococcus infection can take the form of a harmless sore throat – but it can also be life-threatening or result in life-long damage. Experts estimate that streptococcus infections affect some 600 million people each year. In some of these cases, serious complications develop including rheumatic fever. This can often cause serious damage to the heart. In India alone, some 6 million children are suffering from rheumatic heart disease. A quick test for streptococcus could dramatically reduce the number of individuals affected in future.



The German-Indian ASSIST cooperative project will help to treat dangerous strains of streptococcus which may cause rheumatic heart disease by the use of a quick test system. *Photo: HZI*

Indian health minister visits the HZI In May 2007, the Indian health and family minister Dr. Anbumani Ramadoss visited the Helmholtz Centre for Infection Research. The purpose of the visit was to sign a contract of cooperation for the founding of a 'German-Indian scientific centre for infectious diseases'. Here, Indian and German scientists work together in teams. Their aim is to investigate and combat the development of these diseases – a particular focal point in the work of health minister Ramadoss. Ramadoss was accompanied by the director of the Indian Council for Medical Research, Prof. Nirmal K. Ganguly. The Indian delegation also stopped off in Hanover where Ramadoss and Ganguly visited Hannover Medical School (MHH) and the Twincore, the translation centre for the MHH and HZI.

The Indian Minister for Health, Dr. Anbumani Ramadoss and his delegation visited the HZI in May 2007. *Photo: HZI, Krämer*

International congress on complex genetics at the HZI International experts in complex genetics gathered at the Helmholtz Centre for Infection Research in May 2007 to exchange their experience and coordinate their research activities at the 6th annual meeting of the 'Complex Trait Consortium - CTC' (consortium for investigating complex inherited characteristics). No individual researcher or research institute can master the Herculean task of describing the highly complex network of genetic interconnections within an organism or infection – success is only possible through international collaboration. Experts in complex genetics from all over the world therefore decided to set up the Complex Trait Consortium. At the gathering in Braunschweig, organised by HZI department manager Prof. Klaus Schughart, researchers from Germany, Great Britain, Japan and the USA advised how advancements can be made in the new scientific discipline of complex genetics by working together.

Prof. Klaus Schughart organized an international congress on complex genetics at the HZI. *Photo: HZI*

Balling first president of the VBIO The VBIO umbrella organisation was set up in June 2007 to represent biologists and experts in biomedicine throughout Germany. The spokesman for the organisation is Prof. Rudi Balling, the scientific director of the Helmholtz Centre for Infection Research. He was elected as the first president at the inaugural meeting of the VBIO. His aim is to create a strong and influential form of representation for biomedicine in Germany: the VBIO models itself on the associations for chemists and physicists. The VBIO will enable the life sciences, which will play a key role in the 21st century, to speak with one voice. VBIO stands for 'Verband Biologie, Biowissenschaften und Biomedizin in Deutschland e.V.' (association for biology, bio-sciences and biomedicine in Germany). When founded, it comprised 5,000 individual members, more than 80 firms and institutions and numerous associations which themselves represent more than 30,000 members.

Prof. Rudi Balling was elected the first president of VBIO.
Photo: HZI, Gramann

Science in the city: HZI vaccination campaigns in the Burgplatz To mark the 'Braunschweig – city of science' year, experts from the fields of medicine, health care and science gathered in May 2007 in an 'immunisation tent' in the Burgplatz in Braunschweig as part of the 'science in the city' event to provide information on infection research and the essential nature of vaccination. The HZI worked with the Braunschweig City Hospital, Braunschweig Department of Health, Consortium of Braunschweig Health Insurance Funds and Association of Niedersachsen Physicians to devise a comprehensive programme of lectures, films and immunisation advice sessions. Experts from the HZI were given the opportunity to present their work before a large audience and explain how they develop ideas for new vaccines.

During the "Braunschweig – City of Science"-year the HZI provided information on infection research and the importance of vaccination on the Burgplatz in Braunschweig. *Photo: HZI*

Prizes for the battle against cancer and new medical imaging procedures In July 2007, the 'Arbeitskreis Zellbiologie und Biomedizinische Forschung e.V.' (research group for cell biology and biomedical research) awarded prizes to two young HZI scientists who had made significant scientific breakthroughs as part of their PhD work. As part of her PhD, Dr. Kathrin Westphal investigated how pathogenic bacteria could be used to help in the battle against cancer. Scientists have known for hundreds of years that bacteria prefer to colonise cancerous ulcers. Kathrin Westphal demonstrated that salmonella develops specific characteristics in proximity to a tumour which could be used in treating cancer. The second prize-winner, Dr. Bin Ma, was able to develop new techniques to portray the dynamic processes of the individual organs in mice in real time.

Dr. Kathrin Westphal was awarded the prize of the "Arbeitskreis Zellbiologie und Biomedizinische Forschung e.V." for her excellent PhD work. From left to right: Dr. Siegfried Weiß, Dr. Kathrin Westphal, Prof. Jürgen Bode, Dr. Kurt Dittmar.
Photo: HZI, Gramann

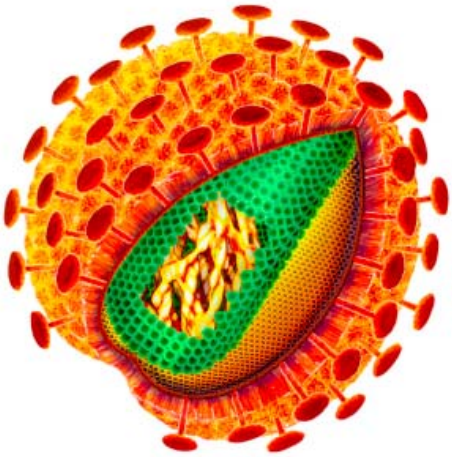
Balling stays in Braunschweig
In October 2007, Professor Rudi Balling decided to remain in his role as scientific director at the Helmholtz Centre for Infection Research. Following his re-appointment, the state of Niedersachsen invested a total of 35 million Euros to enable an expansion of this research into infection. Translational medicine, active substances research and system biology form the focus of this research. The funds are being invested jointly by the research partners at the HZI in Braunschweig and in Hanover to ensure that the fundamental findings of this research can be put to practical medical use more quickly than in the past.

Viruses and the economy
An area which often goes unaddressed was highlighted by international experts at the Helmholtz Centre for Infection Research in October 2007: the interface between infectious diseases and their effects on the economy. The meeting marked the first contact between the two fields: "As scientists, we wanted to broaden our horizons and strengthen our collaboration with the economy. Only then can we manage the problems which may cause infections in the future," Rudi Balling concluded of the event.

Each year, infections are responsible for some 17 million deaths worldwide – and the number is on the increase. In addition to the social consequences, this also has an effect on the economy: infectious diseases require the pharmaceuticals industry to produce ever more new forms of medication and vaccinations for countries which can barely afford to purchase them. Malaria is progressing in the Mediterranean and is threatening to damage the tourism industry. Insurance must be adapted to cover the increased risks of a shortfall in manpower. At the special expertise day at the HZI, these interconnections were outlined by experts for the first time and their effects discussed.

Discussions of experts on the interface between infectious diseases and their effects on the economy at the HZI FORUM.
Photo: HZI, Gramann

Gathering of international AIDS researchers at the HZI
In January 2008, HIV experts gathered at the HZI to discuss the current state of AIDS research. Experts from Israel and the USA attended alongside researchers from Germany and Europe and explained what makes the HIV virus so different, what new approaches to treatment have been developed and what progress has been made in developing a vaccine to protect against HIV. All participants at this public symposium were able to discuss matters with the experts, ask questions and find out more at the various lectures. The international 'Miditrain' doctoral programme and the Helmholtz International Research School for Infection Biology (HIRSIB) were responsible for organising the symposium.



A scheme of the HIV. *Graphic: HZI*

Jürgen Wehland awarded Descartes prize
In March 2008, the EU Commission awarded the Descartes prize of 1.36 million Euros for excellent transnational and cooperative research. One of the three prize-winning groups – a research group from Germany, France and Spain – is investigating the virulence or infectious capabilities of the Listeria monocytogenes pathogen through the 'VIRLIS' project. Jürgen Wehland, head of the Cell and Immune Biology division, has been working successfully with the consortium for many years. He is focusing on how bacteria penetrate the host cells, how they use the cytoskeleton to travel and how they survive inside the affected cells.

Prof. Jürgen Wehland was awarded the Descartes Prize in March 2008. *Photo: HZI, Gramann*

Inhoffen medal 2008 awarded to natural chemist Steven Victor Ley The British researcher Steven Victor Ley was awarded the Inhoffen medal in April 2008 by the Friends of the HZI association for his excellent work on the reproduction of medically relevant natural substances. Ley is a chemist at the University of Cambridge and has been researching the reproduction of complex natural substances for many years. Azadirachtin is one product of this research, a substance isolated from the Indian Neem tree and used as an insect antifeedant. Ley has also established a wide range of new methods which have established themselves in modern synthetic organic chemistry labs. His work is particularly significant as many natural substances are chemically very complex, making them difficult to produce artificially in the laboratory.

Prof. Steven Victor Ley from University of Cambridge, winner of the Inhoffen Medal 2008. *Photo: HZI, Dornbach*

Kenneth Timmis receives highest academic award in Britain The microbiologist Professor Kenneth Timmis of the Helmholtz Centre for Infection Research in Braunschweig received one of the highest awards for science in June 2008: the British-born scientist became a fellow of the British Royal Society. He has been researching at the Helmholtz Centre for Infection Research (formerly the GBF) since 1989 and has proven his great scientific skill in this time through his enormous number of publications, prizes and awards. He was selected as a fellow for his work in the application of genetics to explain complex metabolic pathways in bacteria. He has applied this knowledge to modify bacteria so that they can biologically break down environmental toxins. His technology for removing mercury from wastewater is award-winning. In being selected as a fellow, this Braunschweig-based microbiologist is now part of a sophisticated scientific elite comprising the astrophysicist Stephen Hawking, the developer of the World Wide Web Tim Berners-Lee, Nobel Prize winners Paul Nurse and John Sulston and many other pioneers of science.

Prof. Ken N. Timmis was elected fellow of the British Royal Society in June 2008. *Photo: HZI, Gramann*

International genetics congress in Berlin
The 20th international genetics congress was held in Germany in July 2008 – for the first time in 81 years. Rudi Balling chaired the congress. More than 2,000 researchers came to Berlin to discuss all manner of relevant issues in genetics and their latest discoveries. The participating researchers included six Nobel Prize winners who also gave talks on their work. The return of the congress to Germany had a particular historical significance: July 2008 marked the 75th anniversary of the introduction of the law for the 'prevention of genetically diseased offspring'. The Nazis abused modern genetics to substantiate their views on inferior races. The German Society of Human Genetics offered its views on the behaviour of the scientists involved in this and discussed the current position of German human genetics.

BERLIN 2008
XX INTERNATIONAL CONGRESS OF GENETICS

The logo of the XX International Congress of Genetics in Berlin, July 2008.

The launch of Twincore
In August 2008 Twincore, the centre for experimental and clinical infection research, began work in Hannover. The centre is operated jointly by the Helmholtz Centre for Infection Research in Braunschweig and the Hannover Medical School. It combines the fundamental infection biology research being carried out at the HZI with the clinical infection research of the MHH. In Germany, this is a unique model for the collaboration of university and external research. The research centre aims to be an international leader in infection research. As such, it is a powerful force in channelling the results of research into creating new vaccines, diagnostic products and treatments.

Prof. Dieter Bitter-Suermann (left), President of the MHH, Prof. Rudi Balling (middle), Scientific Director of the HZI and Prof. Ulrich Kalinke (right), the Director of the new established Twincore Research Centre, in front of the entrance of Twincore. *Photo: HZI – MHH*

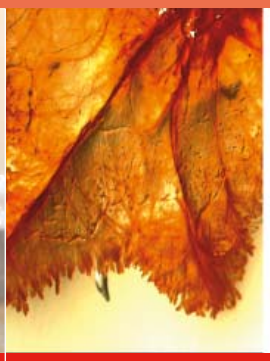
The HZI and 'Braunschweiger Zeitung' newspaper win the Promega prize The Helmholtz Centre for Infection Research (HZI) and 'Braunschweiger Zeitung' (BZ) newspaper were awarded the first prize of 10,000 euros in the 'Key Issue Biology' competition run by the biotech company Promega GmbH. This science-journalism prize is awarded by Promega to particularly successful examples of exciting and informative reports of research results in the regional media. The prize winners were Professors Gerhard Höfle and Hans Reichenbach of the HZI and the Braunschweiger Zeitung's science journalist, Henning Noske. They received their award in November 2008 in Hamburg. The team of three received the prize for their nine-part series 'A cancer medicine is born' which ran from November 2007 to January 2008 in the Braunschweiger Zeitung paper. In it, editor Henning Noske gives an account of the discovery of the active substance epothilone – written in the form of a scientific detective story.

The discovery of epothilone, a cancer medicine, and an informative report on this substance in the Braunschweiger Zeitung resulted in the Promega Prize for HZI and the Braunschweiger Zeitung. *Photo: HZI*

FOCUS RESEARCH REVIEWS SPECIAL FEATURES

Photos from left to right: Prof. Singh Chhatwal discussing with his group results of a gelelectrophoresis | Diana Telkemeyer controlling Petri dishes | Biofilms of myxobacteria | Photos: HZI, Gramann (le, ce) | HZI (ri)

SCIENTIFIC REPORTS FACTS AND FIGURES


Mega-Genomes and Micro-Chemists: New Perspectives for the Production of Bioactive Compounds in Myxobacteria

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For almost 30 years, the myxobacteria have been a subject of research at the Helmholtz-Zentrum für Infektionsforschung (HZI) in Braunschweig – formerly Gesellschaft für Biotechnologische Forschung (GBF). The interest in myxobacteria initially arose from their ability to undergo morphogenetic processes which culminate in fruiting body formation (Fig. 1), a skill which is unique among the prokaryotes. Later it turned out that myxobacteria are also excellent producers of novel bioactive compounds (1). Such substances have a wide variety of applications in the pharmaceutical and agricultural industries, including use as antibiotics, chemotherapeutics, and fungicides.

Myxobacteria as producers of bioactive compounds

The biosynthesis of metabolites with such highly complex structures requires the evolution of equally complicated biosynthetic machineries by myxobacteria. Intricate stereochemistry makes provision by total synthesis difficult if not impossible, so that biotechnological production by means of fermentation of the microorganisms is preferred (Fig 2.). An excess of 100 new metabolites have already been isolated from myxobacteria, and the majority of over 50% were obtained from culture extracts of strains of the species *Sorangium cellulosum* (1,2). The genus *Sorangium* belongs to the suborder Sorangiineae, while *Myxococcus xanthus,* the model organism for studying prokaryotic development, is a member of the Cystobacterineae. Myxobacteria are one of the few new producer groups of bioactive compounds discovered in the last century, and belong to the most prolific producers of secondary metabolites. Astonishingly, with only a few exceptions, these substances exhibit entirely novel basic structures, which often incorporate an unusual combination of peptide- and polyketide building blocks and are often produced as families of structurally-related derivatives.

Fig. 1. Myxobacterial fruiting bodies Photo: HZI

Fig. 2. Bioreactor of the technical school Photo: HZI

Bioactive compounds from *Sorangium cellulosum* and their commercial potential Nearly concurrent with the publication of the complete genome sequence of *Sorangium cellulosum* So ce56 in *Nature Biotechnology* (3), came a second breakthrough in myxobacterial research. In October 2007 Ixabepilone, an active epothilone derivative from *S. cellulosum* So ce90, was granted approval by the US Food and Drug Administration (FDA) as a therapeutic for breast cancer treatment; it is now making its way onto the pharmaceutical market (Fig. 3 Ixabepilone, BMS (4,5)).

Epothilone was isolated from *Sorangium cellulosum* So ce90 at the GBF in 1985, and was originally patented as an agent active against plant pathogenic fungi (1) exhibiting a high cytotoxicity. Subsequently, it was demonstrated that epothilone inhibits division of eukaryotic cells by binding to the tubulin skeleton, a mode of action almost identical to that of the anticancer drug Taxol. HZI/GBF microbiologists have worked intensively on strain optimisation and established an industrial-scale fermentation process. The accumulated "know how" was licensed to Bristol Meyers Squibb in order to initiate clinical trials and facilitate further development of epothilone as an antitumor agent.

Secondary metabolites from the genus *Sorangium* are evidently of high commercial interest (2). One of the first antibiotics discovered from these strains was sorangicin, which exhibits the same activity as the medically-established rifampicin. Unfortunately, the discovery came too late as a second "rifampicin" had no place in the pharmaceutical market. However, such conclusions may have to be re-evaluated, as current rifamycin-based treatment of tuberculosis is hampered by several factors, including the development of drug-resistance and induction of P450 activity. Perhaps the application of sorangicin as a therapeutic agent can circumvent some of these issues in future. Soraphen, another metabolite from *S. cellulosum,* initially showed promise as a plant-protective agent. Field trials demonstrated that this inhibitor of fatty acid biosynthesis acts strongly against fungal pathogens growing on rice and grapes, among others plants. Commercial-scale production of soraphen was almost initiated, but development was halted following studies which revealed teratogenic effects on rat embryos.
There are numerous other products derived from *Sorangium* isolates exhibiting various biological activities which cannot be discussed here in detail due to space limitations. For more information, the reader is referred to a recent review (2).

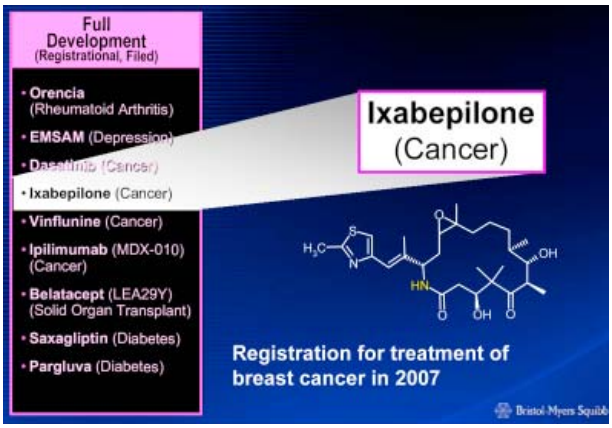

Fig. 3. Ixabepilone Graphic: HZI

Characteristics of the *Sorangium cellulosum* genome The sequencing of the genome of a *Sorangium* strain and the bioinformatic analysis of the resulting data within the scope of the BMBF-sponsored project GenoMik, was accomplished through the cooperative work of the Bielefeld Competence Center, HZI, and numerous research groups both in and outside Germany, with co-ordination by Saarland University. The genome size of *Sorangium cellulosum* and its high GC content of 71 % was a challenge. In total, 9367 genes were annotated in the *Sorangium* genome (Fig. 4), a number which exceeds even that of eukaryotic model organisms such as baker's yeast (*Saccharomyces cerevisiae*). The results of annotation are highly relevant to further efforts to uncover the biotechnological potential of this microorganism. Genome sequence information immediately increases the understanding of secondary metabolite biosynthesis and its regulation, enabling future attempts at targeted expression and production optimization.

A second myxobacterial genome, that of *Myxococcus xanthus,* was also recently sequenced (6). Surprisingly, comparative analysis of both genera showed significant differences in the overall genomic organisation and structure, whereas the distribution of genes into functional categories is globally similar. The *Sorangium* genome contains a remarkably large number of regulatory proteins, including many that were originally identified in eukaryotes (*e.g.* the serine/threonine/tyrosine protein kinases (7)). More than 300 of these proteins (encoded by nearly 1 Mbp of DNA!)

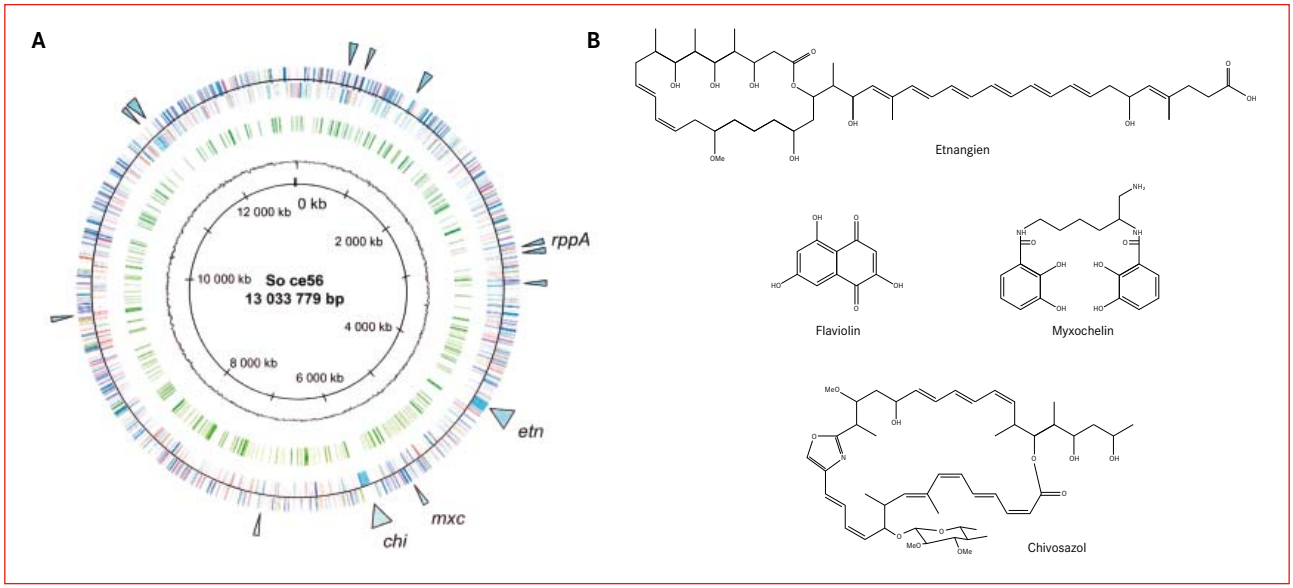

Fig. 4 (A+B). Genome of Sorangium cellulosum *Photo: HZI*

have been annotated (Fig. 4A), and they often exhibit very unusual or, to date, unprecedented genetic architectures. We have also shown in proteomic studies that approximately 40% of the proteins in *S. cellulosum* are in fact phosphorylated and deduced that post-translational modification plays an extremely important role in regulatory processes in *Sorangium* (3). The complex global regulatory network in *S. cellulosum* is significantly different from that in other bacteria. Moreover, the number of two-component regulatory systems and enhancer binding proteins (EBPs) which play a role in external and internal signal transmission and the co-ordination of metabolism, is also unusually high. However, the numerous regulatory genes alone cannot explain the extraordinary size of the genome. The question of whether foreign DNA, including bacteriophages or plasmids, has been integrated into the genome is at present unclear although it is already clear that a few genes have been acquired through horizontal gene transfer (which is another possible explanation for the large genome size). It is highly probable that such genes are also responsible for the biosynthesis of natural products, such as epothilone.

Although hundreds of bacterial genomes have already been sequenced, and intergenome comparison reveals numerous similarities within the highly diverse bacterial groups, much of the genetic information in *S. cellulosum* remains to be decoded: 34.7% of all encoded proteins have no significant similarities to other proteins within sequence databases. A further 13% of proteins exhibit similarity to hypothetical proteins from other organisms whose functions are presently unknown. Surprisingly, the order of magnitude of "novel" genes found in *S. cellulosum* is therefore comparable to complete bacterial genomes.

The lifestyle of myxobacteria: an explanation for genome size? Strains of the genus *Sorangium* are prevalent worldwide and are part of the common soil microflora. *Sorangium cellulosum* strains are among the few aerobic, Gram-negative cellulose degraders, and differ from other gliding cellulose degraders, namely cytophages and sporocytophages by the high GC content of its genome. *S. cellulosum* grows by mineralising plant material and lives, in its natural biotope, in direct competition with cellulose degrading fungi. Whether the common habitat explains the striking abundance of antifungal compounds found in *Sorangium*, remains speculative.

In comparison to almost all other groups of myxobacteria, for example the genus *Myxococcus*, *S. cellulosum* clearly possesses a more versatile metabolism. In addition to amino acids, *Sorangium* can also exploit alternative sources of carbon such as the polysaccharides cellulose and starch, as well as their degradation products. Cellulose, pectin and hemicellulose are typical components of the plant cell

wall. Building blocks of pectin and hemicellulose such as mannose and xylose, are also used as nutritional sources (8). The essential genes (over 40) for the breakdown of these sugars are found to be distributed over the whole *Sorangium* genome. At present, chemically defined media, which contain glucose as carbon source and ammonium or nitrate as nitrogen source, have been developed that enable the growth of *S. cellulosum*. All other primary metabolites, including amino acids, can be synthesised *de novo*.

Myxobacteria are strong only when they are together: they live in biofilms (Fig. 5) and are able to swarm as "packs", a strategy that facilitates the exploitation as food sources of complex and insoluble substrates such as cellulose, or other microorganisms. The cells of one isolate are distinguishable and swarms of different isolates do not intermingle, a behaviour which may arise from communication via signal compounds. This unique survival strategy also manifests itself under starvation conditions: millions of individual cells of myxobacterial swarms gather together and assemble into cell aggregates. A "co-operative morphogenesis" then leads into a more or less complex, genus-specific "fruiting body".

In *S. cellulosum* (Fig. 6) these are made up of masses of sporangioles, wherein the individual vegetative cell transforms into a myxospore, a process of "cellular morphogenesis". Metabolism ceases and the myxospores become impervious to desiccation. Under suitable conditions (*i.e.* when nutrients are again plentiful), vegetative cells emerge from the myxospores and leave the sporangioles to initiate a new life cycle.

The morphogenetic and physiological processes, similar to the production of secondary metabolites, require complex regulation processes that are encoded in the genomic DNA and might explain – at least in part – the extraordinary size of myxobacterial genomes.

Cyanobacteria, actinomycetes and other organisms similarly exhibit complex lifecycles and produce secondary metabolites; among the actinomycetes, the streptomycetes rank as the most prolific producers of active compounds. Thus, there appears to be a direct correlation between the ability to undergo morphogenesis and production of secondary metabolites. Notably, all of these bacteria possess very large chromosomes. Nonetheless, the chromosomal DNA of

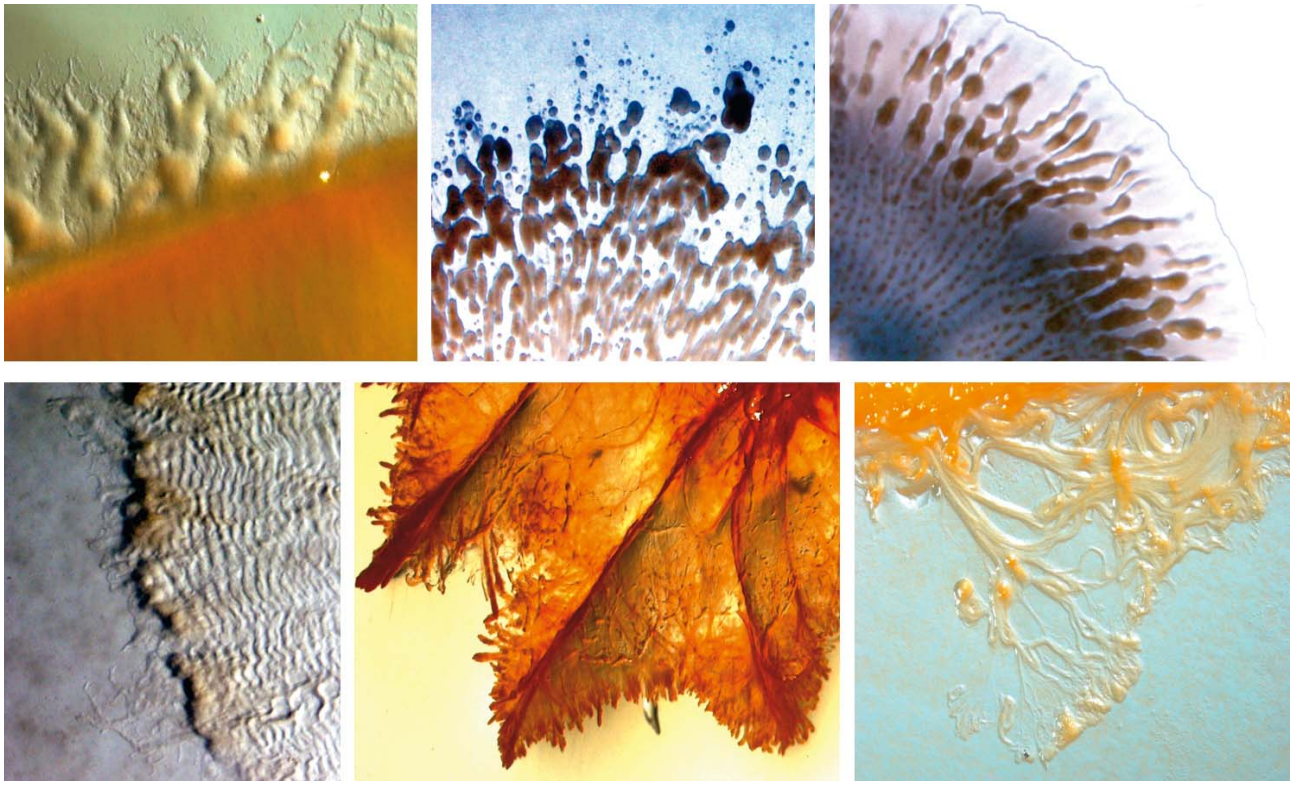

Fig. 5. Biofilms of various myxobacterial species Photos: HZI

Fig. 6. Fruiting bodies of Sorangium cellulosum *Photo: HZI*

S. cellulosum, which comprises 13.1 Mbp, is approximately 4 Mbp larger than all other bacterial genomes sequenced to date – this difference is of the same magnitude as the entire genome of the well-known intestinal bacteria *Escherichia coli.*

The majority of active substances produced by *S. cellulosum* are synthesised by complicated, multimodular enzyme systems, the polyketide synthases and nonribosomal peptide synthetases. These multienzyme complexes are often encoded by biosynthetic gene clusters, which consist of 100 kpb or more of genetic information. Very often, strains of the genus *Sorangium* are multiproducers and are able to biosynthesise up to 12 different metabolites simultaneously (2). In addition, "silent" biosynthetic gene clusters can be found in the genome whose products are to date unknown. *S. cellulosum* So ce56 also produces multiple secondary metabolites at the same time (Fig. 4B). Chivosazol is a cytotoxic and fungicidal compound, while etnangien inhibits the bacterial DNA-dependant RNA polymerase. Furthermore, the iron-chelating myxochelins were isolated from culture extracts of this strain. Genomic analysis of the regions outside the identified biosynthetic gene clusters has revealed that there are additional clusters that encode the biosynthesis of further natural products. Identification of the biosynthetic loci for these compounds as well as genes involved in their regulation should facilitate experiments to produce new and modified antibiotics.

The ability to generate these complex compounds and the underlying regulatory processes are encoded in the *S. cellulosum* genome and therefore enable future directed genetic engineering to produce novel and altered natural products.

New and optimised active substances from myxobacteria: future perspectives A total of 18 gene clusters for secondary metabolite biosynthesis were detected in *M. xanthus* (9). The development and optimisation of analytical chemistry methods for both *M. xanthus* and *S. cellulosum* allowed the identification of other previously unknown secondary metabolites (9-12). To further take advantage of the apparent genetic potential of myxobacteria, work is being carried out to activate "silent" genes to obtain potentially bioactive natural products. The expression of these complex gene clusters is a considerable challenge for secondary metabolite research. One possibility for which proof-of-principle already exists is the heterologous expression of biosynthetic gene clusters in host organisms, which are faster growing and easier to cultivate. An essential prerequisite for these experiments is a detailed knowledge of the biosynthetic mechanisms.

A polyketide synthase of unknown function from *S. cellulosum* was expressed in *Pseudomonas putida*, leading to the production of flaviolin, a compound which had not previously been identified in myxobacteria (13). Large biosynthetic gene clusters from myxobacteria have already been successfully expressed in heterologous hosts. Myxochromide, epothilone, and myxothiazol were produced in *M. xanthus* and *P. putida*, in some cases at higher yield than from the native organisms (8,14-17). Additional methods for optimising the biosynthesis can emerge from investigating regulation in the original producer. An important regulator of chivosazol biosynthesis in *S. cellulosum,* ChiR, was identified by biomagnetic separation, and its function was experimentally verified. ChiR is necessary for the transcription of the biosynthetic genes and overexpression of this regulator led to a five-fold increase in yield (18). This example shows how the availability of genome sequences can be directly exploited for biotechnology.

Genetic information can also support the work of natural product chemists in elucidating the 3D structures of novel molecules. Exact knowledge of the stereochemistry of new molecules underpins the establishment of structure-activity relationships and is required for total synthesis or chemical derivatisation efforts. Detailed analysis of the biosynthetic machineries for both chivosazol and thuggacin enabled the absolute configurations of the molecules to be predicted, hypotheses which were later confirmed by analytical chemistry (19,20).

The availability of the genome sequence of *S. cellulosum* is a milestone in myxobacterial natural product research, having already facilitated the yield optimisation and detection of new substances through genomic mining. Through the planned decoding of the genetic information of other *S. cellulosum* and myxobacterial strains it will soon be possible

to conduct a more targeted search for unknown substances, and to improve production in these promising bacteria. In addition, the genome sequences will obviously provide a wealth of information to study the biological processes described above.

A third emphasis for future efforts, alongside establishing new analytical methods and genome analysis, is to try to isolate novel groups of myxobacteria from unexploited biotopes, by altering the isolation conditions. One successful example of this strategy is the discovery of strains of moderately thermophilic myxobacteria (21). These bacteria thrive in temperatures of 40–45 °C, and derive primarily from soil samples taken in the Mediterranean region. In the case of halophilic myxobacteria, we have concentrated our search on terrestrial, salt-rich biotopes such as potash-mine dumps and inland salt marsh, regions where salt deposits extend to the surface. Both biotopes are even well represented in Lower Saxony. In fact, we have already isolated myxobacteria which can tolerate high salt concentrations but, more importantly, require it for growth. Analysis subsequently showed that, as hoped, this group of myxobacteria produce novel secondary metabolites.

Overall, it appears that the outlook for myxobacterial natural product research has never been better. Classical methods coupled with advances in molecular biology, genomics, screening methods, synthetic chemistry (22-26) and analytics, have significantly increased the future potential for identifying novel compounds, and for better exploiting established substances.

Acknowledgements We would like to thank Niña Socorro d. J. Cortina and Kira J. Weissman for help with the translation of this article. Research in R.M.'s laboratory was supported by grants from the DFG and the BMBF.

The research group of Microbial Drugs at HZI *Photo: HZI*

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Klaus Gerth born in 1946, studied Biology and Chemistry at the University of Freiburg (1967-1973). Thesis at the Institute of Microbiology, Freiburg; Dr. rer nat. 1975. Since 1975 senior scientist at the German Research Centre for Biotechnology (GBF)/ Helmholtz Centre for Infection Research (HZI) where he significantly was involved in the development of myxobacteria as source of secondary metabolites. Highlights were the discovery of the antifungal compound soraphen and the detection and improvement of the antitumor agent epothilone.

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Targeted Diagnosis of Pathogens

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Infections in humans can manifest, spread and reach life-threatening conditions very fast. In cases of sepsis a delay in treatment will dramatically reduce the chance for survival. The speed, in which severe infections progress, generates a necessity for a quick and precise description of the pathogen that accelerates the process of finding the appropriate regimen. Infections that proceed with slower pace also require thorough characterisation, if complications and sequelae can be predicted, they may be avoided by more targeted treatments. Moreover, a diagnostically indicated and optimised medication, instead of an empirical one, promises to slow down the development of antibiotic resistance. The vast variety of pathogens, their high genetic diversity and plasticity are a major challenge for microbiological diagnostics. Different strains belonging to the same species of pathogen can harbour different virulence factors and are therefore capable of causing different diseases. For successful intervention strategies, it is important that the treating physician not only knows which species has caused the disease, but also which particular strains, with information on their pathogenic properties. The emphasis in the future will be on "target diagnosis" to identify the causative strain. To achieve this, a number of different strategies will need to be employed, all of which are based on the basic research dealing with the functional characterisation of virulence factors.

Development of pathogen diagnostics In the second half of the 17th century, Antoni van Leeuwenhoek made use of the young invention the "microscope" to demonstrate the existence of bacteria. Two-hundred years later, at the time when Robert Koch discovered a pathogenic microorganism for the first time – the causative organism of pulmonary tuberculosis - the microscope was a crucial diagnostic instrument. Further differentiation was made possible by staining techniques that were developed by, amongst others, Paul Ehrlich and Hans Christian Gram. Microorganisms have been and still are distinguished and classified on the basis of morphological, phenotypical and sensory attributes and characteristics (Tab. 1).

	attribute
sensory	odour colony consistence
macroscopical	colour colony shape hemolysis
microscopical	shape of the organism nucleus: prokaryotic / eukaryotic flagellated spore forming

Tab 1. Some morphologic, phenotypic and sensory attributes of microorganisms.

Typing, down to the level of subspecies, has been made possible with the invention of biochemical tests based on reactions with chromogenic substrates and immunological methods that detect and discriminate specific surface structures. Immunological tests are used for the detection of infections in blood samples, e.g. infections with *Borrelia burgdorferi*, the causative organism of Lyme-borreliosis. The immune response of the host is the basis for sensitivity tests like the tuberculin skin test that is used for the diagnosis of tuberculosis (*Mycobacterium tuberculosis*). Immunological methods, as well as the electron microscope and modern DNA-based molecular biology methods allow detection and diagnosis of viruses, an inanimate form of pathogens. The polymerase chain reaction (PCR) is an efficient method in routine screening of donor blood for HIV and hepatitis viruses. This method allows pathogen-specific amplification and the detection of genes (Fig. 1).

The invention of the PCR and of automated DNA-sequencing today permit a detailed insight into the genomes of pathogens. Full genome sequencing has broadened the view of the diversity and plasticity of microbial genomes and the dynamics of the constantly ongoing microbial development. Comparative genomics will lead to an understanding of how pathogens develop but also show the limits of contemporary pathogen diagnostics.

Limits of contemporary pathogen diagnostic and novel alternative approaches Many of the classical methods that are mentioned above require isolation of the causative organism. Classical diagnosis is, if feasible at all, very costly in terms of labour and time. Routine diagnostics has to restrict itself to a certain repertoire of culture media in or-

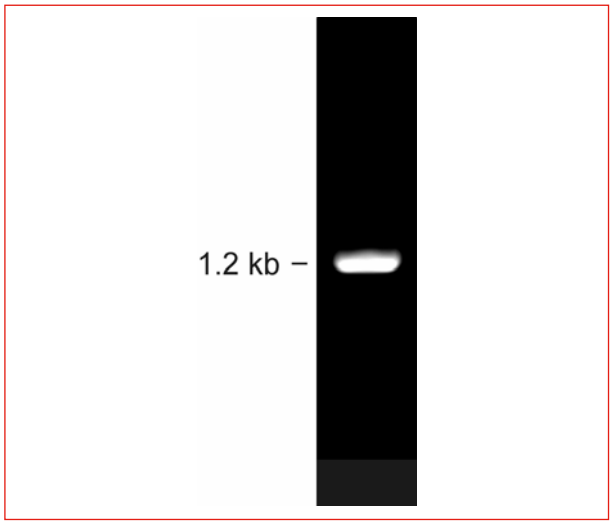

Fig. 1. PCR analysis of emm-*Genes from* S. pyogenes *and* S. dysgalactiae equisimilis. *Agarosegel electrophoretic analysis of the PCR for the* emm3-*gene of* S. pyogenes

der to remain feasible. Therefore, fastidious pathogens with very special nutrition and culture requirements remain undetected. Phenotypical, morphological and biochemical characterisation of cultivable pathogens often allows a detailed classification and is a sound basis for selection of a suitable therapy. In certain cases, however, these methods fail and the causative agent remains inadequately characterised. Pathogenicity of enterohemorrhagic C (EHEC) greatly depends on shiga-toxin genes in different variants. EHEC are food-borne pathogens and therefore an important matter in control screening for food safety. Presence of shiga-toxin genes in *E. coli* correlates widely with certain "immunological fingerprints" called serotypes. The immunological tests, however, are costly and shiga-toxin positive strains of atypical serotypes are not detected . Reliable and cost-effective assessment of the pathogenicity of *E. coli* strains requires the support of newly developed methods. The same applies for other pathogens.

PCR-based methods allow culture-independent tests in which fastidious and non-culturable pathogens can be detected which greatly increase both the time and cost efficiency of the examination. Stable marker-genes that allow determination of the species and that are suitable for PCR-based typing systems (genotyping) have been identified and evaluated and more of these will follow. Diagnostic genotyping that is based on virulence-associated genes like the shiga-toxin-genes of EHEC or emm-typing of *Streptococcus pyogenes* are promising approaches to gain fast insights into the pathogenic potential of a microorganism. But the informative value of genotyping also, has its limits. Full-genome analysis and the discovery of mobile genetic elements shed light onto the fast and extensive changes in the genomes of microorganisms. An intensive exchange of genes occurs in bacteria of the genus *Neisseria*. This complicates the diagnostics of gonorrhea (*Neisseria gonorrhoehae*). Transfer of marker genes to apathogenic *Neisseria* species can lead to false-positive results. Loss of marker genes in pathogenic species causes false-negative diagnoses. Bacteriophages and plasmids that carry virulence genes are transferred – also across species borders – and confer pathogenic properties to the recipient strain. Correlations between pathogenicity and certain genotypes are not fixed, they dissolve and lose their diagnostic value. Indications for gene transfer between different species have been found in streptococci . *S. pyogenes* and *Streptococcus dysgalactiae* equisimilis share a variety of virulence genes, among them is the emm-gene (Fig. 1). Emm-genes are a prominent example for virulence genes that encode a sole protein that has a crucial influence on pathogenesis, without being limited in its presence to a single species. Emm-genes code for a streptococcal surface protein, the M protein . Only recently a collagen binding motif of M proteins has been described (Fig. 2 and 3). This motif has substantial influence on the generation of autoimmunity against collagen, which leads to a typical and severe sequela of streptococcal infections, acute rheumatic fever. Therefore, the motif was named PARF (peptide associated with rheumatic fever). Not all M proteins have the ability to elicit such a rheumatogenic auto-immune response. This is a property that is limited to PARF-positive M proteins. Thus, PARF is a valuable marker for rheumatogenic strains

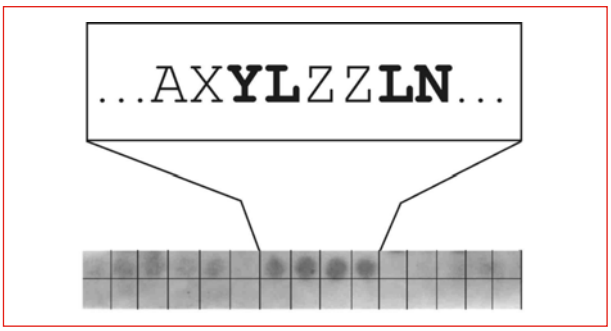

Fig 2. Peptide associated with Rheumatic Fever – the PARF-motif. In a peptide-array-experiment (lower part) collagen binds exclusively to the four immobilized PARF-peptides and appears as dark spot after detection. The PARF consensus motif is given above.

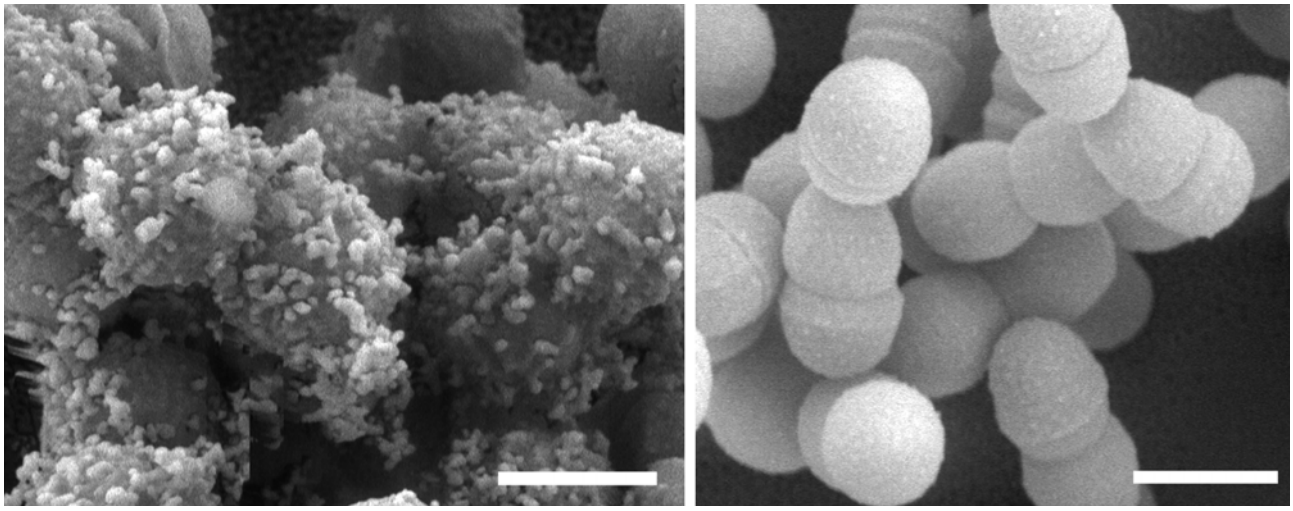

Fig 3. Collagen-binding rheumatogenic streptococci. Rheumatogenic streptococci bare M protein with a PARF-motif. This leads to binding and aggregation of collagen on the bacterial surface (left). The picture on the right for comparison shows streptococci without collagen-aggregates. *Photo: HZI, Rohde*

and diagnosis of infections with PARF positive strains is an indication for prolonged and intensive antibiotic treatment. An advantage of such markers that are directly involved in pathogenesis is a stable correlation with the pathogenic potential. Moreover such diagnostic tests are largely detached from the species and genotype of the organism.

Only in rare cases the pathogenic potential of a microorganism can be linked to only one virulence factor. Because of the high genetic variability of pathogens and because virulence is often determined by a variety of factors, progressive diagnostics research strives for tests that allow the determination of whole virulence factor profiles. Such tests utilise the DNA-microarray technique, which is able to detect the presence of several thousand genes in parallel. This technique is based on the immobilisation of gene-probes onto a support. In case of the presence of the specific marker, virulence or resistance gene, it will be bound by the corresponding probe and than visualised (Fig. 4). DNA-microarray allows extensive genotyping but, beyond this, information about the virulence factor profile promises insights into the individual character of the pathogen and a tailored treatment. A comprehensive knowledge about the functions of virulence genes and targeted therapeutics remain a prerequisite for such in-depth diagnostic approaches.

Outlook With the growing knowledge about the repertoire of virulence and resistance genes and about how the different gene-predicts influence each other, diagnostics can focus more on the detection of such factors. The required techniques are still costly and require considerable specific expertise. A lot of effort will be necessary to optimise these techniques for routine application. But in some cases, like the PARF-motif – it will be possible to translate our knowledge for use in simple tests that, perhaps soon, will allow a rapid and inexpensive diagnosis in the medical practice.

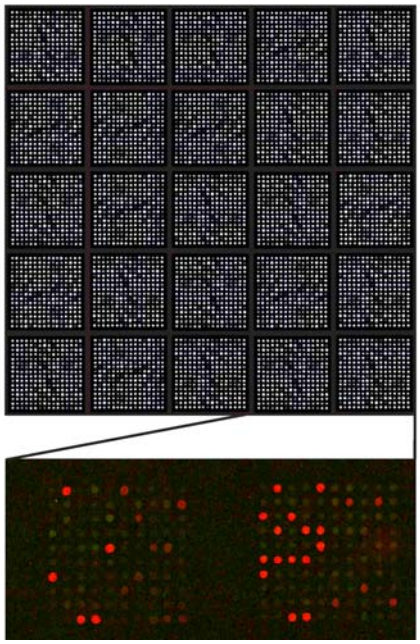

Fig 4. DNA-microarray for detection of streptococcal virulence factors. The 6400 oligonucleotide-probes of the DNA-microarray are visualized in the upper part of the figure. The detection of virulence associated streptococcal genes is shown in a magnified section in the lower part of the figure.

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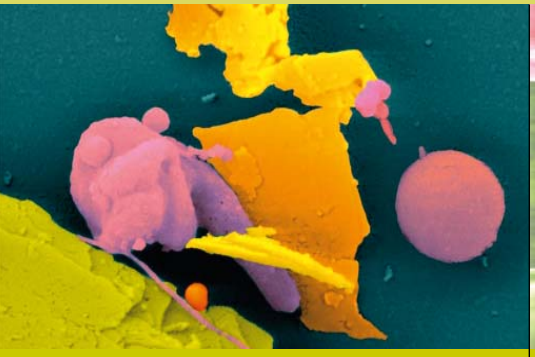
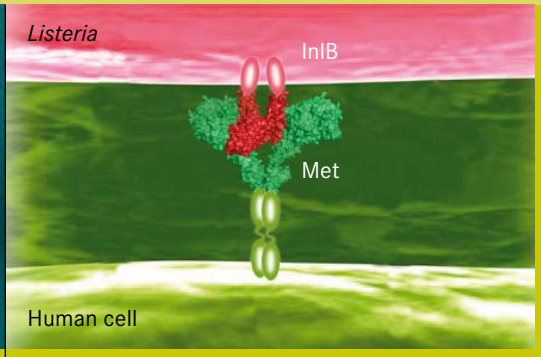
Photos from left to right: Dr. Olga Golyshina and Prof. Dr. Peter Golyshin controlling the growth of microorganisms | Ferroplasma acidiphilum *on pyrite* | *Schematic representation of InlB-mediated interactions between Listeria and human cells*

Photos: HZI, Gramann (le) | *HZI (ce)*





Ferroplasma acidiphilum: a Novel Microbe with a Unique Iron:Protein-Dominated Metabolic Apparatus

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Extremophilic microorganisms live in environments characterised by physico-chemical conditions hostile to life that exist at all biosphere:geosphere interfaces and at the vast range of other sites worldwide. Such extremophiles, which often belong to the *Archaea*, one of the three branches of the "tree of life", are phenotypically classified according to the extreme parameter that characterises their habitat: thermophiles, acidophiles, piezophiles, halophiles, and so on. Extremophiles exhibit defining physiological, metabolic and structural characteristics that enable them to tolerate the extreme conditions in which they live, and that are not found in mesophiles, organisms that live under what humans consider to be non-extreme conditions (though note that "extreme" conditions under which extremophiles live are, for them, "normal", whereas mesophilic conditions may be hostile, i.e. "extreme": it is all a question of individual perception!). Some extremophiles, such as acidophilic sulfur-oxidizing microbes, actually create and/or maintain the extreme conditions that characterise their habitat, through their metabolic activities. Whereas some conditions, *e.g.* high/low pH, presence of solvents, heavy metals, etc., can be excluded from the cytoplasm by specialised cell surface barriers, and thus prevented from affecting metabolic activities, others, *e.g.* extremes of temperature, pressure, ionising radiation, etc., cannot be excluded and must be specifically counteracted by specially adapted physiologies and metabolisms. Characterisation of the unique cellular and biochemical characteristics of extremophiles and causally linking these features to the hostile environmental conditions in which they live not only expands our knowledge of the range of life processes, but also defines and provides an understanding of the physical-chemical boundaries that limit life on Earth (and, by extrapolation, to possible life on other planets). Such research also reveals the contribution of extremophiles to the biogeochemical cycles that shape the biosphere, maintain biological elements in a life-sustaining balance, and regulate its functioning. Furthermore, extremophiles constitute a treasure trove for biotechnological applications that is only just beginning to be explored, although the economic importance of the so-called "extremozymes" produced by such microbes, enzymes tolerant of high temperature (*e.g.* Taq polymerase, used in PCR), low temperature, high/low pH, high salinity, presence of organic solvents, etc., is well established (Table 1). As a consequence, extremophile biology is currently one of the most discovery-rich study topics in life sciences research, and is not only experimentally exciting but also of great scientific and social importance.

Acidophiles Acidic environments are widespread in nature and hostile to life. A major one is the gastric content of mammals, previously thought to lack an indigenous flora, but now known to harbour acid-tolerant microbes, including *Helicobacter pylori*, a causative agent of gastric ulcers, and to permit the passage of certain acid tolerant microbes. Other such environments are geothermally-influenced waters, like acidic volcanic pools and geysers. One of the most extreme acidic environments is mine drainage streams made acidic by sulphur-oxidising microbes: these can reach a pH of 0. Acidophilic microbes, *i.e.* those having optima for growth lower than pH 3, play important ecological roles in such environments and are distributed among all domains of life, including the *Eukarya* (fungi), autotrophic and heterotrophic members of *Bacteria* (e.g. *Helicobacter pylori*) and members of the *Archaea*.

Although most microbes have intracellular cytoplasmic pH values around neutral, acidophiles tend to have acidic cytoplasmic pH values of 5-6 or below. Such values are maintained, even if external values are below 1, by cell surface structures and activities that restrict proton passage into the cytoplasm and transport protons from the cytoplasm across the membrane up the proton gradient. One important mechanism exploited by acidophilic organisms is the generation of a positive potential on the inner face of the cytoplasmic membrane, which creates a proton diffusion barrier and a Donnan potential. Another mechanism involves special

Extremophile Types	Extremozymes	Requirements, tolerance	Applications
Halophiles	• amylases • proteases	2-5 M NaCl	• manufacture of chemicals (pesticides, herbicides) • bioremediation • peptide synthesis
Thermophiles	• glycosyl hydrolases • xylanases • lipases • esterases • dehydrogenases • proreases • DNA polymerases	up to 120°C	• starch • chitin • cellulose pectin processing • paper bleaching • detergents • hydrolysis in food and feed • molecular biology purposes (e.g. PCR)
Psychrophiles	• glycosyl hydrolases • proteases • dehydrogenases • esterases • lipases • oxidases • peroxidases • catalases	down to 5°C	• detergents • food applications • cosmetics • biosensors • bioremediation
Alkaliphiles	• glycosyl hydrolases • proteases • lipases	up to pH 11.5	• detergents • food industry • cosmetics
Acidophiles	• glycosyl hydrolases • proteases • oxidases • esterases	down to pH 1.0	• starch production • desulfurization of coal • feed components
Piezophiles	• glycosyl hydrolases • hydrogenases • dehydrogenases	up to130 MPa	• antibiotic production • food industry

Table 1. Extremophiles, extremozymes and some examples of their applications.

chloride transporters, H^+/K^+ $(Na)^+$ anti-porters, and multiple secondary transporters. A third is the ether-linkages in archaeal cell membrane lipids, which make them less sensitive to acid hydrolysis compared with the ester linkages in bacterial membranes, and a bulky isoprenoid core, which is highly impermeable to protons.

Despite cytoplasmic pH homeostasis, acidophilic microorganisms with cytoplasmic pH values of 6 and below not only require low pH-adapted enzymes but are also confronted with a constant challenge to their genome stability, since deamination, oxidation and depurination of nucleotides – all mutagenic reactions – are favoured by low pH. Organisms with low cytoplasmic pH values seem to have evolved efficient mechanisms of DNA repair and the unusual feature of archaeal Y-family DNA polymerases of low fidelity on undamaged templates, together with an ability to replicate through regions of damaged DNA, are well documented. It should be noted that the genomes of extreme acidophiles contain a large number of determinants for chaperones, which suggests enhanced protein stabilisation activities are needed by and characteristic of such cells. It is also worth mentioning that some bacteria, such as *E. coli* and *Helicobacter pylori*, that grow normally at neutral pH values are also able to tolerate acidic conditions as a result of three acid resistance systems, namely up-regulation of chaperone-like proteins, changes in lipopolysaccharide composition and induction of ammonia-producing pathways.

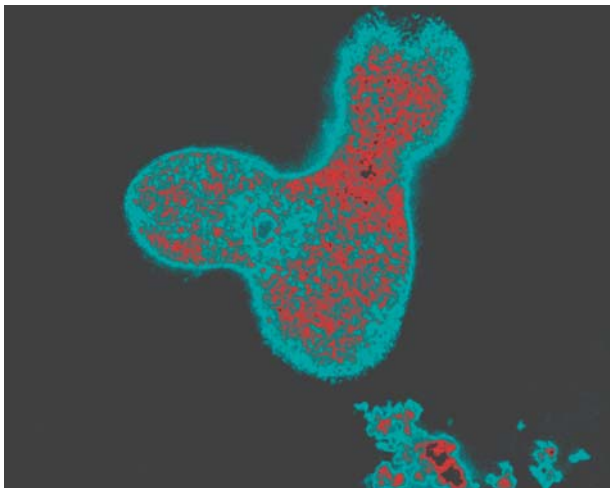

Fig. 1. Morphology of cells of F. acidiphilum: *pleomorphic shapes typical of cell wall-less microbes.* *Photograph courtesy of Heinrich Lünsdorf, HZI.*

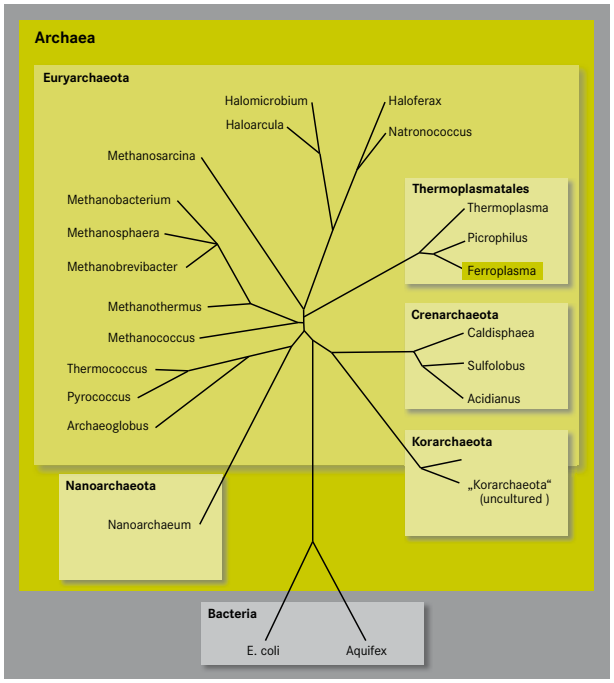

Fig. 2. Unrooted phylogenetic tree showing 16S rRNA gene sequence relatedness of major evolutionary lineages of Archaea.

Ferroplasma acidiphilum *Ferroplasma acidiphilum* (Fa) was isolated by us from a pilot bioreactor set up to study metal bioleaching by acid-producing microbes and fed with pyrite ores from Bakyrchik (Kazakhstan). It is an acidophilic, mesophilic, ferrous-iron oxidising, cell-wall lacking microbe (Fig. 1) that becomes the basis of a new family, the *Ferroplasmaceae*, of the Thermoplasmates, a branch of the *Archaea*, and its first non-thermophilic member (Fig. 2). It is chemoautotrophic: it obtains carbon by fixing CO_2 and energy by oxidising ferrous iron to ferric. Fa is doubly extremophilic, in that it requires in its habitat extremely low pH values and high concentrations of ferrous iron, which in practice means tolerance of high concentrations of the heavy metals that characterise iron-containing metal ores. The principal constituents of the cytoplasmic membranes of *Ferroplasma* are caldarchaetidylglycerol tetraether lipids (Fig. 3), which exhibit low proton permeability, as a result of the bulky isoprenoid core, and are probably a major contributor to the extreme acid tolerance of this cell wall-less microbe.

Members of the *Ferroplasmaceae* belong to the most acidophilic microbes investigated so far, and may be mesophilic or moderately thermophilic, autotrophic or heterotrophic. They are numerically significant members of microbial consortia of acidic environments in diverse geographical locations worldwide.

Unique acidophilic intracellular proteins Acidophilic proteins have applications in a number of biotechnological processes (e.g. xylanases in the bleaching of Kraft pulps, proteases and cellulases in the nutritional upgrading of animal feeds, amylases/glucoamylases in starch processing, oxidases in the desulphurisation of coal, etc.). *Ferroplasma* should *a priori* be a good source of acid tolerant enzymes, since at least those enzymes excreted into the external medium must be acid tolerant. In order to assess this possibility, we cloned and expressed a number of Fa proteins in *E.coli* and determined the cellular location of the native proteins in *Ferroplasma*. In fact, we did not detect any extracellular proteins from *Ferroplasma* and all those cloned and expressed in *E. coli* were either cytoplasmic or membrane located. These were 3 α-lycosidases and 1 esterase, enzymes that play central roles in metabolism: glycosidases are involved in carbohydrate metabolism, glycosylation of lipids (most archaeal lipids are glycosylated) and in energy processing, whereas esterases participate in the synthesis of cofactors and precursors of macromolecules and in energetic processes. Surprisingly, given that all of the enzymes studied were cytoplasmic or membrane bound, and that the mean pH of the cytoplasm of other acidophilic organisms was measured as neutral or slightly acidic, all of the cloned enzymes from *Ferroplasma* had *in vitro* activity optima in the pH range 1.7-4.0, *i.e.* up to 3 pH units lower than that of the cytoplasm (Fig. 4). These findings are not only *a priori* surprising but they stand in stark contrast to intracellular enzymes cloned from other extremophilic microorganisms (bacteria and archaea) which have *in vitro* pH activity optima close to the measured intracellular pH values.

In addition to these enzymes, DNA ligase (see below) also has an extremely low pH activity optimum. Thus, 5/5 enzymes purified and characterised from Fa have pH optima

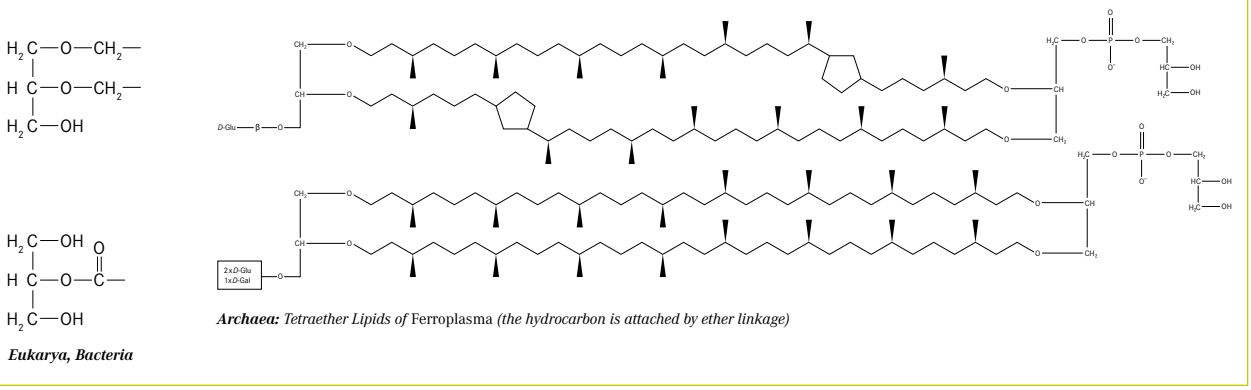

Fig. 3. Microbial membrane lipids. The main defining features of lipids in Archaea *are ether-linked molecules in contrast to the ester-linkages in lipids of Eukarya and Bacteria. Tetraether lipids of* Ferroplasma *and other acidophilic archaea are probably a key means of survival in conditions of extreme acidity.*

far below that reported for the cytoplasm. Although this is 100%, 5 of perhaps 3000 proteins is not statistically robust. Nevertheless, we assume at this point that the proportion of *Ferroplasma* proteins active at very acidic pH is substantial. Although the reason for this "pH optimum anomaly" is presently unknown, one possible explanation might be cytoplasmic heterogeneity, since pH measurements for cytoplasm provide average values, and distinct pH values might be present in different cellular compartments with distinct local physical-chemical environment for the proteins. Another reason may be that these proteins possess high surface densities of positively charged amino acids, since it is known that one mechanism by which acidophiles counteract the large transmembrane pH gradient to prevent proton influx is the generation of a positive intracellular potential. Known examples of acidic eukaryotic cellular compartments having a net positive charge include mitochondria, lysosomes, erythrocytes, collagen fibres, and secretory vacuoles, in which an acidic pH is required for organelle functions, such as hydrolysis of macromolecules, release of ligands from receptors, processing of hormones and protein sorting. In bacteria, acidocalcisomes, organelles whose function is that of energy storage and acidity control, exist. It cannot at present be excluded that *Ferroplasma* has evolved some unique, so far undescribed mechanism to survive in extremely acidic environments.

All proteins homologous to the Fa α-glucosidases that can be predicted from the genome sequences of other archaea have been previously annotated as having functions different to those experimentally determined in our study, reflecting a certain inadequacy of reliance on homology-based genome annotations. For instance, α-GluFa, one of the Fa α-glucosidases, exhibited a high similarity to "uncharacterised membrane protein" belonging to the COG1287 found in almost all archaeal genomes and in the pathogenic bacteria *Helicobacter* spp., *Campylobacter* spp. and in the yeast *Saccharomyces cerevisiae*. Thus, we have shown that α-GluFa is a new member of the glycosyl hydrolase family exhibiting a new mechanism for sugar glycosylation and transglycosylation.

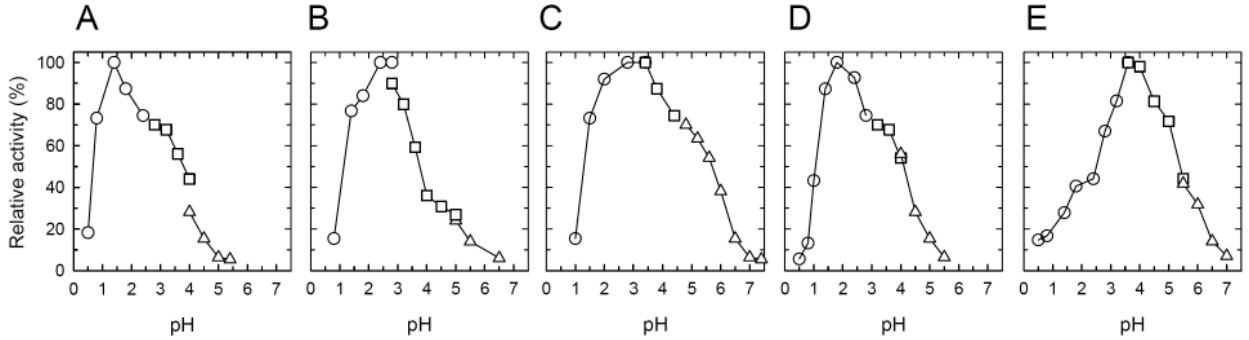

Fig. 4. "pH optima anomaly" of intracellular enzymes of Ferroplasma in vitro *(s.* Environmental Microbiology *2006,* ***8(3),*** *416-25).*

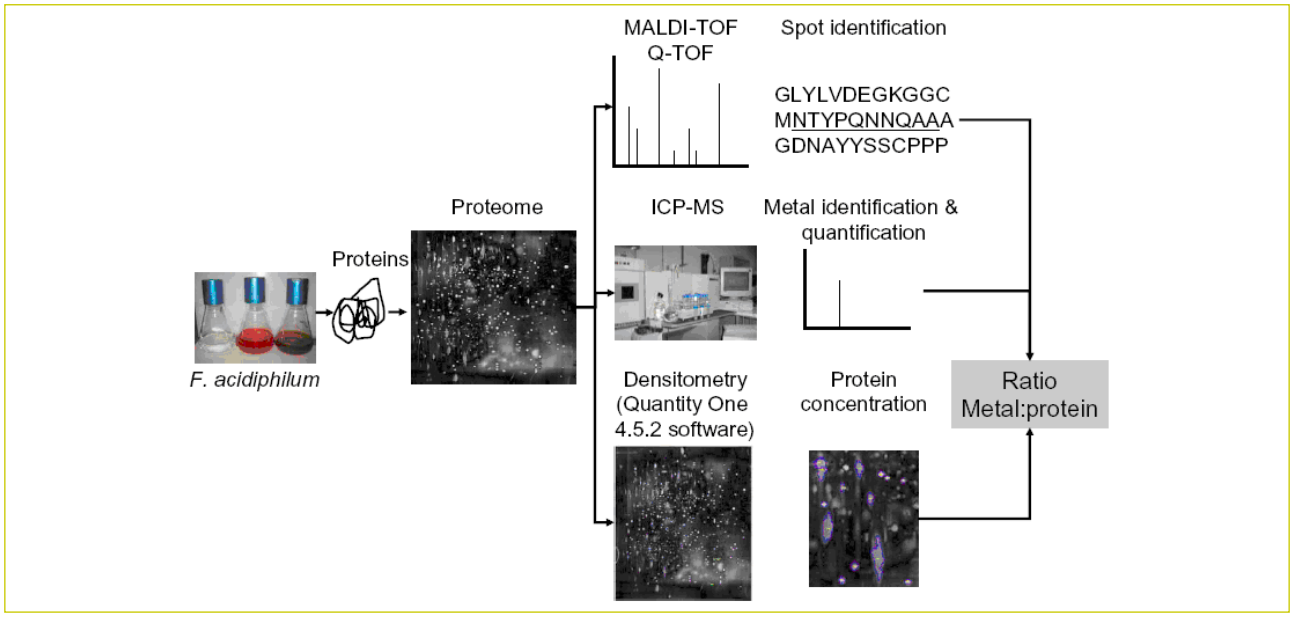

Fig. 5. Proteomics combined with ICP-MS to identify metalloproteins in Ferroplasma *and determine the metal:protein stoichiometry (s.* Nature *2007,* ***445****, 91-94).*

***Ferroplasma* has an iron-metalloprotein dominated metabolic machin ery** Another surprising feature of the purified *Ferroplasma* proteins is that they all contain iron in stoichiometric amounts: no related enzymes from other organisms have previously been shown to contain iron. Removal of the iron led to unfolding of the proteins and loss of activity, so iron is crucial for maintenance of their three-dimensional structures, and hence their activities. Multivalent iron plays a key catalytic role in a number of proteins, like oxygenases, in which it activates the oxygen co-substrate, and is held in the enzyme reaction centres by strategically placed amino acid ligands. In the Fa proteins, it seems to play the contrary role, namely to hold the amino acid ligands in a strategic 3-D formation, i.e. to organise the 3-D structure of the enzymes, a function we designated as the "iron rivet".

Again, 5/5 is 100%, but not statistically robust. In order to obtain statistically better numbers of iron-containing proteins of *F. acidiphilum*, we displayed the Fa proteome on 2-D gels, cored discrete spots, and determined their sequences, amounts, and amounts of metals (Fig. 5). Incredibly, 86% of 189 distinct cellular proteins were identified as iron-metalloproteins containing stochiometric amounts of iron. These included many housekeeping proteins with catalytic, chaperone and structural roles, homologues of which from other organisms are not known to contain either iron or other metals. Analysis of the proteomes of the closest phylogenetic neighbour of *Ferroplasma acidiphilum*, *Picrophilus torridus*, and of a habitat neighbour, the bacterium *Acidithibacillus ferrooxidans*, revealed that they contained far fewer and only typical metalloproteins. *F. acidiphilum* has therefore a unique iron-protein-dominated cellular machinery and biochemical phylogeny.

What could be the explanation of the uniqueness of the iron rivet to Fa? Iron is the fourth most abundant chemical element on the Earth, and is crucial for diverse physiological, metabolic and catalytic functions. It is, however, poorly water soluble and thus poorly bioavailable, and, in the nutrient-poor open ocean, is often the main factor limiting primary production (biomass production) by photosynthesis (thus, efforts to reduce greenhouse gases by increasing CO_2 fixation in marine systems involve iron addition: iron "fertilization"). Organisms invest significant energy to acquire and retain iron for their metabolic needs and competition for bioavailable iron between organisms, involving extremely high affinity iron capture, uptake and complexing/transport systems, is fierce, involving theft from one by another, and often determining the outcome of "infection battles" between parasitic organisms and their hosts. The one exception in the global iron-limited biosphere is acidic iron-rich environments, which, because of the increasing solubility of iron with decreasing pH, contain bioavailable iron in abundance. However, the simple notion that the iron rivet is a preferred protein organiser, and only lacking in most organisms because they are iron limited, cannot be true because other iron-oxidising microbes, such as the bacterium *Acidithiobacillus ferrooxidans*, inhabiting the same iron-replete habitats have normal proteins. Thus, the hypothesis that an ancestor of *F. acidiphilum* evolved a new iron-based protein stabiliser after colonising acidic pyrite-rich environments would seem to be unlikely, since others did not. More probable, therefore,

is a contrary hypothesis that assumes the iron rivet to be an ancient property that predated currently prevalent protein organisers – ionic interactions, hydrophobic interactions and leucine zippers, α-helices, ß sheets, S-S bridges, etc. – which evolved later in response to poor iron bioavailability. This would imply that both iron riveted proteins and *F. acidiphilum* are so far rather unique.

An intriguing possibility we have therefore considered is that iron rivets are an ancient property that has been retained solely by *F. acidiphilum*, and that Fa has a thus far unique evolutionary trajectory. This possibility is based on one of the theories of the origin of life, proposed by Günther Wächtershäuser, which invokes iron-sulphur-catalysed chemistry on iron-sulphur-rich, energy-rich surfaces, like pyrite, catalysing the initial formation of simple organic molecules, their diversification, and the subsequent formation of the complex monomeric and polymeric molecules characteristic of life. As reaction specificity evolved through the replacement of rigid small molecule catalysts by flexible protein catalysts (enzymes), some of which retained iron-sulphur catalytic centres, so the need arose to stabilise the inherently flexible long polypeptides. Multivalent iron, abundant in these sites where early life may have evolved, may have became the first effective structure-organiser and stabilising element in proteins and the forerunner of other protein organisers. It may be noted that acidic, iron-rich conditions were characteristic of prevailing volcanic conditions during the Archaean and early Proterozoic periods.

The subsequent radiation of early cellular forms of life, the prokaryotic microbes, from environments rich in iron and sulphur inhabited by low diversity microbial communities to other environments of the planet offering an enormous range of nutritional opportunities that drove exceptional diversification of early microbes, and characterised by higher pH conditions and low iron bioavailability, would have been a powerful selective force for the evolution of an iron-independent protein machinery. As a result, iron would have been retained only for functions such as oxygen activation/co-ordination by iron-sulphur clusters and haem, which cannot be accomplished as effectively by other structural elements. This suggests the possibility that, unlike other habitat and phylogenetic neighbours, the ancestors of organisms related to *Ferroplasma* might have evolved entirely within acidic pyrite habitats and their protein repertoire might represent a unique ancient form of life.

On the other hand, we found that approximately 15% of Fa proteins lack iron, suggesting that these have normal protein organisers which are presumably more effective than iron. Such proteins could have been acquired by horizontal gene transfer from habitat neighbours, like *Acidithibacillus ferrooxidans*, whose evolutionary trajectory involved a non-pyrite phase.

LigFa: a purple, acidophilic, iron-containing DNA ligase

Acidophiles tend to have cytoplasmic pH values below neutral. DNA ligases are key players in genome replication, recombination and repair, and are cardinal enzymes in the central processes of cell division and maintenance of genome integrity in all cellular systems. However, available information indicates that DNA ligases function suboptimally at lower than neutral pH levels. It was therefore a major surprise that purified DNA ligase of *Ferroplasma*, LigFa, has an *in vitro* activity optimum at pH 2.5-3.0, an activity range from pH 1-4, with 80% maximal activity at pH 1.5-2.0, and no detectable activity above pH 5.0. No other DNA ligase exhibits this property. Also surprising was that the catalytic activity of LigFa neither depended on nor was stimulated by the addition of magnesium or potassium, obligatory cofactors of all other known DNA ligases. LigFa has a beautiful purple colour (Fig. 6), again unique among DNA ligases, and a corresponding absorption spectrum, indicative of iron-tyrosinate interactions. Mössbauer spectroscopy confirmed that LigFa contains two ferric ions per molecule that undergo local electronic changes during substrate DNA binding.

Fig. 6. Purified DNA ligase of Ferroplasma acidiphilum (s. Proceedings of the National Academy of the Sciences of the USA 2008, 105(26), 8878-8883). Photo: HZI

A possible non-specific binding of iron to the proteins of *Ferroplasma* was excluded by site-directed mutagenesis studies that revealed a specific genetic basis of iron as an essential component of LigFa and of acidophily/acid tolerance. These studies identified phenylalanine-192 and glutamic acid-134 as key amino acids in the acidophilic phenotype of the enzyme, and tyrosines-55 and -129 to be involved in two ferric-tyrosinate interactions, determining the characteristic purple colour of *Ferroplasma* ligase. These latter two amino acids, and Phe-192, are probably ferric iron ligands in LigFa.

Low pH promotes depurination and hence mutational change, and iron readily mediates redox reactions that generate mutagenic oxygen radicals, which also cause mutagenic modifications of DNA bases, as well as enhancing lipid peroxidation, and alteration of calcium and sulfhydryl homeostasis. The low pH activity optimum and essentiality of ferric iron are thus biochemically counter-intuitive properties of an enzyme responsible for genome integrity and stability.

However, it remains to be determined whether or not the *in vitro* activities of LigFa reflect its *in vivo* activities. In any case, it seems likely that a restrictive co-ordination of the ferric iron centres in LigFa minimise its redox activity. It is interesting to note that a number of disease states, such as tumour initiation, atherosclerosis, inflammation, heart infarction and stroke, are either characterised by or may result from cellular acidosis. Knowledge of DNA repair processes at low pH and, in particular, the availability of enzymes like LigFa able to carry out such processes, may ultimately provide new strategies and therapeutic agents to mitigate the DNA-damaging effects of cytoplasmic acidification and the resulting disease initiation in humans.

Given the extraordinary in vitro properties of LigFa, the question arises as to whether other DNA ligases from phylogenetically-related and/or ecophysiologically similar, acidophilic habitat neighbours of *Ferroplasma* are similar to LigFa. We therefore isolated and characterised DNA ligases from the hyperacidophilic microbes *Thermoplasma acidophilum* and *Picrophilus torridus* (the closest phylogenetic relatives of *Ferroplasma* – all three from the order *Thermoplasmatales*, phylum *Euryarchaeota*), *Sulfolobus acidocaldarius* (an archaeon from the phylum *Crenarchaeota* inhabiting sulfur- and metal-rich acidic high-temperature environments), and *Acidithiobacillus ferrooxidans* (an iron-oxidizing bacterium inhabiting the same ecological niches as *Ferroplasma*). In contrast to LigFa, all DNA ligases from these phylogenetic and habitat neighbours were unremarkable in terms of pH activity optima (around neutral) and metal requirement (no iron in the native enzyme and a strict requirement for added Mg or K for catalysis), and thus typical of other DNA ligases characterised thus far from all domains of life. LigFa thus remains so far unique: it is the paradigm of a new type of DNA ligase, other members of which await discovery, the characterisation of which will open new windows of knowledge in biology and may also have interesting new applications in medicine and biotechnology.

Concluding remarks *Ferroplasma acidiphilum* is a thus far unique form of life with a cellular metabolic machinery dominated by iron-metalloproteins, those of which have been characterised having extremely acidic pH activity optima. Since Fa grows only very slowly and does not form individual colonies on solid media, it is arduous to carry out physiological experiments and thus far impossible to conduct genetics with it. Much of the detailed work described here has involved proteins expressed in *E. coli* and future work will continue to exploit this genetic workhorse. However, the current sequencing of the Fa genome in the HZI will open new functional genomics perspectives for investigating the physiology and biochemistry of this fascinating organism. In addition, our recent isolation of new *Ferroplasma*-like microbes from volcanic environments will allow us to assess how unique this organism is and how it has evolved in relation to its close phylogenetic neighbours. More generally, our understanding of the physiology and biochemistry underlying the lifestyles of acidophiles and S- and Fe-oxidisers will be significantly advanced through the study of *Ferroplasma* and its relatives. And *Ferroplasma* will undoubtedly prove to be a treasure trove of acidophilic and acid tolerant enzymes for biotechnological application in a wide range of spheres.

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The Internalin Story – Lessons from Structural Infection Biology

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Structural biology is a key discipline in modern biology that provides detailed information on biomacromolecules and their three-dimensional structure. Such detailed structural data is crucial in understanding the molecular principles and interactions that underlie physiological and pathological processes of cells and organisms.

Research within the Division of Structural Biology at the HZI is focused on the structural elucidation of proteins central to infection processes. Techniques employed include X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. The interactions of the microbial pathogen with its host organism at the molecular level are of particular interest. In other words, we would like to understand how individual microbial pathogenic factors are able to specifically interact with their respective host cell receptors and how this interaction is able to initiate a course of events that is central to survival and propagation of the pathogen - and conversely to the detriment of the host.

For some time, we have concentrated on one family of proteins, namely internalins from *Listeria monocytogenes.* By investigating the delicate interplay of individual members of this family of invasion proteins with their human receptors at the atomic level, we have described these protein complexes in unprecedented detail. Based on these data, we have developed new strategies and tools to broaden our understanding of bacterial adhesion and invasion as well as human receptor signalling.

Introduction The Division of Structural Biology (SB) at the HZI is optimally equipped to pursue its primary goal of elucidating the three-dimensional structures of proteins involved in infection processes. A major area of focus within the division is the field of bacterial adhesion and invasion. Other topics include bacterial survival within the host cell, viral maturation and the formation of physiological and pathological amyloid fibrils.

Pathogenic microorganisms characteristically produce dedicated molecules or so-called virulence factors that are able to subvert host cell physiological processes as part of an infection. Such virulence factors (mostly proteins) emulate, modify, redirect or suppress individual host cell processes or entire signalling cascades to benefit the invading pathogen. By employing a limited arsenal of host-specific virulence factors the pathogen is often able to attach to specific cells of the host and then invade and spread systemically within the host cell, leading to disease or possibly even death of the host organism.

The principal goal of SB is the structural elucidation of recognized microbial virulence factors from various pathogens, ideally in complex with their respective host cell receptors. The structures of these proteins and protein complexes not only shed light on the molecular mechanisms of infection but also provide a basis for the development of small molecule compounds that specifically interfere with these processes and eventually pave the way to the desig of new pathogen-specific antibiotics.

Seeing is believing: from crystals to atomic structures
The roots of modern X-ray crystallography stretch back more than a hundred years to the discovery of X-rays themselves and to the realisation that crystals interact and deflect these rays according to defined rules. Today, X-ray crystallography is a powerful technique that allows the *de novo* determination of protein or nucleic acid structures at high resolution. Before the X-ray diffraction experiment itself can begin, however, the molecule or complex of interest must first be purified to near absolute homogeneity and crystallised – a procedure that can take anywhere from hours to months. Micrometer sized protein crystals are grown from supersaturated protein solutions to which suitable salts and other precipitating agents have been added.

A single, fragile protein crystal is then placed into the path of an intense X-ray beam, ideally produced by a powerful synchrotron source, and slowly rotated during exposure. Using suitable detectors the resulting X-ray diffraction

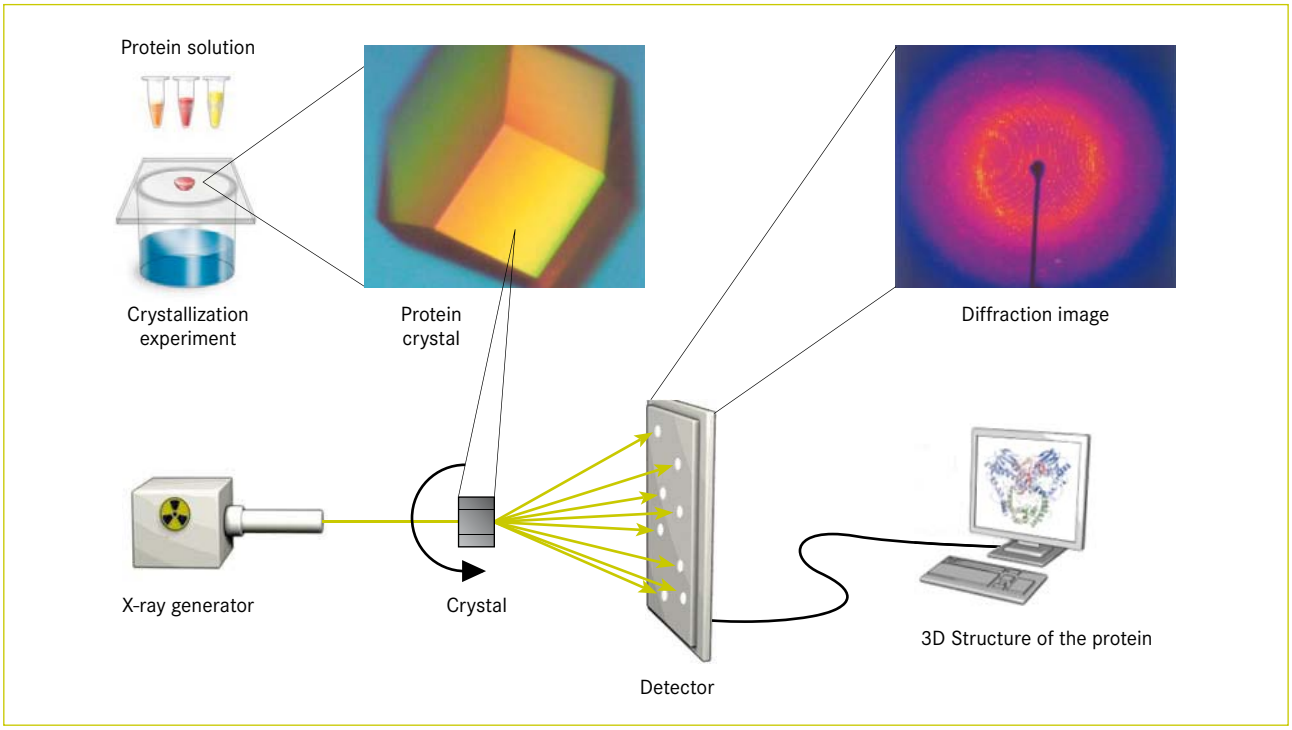

Fig. 1. From crystals to structures

patterns are recorded and stored electronically. The patterns are evaluated by specialised computer programmes and the data are used to re-create the distribution of the electrons within the crystals. Based on this distribution each atom of a protein may be identified and placed in its correct position within the crystal, generating a complete and precise description of the three-dimensional structure.

The bacterial intruder *Listeria monocytogenes* *Listeria monocytogenes* is a bacterium that infects both humans and other mammals. It is spread by contaminated food and is of particular danger to people with a weakened immune system. These include neonates, pregnant women and the elderly. *L. monocytogenes* is a well established model system for intracellular bacterial pathogens and is frequently used to investigate the cellular response of the immune system. Many research groups, some of them located at the HZI, have investigated the infection process of *L. monocytogenes* and the resulting immune response over many years. The bacterium is able to breach the intestinal barrier mainly constituted by the gut epithelium. It then spreads within the body, giving rise to listeriosis, a systemic and often fatal disease. *L. monocytogenes* can force its way into and survive within individual host cells, avoiding inter alia detection by antibodies. In fact, the pathogen uses a limited set of dedicated virulence factors to divert host cell processes, which result in host cells actively participating in the uptake of bacteria.

In *L. monocytogenes* two proteins, Internalins A (InlA) and B (InlB), are involved in cellular invasion. InlA is specifically required for the first step of the infection, the breaching of the intestinal barrier. As a result InlA is specific to epithelial cells. InlB, by contrast, has a much wider host cell specificity and is essential to establish a systemic infection. Both proteins are members of a larger group of related surface proteins in this organism, appropriately named the internalin family. All family members share a central region created by tandem sequence repeats of 22 amino acids known as leucine-rich-repeats (LRR). These repeats form a curved, tube-like structure that in the case of InlA and InlB specifically interacts with receptor proteins on the host cell. InlA recognises E-cadherin, a transmembrane protein that normally ensures the tight interaction of neighbouring cells in the human intestinal epithelium. InlB binds the human cell-surface protein Met, whose natural responsibility is

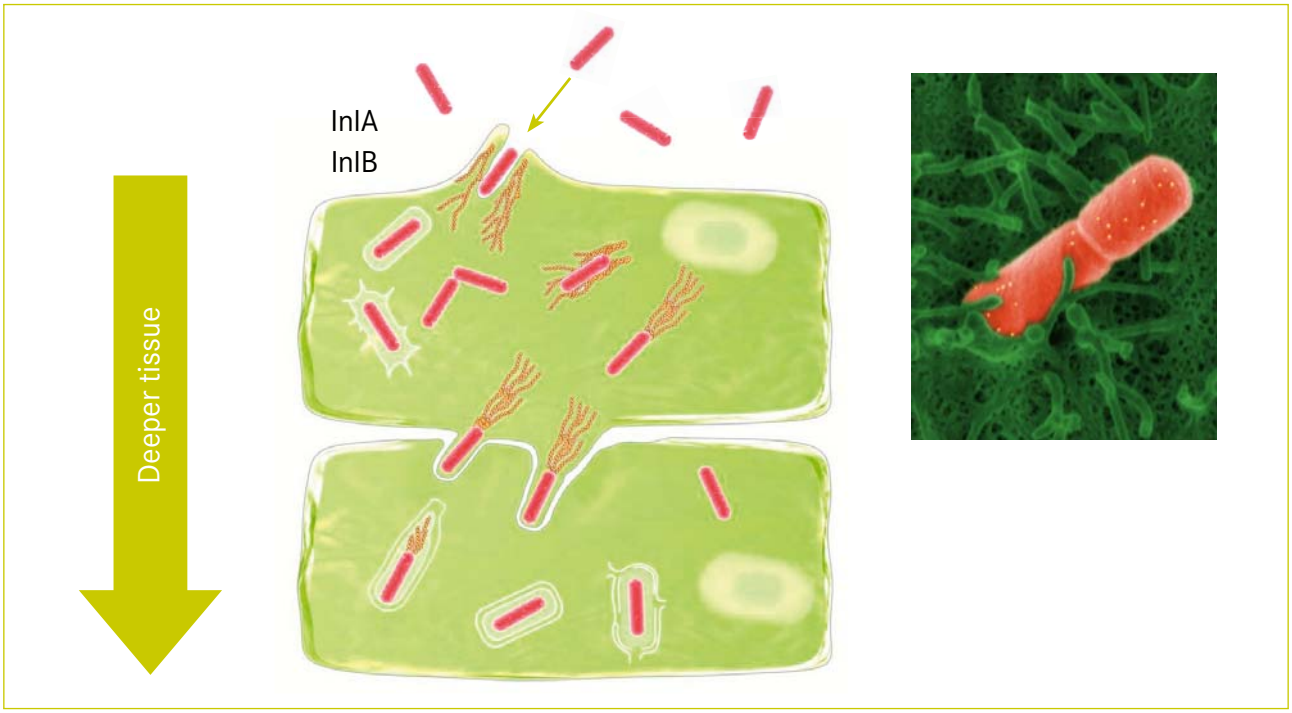

Fig. 2. Listeria *infection process*

to transmit signals of the human hepatocyte growth factor (HGF) across the plasma membrane into the cell interior. Met signalling is critical during embryogenesis, tissue regeneration and tumour metastasis. Remarkably, InlB is an almost perfect functional mimic of HGF, despite the lack of sequence and structural similarity.

A closer view at bacterial invasion In 2002 we were able to solve the crystal structure of InlA in complex with the N-terminal domain (hEC1) of human E-cadherin. This provided a first detailed picture of the initial step of human infection by *L. monocytogenes*. In this structure the curved LRR-domain laterally siezes hold of the smaller hEC1 domain. However, despite many direct and water-mediated interactions linking the two proteins, the overall affinity of InlA for E-cadherin is surprisingly weak. This structure also revealed on a molecular level why *L. monocytogenes* is unable to reproducibly infect mice via the oral/intestinal route. This question had puzzled experts in the field for many years and had forced researchers to inject bacteria intravenously to simulate human listeriosis in mice. Intravenous injection of bacteria is, however, a poor model of human listeriosis, as it only mimics the very late stage of this disease. The reason for the species specificity of *L. monocytogenes* can be mapped to the amino acid at position 16 of E-cadherin. The human protein has a proline at this position comfortably accommodated by a hydrophobic pocket on the concave interface of the LRR-domain of InlA. In murine E-cadherin, the small, hydrophobic proline is replaced by a longer, negatively charged glutamate giving rise to both steric and electrostatic repulsive forces between the two proteins, preventing their mutual recognition and interaction.

The structure of InlB in complex with the extracellular domain of human Met was solved and published in 2007. Some analogies with the complex of InlA-E-cadherin are apparent, but mostly the mode of interaction and activation are entirely unrelated. InlB, like InlA, binds the receptor predominantly through the concave face of the curved LRR. The extracellular part of Met consists of six domains and the one recognised by InlB is a small β-sandwich domain, comparable to the hEC1 domain of E-cadherin. However, whereas the contact surface area between InlB and Met is less than half the size of the InlA/E-cadherin-complex, the interaction of the first two proteins is some 1000-fold tighter than that of the latter pair.

In binding to Met, InlB contacts two of six domains of the extracellular domain of Met. As a result, the overall conformation of the extracellular domain is forced to change, presumably initiating signal transmission across the membrane. Comparing the interaction of Met and InlB to that of Met and its natural human ligand HGF reveals distinct binding sites and indicates that the receptor Met can be activated in very different ways.

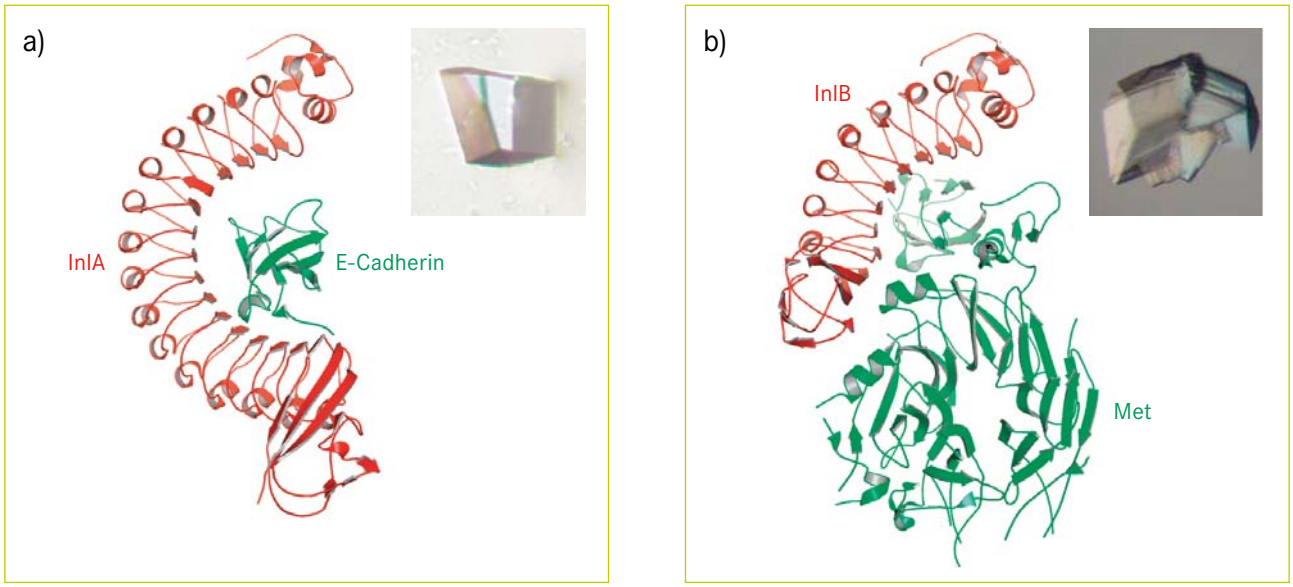

Fig. 3. Structures of the complexes between a) InlA/E-cadherin and b) InlB/Met. Inset: Crystals of complexes

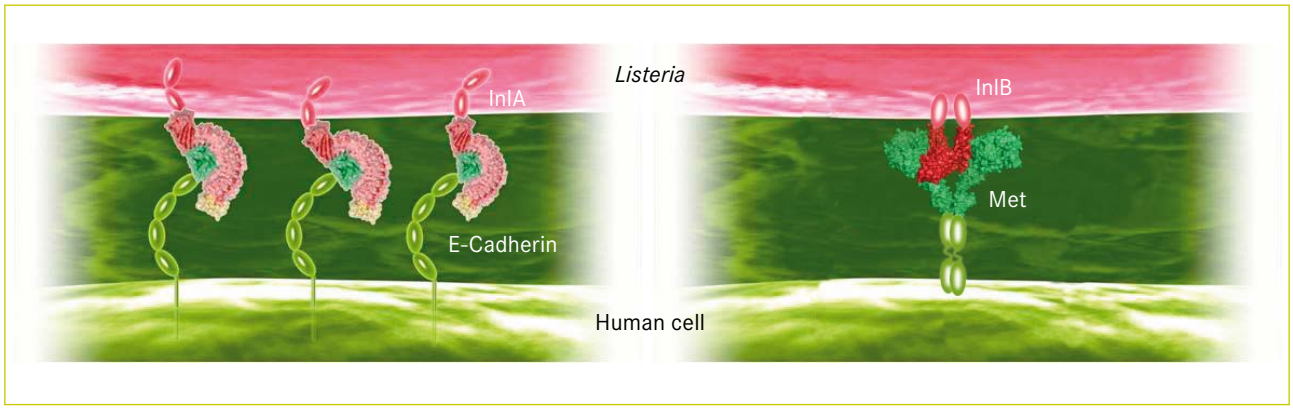

Fig. 4. Schematic representation of InlA- and InlB-mediated interactions between Listeria *and human cells*

Of mice and men The unexpectedly weak affinity of InlA towards human E-cadherin prompted us to investigate whether the interaction of the two proteins could be strengthened by substituting individual amino acids of InlA lying in the interprotein contact surface. A detailed analysis of the interface indicated four potential single residues which, when replaced by more suitable amino acids, could improve the intermolecular contact. A combination of two of these substitutions (serine 192 substituted by asparagine and tyrosine369 substituted by serine) indeed proved to dramatically increase the affinity of the two proteins by more than four orders of magnitude.

Perhaps even more striking was the observation that affinity of this InlA variant (termed $InlA^m$) towards the N-terminal domain of murine E-cadherin (mEC1) is comparable to that of wild-type InlA for human E-cadherin. The crystal structure of the complex of $InlA^m$ and mEC1 revealed that the unfavourable glutamate at position 16 of mEC1 (that prevents the contact with InlA, see above) is efficiently outweighed by the two additional points of contact between the two proteins.

We replaced the gene *inlA* of wild-type *L. monocytogenes EGDe* with a correspondingly modified gene $inlA^m$ to generate a modified bacterial strain named Lmo-$InlA^m$. This strain displays increased adhesion and uptake into a human epithelial cell line – however, only by a factor of two. Higher binding affinity does not automatically lead to higher infectivity. In addition, this strain is thus not significantly more infectious to humans than the wild-type strain is. Perhaps more interestingly, however, this strain is able to infect mice when applied by the intestinal route. By altering or "murinising" a bacterium, we have thus generated a widely applicable small animal model system to study human listeriosis. The structure-guided introduction of a mere two mutations in the gene encoding the protein InlA is thus sufficient to extend the range of the human pathogen *L. monocytogenes* to the mouse – the first time that such an approach has been successfully attempted. This experiment furthermore demonstrates the remarkable potential of structural biology to advance far beyond the sole determination of biomolecular structures.

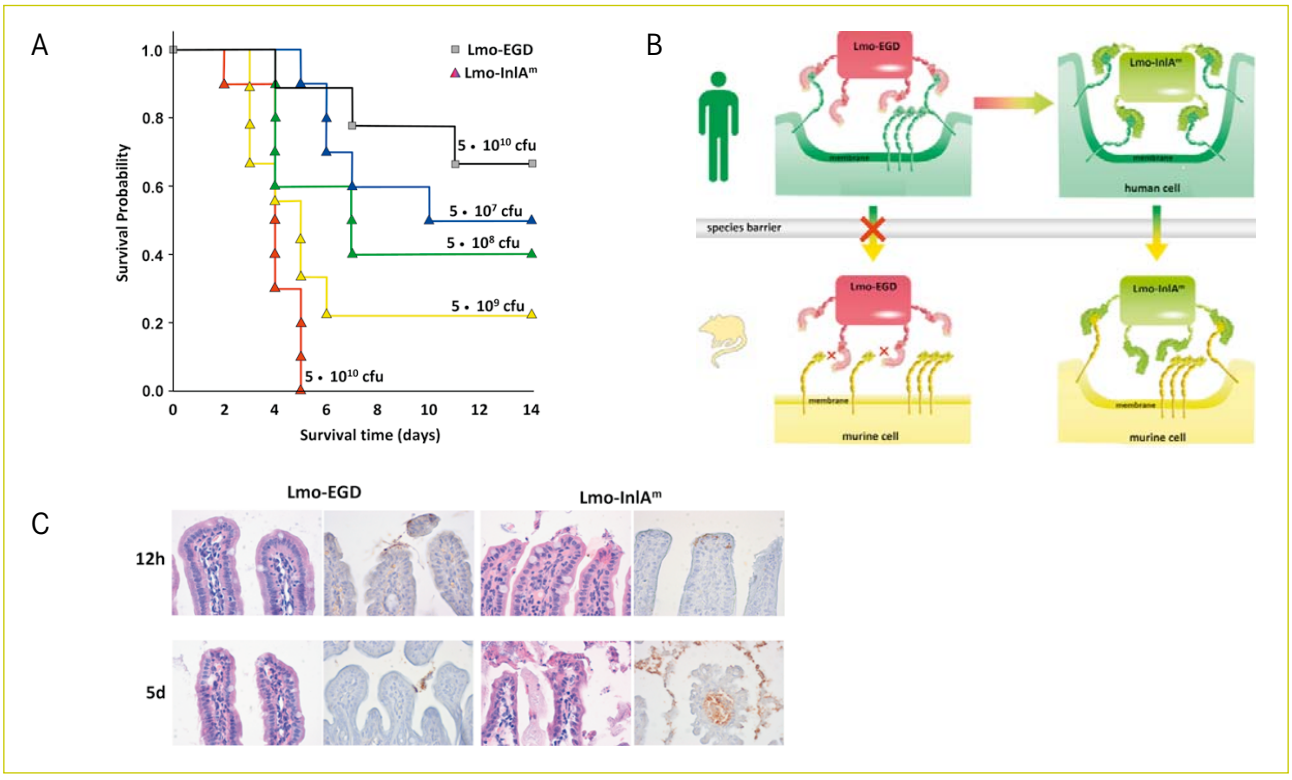

Fig. 5. Mouse infection studies using $InlA^m$. A)The Listeria strain carrying the mutant InlA (Lmo-InlAm) showed a drastic increase in mouse lethality when compared to wild-type Listeria (Lmo-EGD), B)Breaching the species barrier by rational protein design, C) Lmo-InlAm infection leads to massive destruction of intestinal epithelium.

New tools for studying receptor signalling When first solved, the crystal structure of the InlB/Met-complex (see above) did not obviously reveal whether the receptor is activated by dimerisation – a common activation mechanism previously demonstrated for a range of growth factor receptors. This scenario would require the formation of a complex consisting of two InlB and two Met molecules. In a second crystal form of InlB/Met an arrangement was observed in which InlB-molecules dimerized head-to-tail via the convex side of their LRR-domain. This generates a physiologically plausible Met/InlB/InlB/Met-complex in which the C-terminal ends of the Met molecules both point in the same direction – presumably towards the cell membrane. Strikingly, this dimeric InlB arrangement is also present in several different crystal forms *(i.e.* molecular packing arrangements) of isolated InlB. Dimerisation of InlB could thus be physiological. Inspection of the InlB dimer interface revealed weak but specific interactions between both InlB monomers.

To confirm a potential role of InlB-mediated dimerisation for Met activation, two InlB molecules were covalently linked by introducing cysteine residues on the convex surface that would promote the formation of disulfide bridges between both InlB monomers in an arrangement similar to the observed InlB dimers. Strikingly, the resulting constitutive InlB-dimer causes a dramatic activation of Met, outperforming even the natural Met-ligand HGF. These results not only demonstrate that Met can be activated by InlB-mediated dimerisation, but provide an elegant "molecular tool" to study cellular Met signalling, which is crucial to tissue regeneration and cancer.

Conclusions The structural analysis of InlA and InlB in complex with their human receptors not only provides a precise (*i.e.* atomic resolution) description of the first step of listerial adhesion and invasion, but also affords the possibility of novel functional applications based on structure-guided protein design. We generated InlA variants and a mutant strain of *L. monocytogenes* that can be used to study listerial invasion in a long-awaited experimental mouse model. The artificially cross-linked dimeric InlB-variants can now be exploited to study Met receptor signalling events in physiological *(i.e.* wound healing and tissue regeneration) as well as pathological processes (*i.e.* cancer metastasis).

Acknowledgements
We are most grateful to our numerous co-workers, PhD and diploma students, and the technical staff for their invaluable contributions to the internalin project.

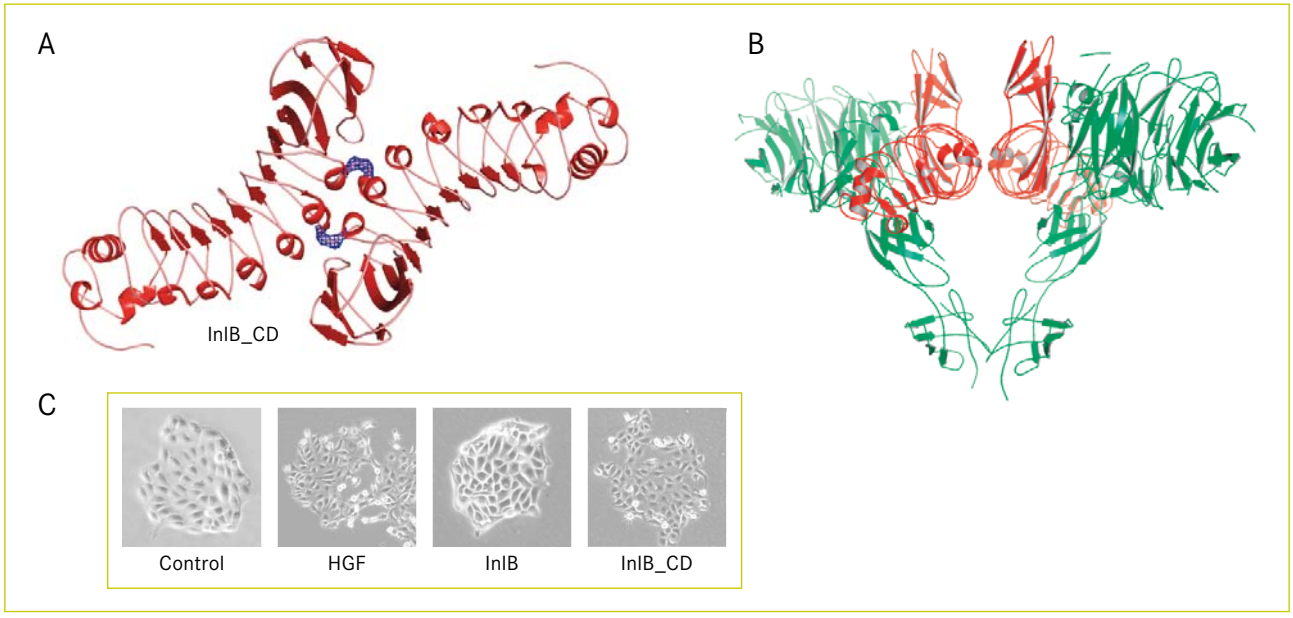

Fig. 6. Artificial dimerization of InlB to study Met-signalling in the host cell. A) Cross-linking of two InlB-molecules by intermolecular disulfide bridges (InlB_CD), B) Structure of the dimeric InlB/Met complex, C) InlB_CD leads to cell scattering as a result of Met activation by dimerization.

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Wolf-Dieter Schubert born 1966, studied Chemistry at the University of Cape Town (South Africa) completing his MSc in Chemistry in 1991. This was followed by a PhD at the Free University of Berlin, 1997. A first postdoctoral position was at the Institute of Biotechnology and Human Science, Tsukuba, Japan, 1998 before moving to the GBF in the same year to a second postdoctoral position. Since 2005, Group Leader in the Division of Structural Biology at the HZI.

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FOCUS RESEARCH REVIEWS SPECIAL FEATURES

Photos from left to right: Ute Widow preparing for a preparative chromatographic separation | Dr. Theresia Stradal during her work at a clean bench | Dr. Heinrich Lünsdorf looking for bacterial structures using an electron microscope

Photos: HZI, Bierstedt (le) | HZI (ce) | HZI, Hübner (ri)

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INFECTION AND IMMUNITY

PROGRAMME SPEAKER | **Prof. Dr. Jürgen Wehland | Division of Cell and Immune Biology | jwe@helmholtz-hzi.de**

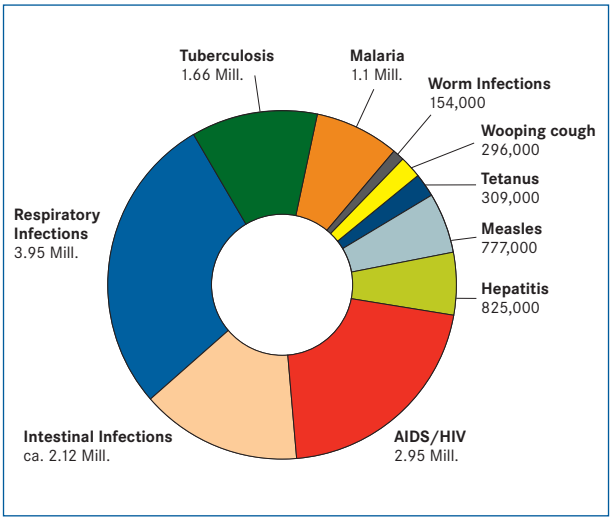
Infections cause one third of all disease-related death cases worldwide. Even though improved hygiene and the availability of antibiotics and vaccines have lead to a continuous decline in the outbreak of such diseases in recent decades, we are confronted with the fact that many infectious diseases are not only becoming resistant to medication but are also re-emerging. Worldwide travel and global exchange of goods have lead to epidemic outbreaks of previously unknown diseases, best illustrated by novel zoonotic infections such as HIV, SARS and avian influenza. Moreover, infections that were thought to be eradicated, such as tuberculosis, have become a global threat once again.

In industrial countries infectious diseases have become very challenging due to modern high-tech medicine: transplant patients or those under intensive care are highly susceptible to opportunistic infections as a result of immune-suppressive medication. Furthermore, new resistances continue to emerge and the chances of curing not only acute diseases, but also chronic or persistent infections, are becoming greatly reduced. This situation emphasizes the urgent need for developing new strategies for diagnosis, prevention and therapy of infectious diseases. Therefore, it is imperative that basic research of infection processes focuses on mechanisms underlying pathogen/host interactions. In order to develop new, innovative vaccines we must examine how the host's immune system reacts to the invasion of a pathogen and how an immune response is initiated. Last but not least, one has to know what influence the environment, *e.g.* food and pathogenic reservoirs, has on the course of an infection and the defence against it.

Opportunistic infections are a serious problem not only for immune-compromised patients, as mentioned above, but also for the aging population. Despite these alarming developments, the chances of establishing new diagnostic and effective therapeutic strategies are very good. Systematic genome analysis provides information on potential drug targets, thus aiding the development of new antibiotics. A better understanding of the functions of individual products, combined with knowledge of the interactions of microbial factors with host cellular genes, is an excellent basis for the directed design of chemotherapeutic strategies against microbial pathogens. Functional genome analysis also provides insights into the molecular basis of immune responses and the genetic susceptibility and resistance to infectious diseases.

Our increased knowledge of the molecular and cellular components of the immune system has opened up new possibilities of clinical intervention that will allow immune therapies extending beyond prophylaxis and will include therapeutic intervention. Today, our understanding of immunity extends far beyond its protective role against infectious diseases. We know that the immune system not only protects the host against microorganisms, but that it also specialises in surveillance, detecting altered cellular antigens and thus monitoring and eliminating detrimental changes in the tissues and organs of the body. Nevertheless, the precise mechanisms by which the immune system is continuously undermined by various microorganisms, resulting in latent or chronic infections, are still only barely understood.

The Infection and Immunity research programme of the Helmholtz Centre for Infection Research is based on basic research in the area of infectious diseases and immunity. It is at the interface of these two fields where we expect the greatest potential for the development of new compounds and strategies for the prevention and treatment of diseases. The main objective of the programme is to understand the principle mechanisms that underlie the development of infectious diseases. This involves basic research on model microorganisms and their pathogenicity as well as detailed analysis of the mechanisms of immunity. Our aim is to understand the individual molecular and cellular steps that occur during the process of an infection, the mechanisms of how selected microorganisms cause disease and the basic principles of defence mechanisms that are used by the host to resist and control infections. This knowledge will be used to develop new strategies and tools to treat and prevent infectious diseases.



Infectious diseaes worldwide: Death cases per year
Source: PathoGenoMik Report 2003

Topics of the research programme

- Microorganisms
- Pathogenesis
- Inflammation and Immunity
- Prevention and Therapy

01 Microorganisms

TOPIC SPEAKER | **Prof. Dr. G. Singh Chhatwal** | **Department of Microbial Pathogenicity** | **gsc@helmholtz-hzi.de**

In spite of the availability of a large number of different antibiotic and antiviral agents, the disease burden caused by infections is continuously increasing and markedly impairs the achievements of medical care. Due to advanced medical practises chronic persistent infections are becoming more and more apparent, and constitute a major challenge for the medical profession. Furthermore, the increasing emergence of antimicrobial resistant bacterial isolates is a major concern and multi- and pan-resistant pathogens are frequently identified especially in the clinical setting. Due to the decreasing therapeutic options the development of new treatment strategies is urgently required. To meet these challenges a detailed understanding of the mechanisms of pathogenicity is of utmost importance. Adherence to and invasion of the host cells, intracellular survival, dissemination, immune evasion and persistence within biofilms are just a few of the strategies used by microorganisms to establish an infection in the host. Another area of concern is the diversity of many infectious agents. Many bacterial and viral species have hundreds of different serotypes with strong antigen variation, which impedes development of effective therapies. Therefore, a multi-disciplinary approach has to be applied in order to fully understand the pathogenic mechanisms of microorganisms.

The main objective of this topic is the in-depth study of the biology of pathogenic microorganisms and their interaction with the host cell. The topic deals with the following research themes.

Study of the host cell-pathogen interactions at the molecular and cellular level

In the course of their co-evolution with their hosts bacterial pathogens have developed sophisticated strategies to exploit the host cell and immune system for their own benefit. Intracellular survival, dissemination in the host and pathogen persistence requires a complex series of interactions within the host cell. A prime target is the cytoskeleton that is involved in numerous cellular functions and processes that depend on the intrinsic dynamics of its constituents, in particular the actin system. A large number of actin binding proteins have been described, which regulate the dynamic reorganisation of the cytoskeleton. This research area deals with the elucidation of exact signalling pathways and principles of the control of actin assembly. Whereas the subversion of the actin system by a pathogen has been a topic of interest for a long time, the contribution of the dynamic microtubule system to bacterial pathogenicity is emerging as an important new area of infection research and has been included in this topic.

Analysis of biofilm formation and its regulation

Biofilm formation by bacterial communities represents an important process in many infectious diseases. Biofilms can develop in many organs such as lungs, urinary bladder and heart valves as well as on various implanted prosthetic devices and in the oral cavity. The microbes in biofilms are often resistant to the action of anti-infectives and are inaccessible to the host's immune defence system leading to chronic and potentially life-threatening clinical manifestations. In this research area the focus is on the role of biofilm formation and bacterial communication in the chronic infection caused by *Pseudomonas aeruginosa*. The topic also deals with the role of quorum sensing in biofilm formation by oral streptococci.

Identification of novel virulence factors from pathogenic bacteria

Pathogenic microorganisms produce a variety of virulence factors with diverse functions in processes such as adherence, invasion, intracellular survival, evasion of the host immune response and bacterial communication. Their functional characterisation not only contributes towards our understanding of pathogenicity, but also helps in identifying promising candidates for the development of vaccines, diagnostics and novel therapeutics. Fibronectin, collagen and plasminogen are important constituents of the extracellular matrix shown to be directly involved in host-pathogen interactions. Fibronectin binding proteins of pathogenic bacteria are important factors in adherence, invasion and also intracellular survival. Collagen binding to bacteria plays a role in certain autoimmune manifestations and the activation of plasminogen induced by bacterial proteins is crucial for tissue invasion. The main focus of this research area is the virulence factors of different streptococci, especially those that interact with extracellular matrix components.

Analysis of bacterial virulence using proteomics

In this theme, the model organism *L. monocytogenes* is used to unravel the spatial and temporal dynamics of host-pathogen interactions at the protein level. In particular effector mediated host cell signalling is studied using a qualitative phosphokinome analysis. Since the progression from first *P. aeruginosa* acquisition until sustained colonisation of the lung of CF patients correlates directly with general life expectancy, a highly sensitive immunoproteome workflow for the identification of diagnostic and prognostic antigenic markers is being established together with the outpatient clinic of the Hannover Medical School. Furthermore, the project investigates signal transduction processes both specific for the bacterial pathogen *P. aeruginosa* and specific for lung epithelial cells derived from CF patients.

Structural analysis of proteins involved in host-pathogen interactions

The elucidation of host-pathogen interactions at the atomic level provides mechanistic insights for the development of new approaches to specifically interfere with infection processes. The 3D-structures of microbial virulence factors and, if known, their complexes with host cell interaction partners and receptors are determined at high resolution using the well-established techniques of X-ray crystallography and NMR spectroscopy. The main emphasis is placed on virulence factors that contribute to microbial adherence and invasion, remodelling of the host cell cytoskeleton, components and effectors of type III secretion systems and key gene regulators of microbial virulence, as well as heme and iron-uptake systems.

The above-mentioned research areas are being handled at project level by a multi-disciplinary team consisting of 14 scientists. These scientists belong to different departments and research groups at HZI and bring with them a variety of different expertise. The highlights of the results obtained at project level are given in the following project reports.

01.1 Structural Analysis of Virulence Factors

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A characteristic feature of pathogenic microorganisms is their use of so-called virulence factors to take control of various host-cell processes during infection. The aim of this project is the structural analysis of microbial virulence proteins using X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy to gain a precise understanding of how these proteins interact with host-cell factors during infection. From this knowledge new strategies can be developed to defend and protect against microbial infections.

Receptor tyrosine kinase signalling exploited during bacterial uptake The bacterium *Listeria monocytogenes* is a harmful food pathogen which can cause listeriosis, a systemic disease with high mortality rates in neonates, the elderly and other immuno-compromised individuals. The unique capability of the invasive bacterium to breach several important host-cell barriers critically depends on two invasins, internalin A and B (InlB), located at the bacterial surface. InlB specifically interacts with the receptor tyrosine kinase Met, leading to bacterial uptake. Met itself is the cellular receptor for hepatocyte growth factor (HGF), making it a key player in cell growth, cell migration and cancer metastasis. The recently determined crystal structure of the complex between InlB and Met (Fig. 1) rationalizes how the bacterial protein exploits Met signalling. InlB allows receptor oligomerisation, and consequently receptor activation, by locking its flexible ectodomain into a rigid conformation. Despite being a functional mimic of HGF, InlB interacts with Met in a very different fashion, showing the striking promiscuity of this receptor towards different protein ligands.

Correct needle length of the type-III secretion system Gram-negative pathogenic bacteria, such as the plague causing *Yersinia pestis,* possess a type-III secretion system (T3SS) or injectisome, a needle-like assembly which is needed to transfer virulence factors into the host cell during infection. The inner-membrane protein YscU plays an important role during the assembly of the *Yersinia enterocolitica* injectisome and undergoes an unusual autocleavage at a conserved tetrapeptide sequence motif during the infection process. Mutations of this motif cause marked changes in the properties of the protein resulting in the cessation of protein export and the occurrence of longer needle phenotypes. The structure of an uncleaved YscU variant (Fig. 2) reveals that the protein is poised for autocleavage due to an optimal reaction geometry for nucleophilic attack of the scissile bond by a reactive asparagine side-chain.

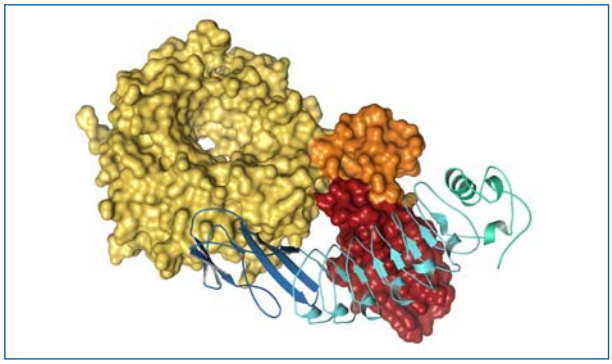

Fig. 1. Structure of the InlB-Met complex. Met domains are shown as surface representation (yellow to red), InlB domains as diagram (green to blue).

Structural comparisons of viral virulence factors The structure of the equine infection anaemia virus Gag protein p9 has been determined for comparison with that of p6 from human immune deficiency virus 1 (HIV-1) using NMR spectroscopy. Structural differences are related to the different types of late domain motifs that interact with proteins of the endosomal sorting complex required for transport complex. Similar comparative studies of two sets of synthetic peptides of PB1-F2 corresponding to “Spanish flu” and “bird flu” influenza A virus isolates have revealed structural differences between these and those of our recently published data for the original H1N1 isolate. Such differences appear to influence the pathogenesis of viral and secondary bacterial pneumonia.

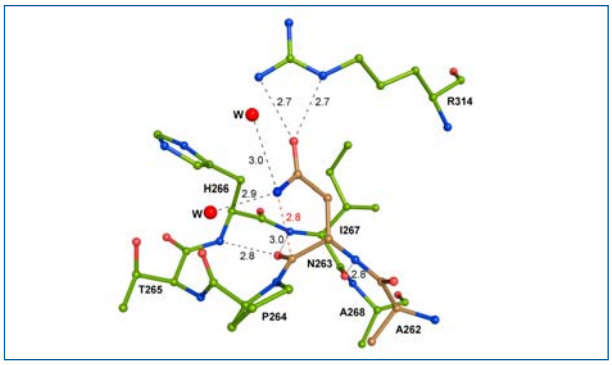

Fig. 2. YscU self cleavage. The side-chain of ^{263}Asn is ideally positioned to attack its own carbonyl carbon.

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01.2 Pathogenesis of Chronic *Pseudomonas aeruginosa* Infections

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PROJECT MEMBERS | Andreas Dötsch | Ahmed Haddad | Vanessa Jensen | Mathias Müsken | Yusuf Nalca | Claudia Pommerenke | Juliane Schmidt | Caroline Zaoui

The diagnostic and therapeutic strategies that have served us so well in the treatment of acute bacterial diseases have not yielded favourable outcomes when applied to chronic infections where bacteria grow in matrix-enclosed sessile biofilm communities. Although every single cell is able to induce a stress response with a characteristic change of the gene expression pattern, living in populations provides a species with additional mechanisms of survival, the most obvious being heterogeneity and co-operation.

Diversity facilitates survival *Pseudomonas aeruginosa* is the most dominant bacterial pathogen causing chronic lung infection in cystic fibrosis (CF) patients. Although most patients are colonised with only one *P. aeruginosa* clone, the isolation of various morphotypes is a very characteristic microbiological finding. This diversity seems to play a key role in the persistence of chronic infections. Our research focuses on the elucidation of the molecular mechanisms responsible for this diversity.

Mutation and selection – keys to the establishment of diversity In CF patients who are chronically colonized with *P. aeruginosa* so-called small colony variants (SCVs) are frequently recovered from the respiratory tract, which form particularly efficient biofilms.

The biofilm-forming SCV phenotype is characterised by the expression of the "Chaperone Usher Pathway" *(cupA)* gene cluster. This gene cluster encodes for bacterial fimbriae and is regulated via the modulation of a bacterial signal molecule, cyclic di-GMP (c-di-GMP).

In collaboration with Affymetrix we have developed a *P. aeruginosa* genome array in order to identify the mutations that form the basis for the generation of the SCV phenotype.

Comparative hybridisation of chromosomal DNA from two *P. aeruginosa* strains onto this array was demonstrated to successfully identify even single nucleotid exchanges. In the future we aim to employ this to identify clinically relevant adaptive mutations that occur in *P. aeruginosa* under *in vitro* biofilm growth conditions and *in vivo* in the course of a chronic infection. The knowledge of the genotypes that are selected at different stages of infection should help us to develop new, promising therapy strategies.

Interbacterial communication plays a key role in the establishemnt of bacterial diversity In addition to two well-characterised homoserine lactones, *P. aeruginosa* also produces a third interbacterial signal molecule that is referred to as the Pseudomonas quinolone signal (PQS). PQS is involved in cell density dependent virulence factor regulation and the establishment of *P. aeruginosa* biofilms. We have also been able to show that PQS plays a key role in the generation of morphological diversity under biofilm conditions. PQS is an anti-oxidant but at the same time produces oxygen radicals via the activation of the Fenton reaction. This pro-oxidant activity damages the DNA, resulting in double strand breaks that are only insufficiantly repaired. The consequence is the evolution of genetic variants that are then selected under biofilm conditions.

The investigation of the link between interbacterial communication and the generation of specific biofilm phenotypes will be an interesting challenge for the future.

Prof. Susanne Häußler and Yusuf Nalca discussing results of the growth of Pseudomonas aeruginosa. *Photo: HZI, Gramann*

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Jensen,V., Löns,D., Zaoui,C., Bredenbruch,F., Meissner,A., Dieterich,G., Münch,R. & Häußler,S. (2006) RhlR Expression in *Pseudomonas aeruginosa* Is Modulated by the Pseudomonas Quinolone Signal via PhoB-Dependent and -Independent Pathways. *Journal of Bacteriology* **188**, 8601-8606.

01.3 Virulence Factors of Streptococci and Pneumococci

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Group A streptococci and pneumococci are important human pathogens capable of causing a wide spectrum of diseases and sequelae. Group C and G streptococci have recently been associated with rheumatic fever. Oral streptococci, the caries causing bacteria, are also capable of causing life-threatening systemic diseases. In spite of the availability of antibiotics, the mortality and morbidity due to these infections remains very high. In 2008, streptococcal infections and their sequelae were listed among the neglected infectious diseases. Identification, molecular characterisation and structure function analysis of the streptococcal pathogenicity factors are a prerequisite to design and develop novel control strategies.

Mechanisms of streptococcal invasive diseases Most invasive bacterial infections are caused by species that more commonly colonise the human host with minimal symptoms. The globally disseminated M1T1 clone of group A streptococcus (GAS) is linked with the rare but life-threatening syndromes of necrotising fasciitis and toxic shock syndrome. Mutations in the GAS control of virulence regulatory sensor kinase (covRS) operon are associated with severe invasive disease. The human protease plasmin plays a crucial role in the capacity of the group A streptococcus to initiate invasive disease. Utilising a humanised plasminogen mouse model of invasive infection, we demonstrated that the capacity to bind plasminogen and accumulate surface plasmin activity plays an essential role in GAS virulence.

Acute rheumatic fever Acute rheumatic fever is a serious autoimmune sequela of pharyngitis caused by certain group A streptococci. One mechanism is formation of an autoantigenic complex with human collagen IV. We demonstrated that formation of collagen complexes during streptococcal infections depends on an octapeptide motif, which is present in collagen binding M and M-like proteins of different beta-hemolytic streptococcal species. Mice immunised with streptococcal proteins that contain the collagen binding octapeptide motif developed high serum titers of anti-collagen antibodies. In sera of rheumatic fever patients such a collagen autoimmune response was accompanied by specific reactivity against the collagen-binding proteins.

Signal transduction in endothelial cells The uptake of M3-type streptococci into human umbilical endothelial cells was analysed in order to identify host signalling factors that are required for early steps in the uptake process. We found that Src family protein-tyrosine kinases (PTKs) and the small GTPase Rac1 are essential in mediating *S. pyogenes* internalisation. We showed that Src PTKs are activated in a time-dependent manner in response to M3-type streptococci. Beside Src PTKs, the small GTPase Rac1 was found to be activated in infected cells and accumulated with F-actin at the bacterial entry site. In addition to this, for the first time we demonstrated accumulation of the actin nucleation complex Arp2/3 at the entry port of invading streptococci.

A novel marker uncovers the role of *S. anginosus* in clinical group C and G streptococcal infections. Although streptococci of the anginosus group *(S. milleri)* are recognised as significant human pathogens, their epidemiology is far from being resolved. One reason is the protean character of these organisms, which complicates diagnosis. We have identified a novel marker for the species *S. anginosus* and *S. constellatus* and deliver a fast and reliable PCR-method to distinguish these species from other members of the genus. The marker gene is predicted to code for a surface localised protein and thus may be suitable for the development of a fast, inexpensive and accurate field test for improving diagnosis and may lead to a more targeted therapy.

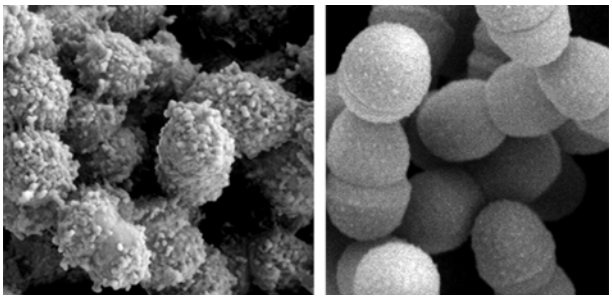

Fig. 1. A streptococcal octa-peptide, PARF (AXYLZZLN), aggregates human collagen and thereby plays a crucial role in induction of acute rheumatic fever. The figure shows the aggregation of collagen on streptococcal surface (left panel) as compared to streptococci without collagen (right panel). *Photo: HZI, Rohde*

Walker,M.J., Hollands,A., Sanderson-Smith,M.L., Cole,J.N., Kirk,J.K., Henningham,A., McArthur,J.D., Dinkla,K., Aziz,R.K., Kansal,R.G., Simpson,A.J., Buchanan,J.T., Chhatwal,G.S., Kotb,M. & Nizet,V. (2007) Dnase-mediated resistance to neutrophil killing provides selection pressure for a genetic and phenotypic switch promoting invasive group A streptococcal infection. *Nature Medicine* **13**, 981-985.

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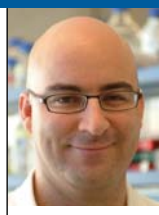

01.4 Systems Biology of *Pseudomonas*

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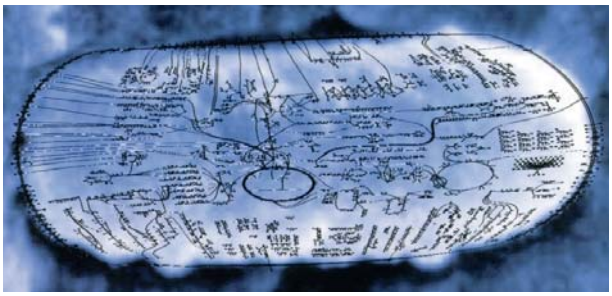
With the recent advances in high-throughput analytical technologies, we are gaining insights into how an organism utilises its genome under diverse environmental conditions. The question is how can we capitalise on these insights to design intervention strategies against pathogens or to exploit the biotechnological capabilities of relevant microorganisms? While understanding individual genes and proteins is important, understanding how cells function requires every gene to be placed in its dynamic context. This requires the integrated consideration of many interacting components, which is best done by relying on sound modelling frameworks.



Depiction of the reconstructed genome-scale metabolic network of Pseudomonas aeruginosa

***Pseudomonas* as model microorganisms** *Pseudomonas aeruginosa* is a major life-threatening, opportunistic pathogen. Its infections are multifactorial and combinatorial and this bacterium is generally considered to possess global, intertwined traits that render it highly competitive and effective in a variety of environments. It is unclear what the genomic repertoire is underlying its success as a pathogen. The non-pathogenic *P. putida* is a versatile microorganism of great biotechnological importance. Our goals are to understand how the genomes of *P. aeruginosa* and *P. putida* determine their behaviour within the context of relevant environmental and host-related constraints.

Genomes determine behaviour To gain insight into the genomic programmes underlying infection, we conducted an extensive comparative genomic study and generated a statistically significant number of *P. aeruginosa* genes that are believed to be specifically involved in pathogenesis. Although a large core set of genes was common to different infection settings, smaller subsets thereof were expressed differentially. Furthermore, computational analysis has revealed a large number of potentially new virulence genes of *P. aeruginosa*.

We developed a computational platform to retrieve heterogeneous data. Using annotated genome sequence data, biochemical information and strain-specific knowledge, we developed a preliminary genome-scale, constrained-based modelling framework for *P. aeruginosa* and *P. putida*. The networks comprise between 650 and 950 genes, which is about 10 to 15% of the genome of these bacteria. Preliminary results also show that the number of extreme pathways that represent the metabolic potential of *P. aeruginosa* is considerably higher than those for *P. putida*. This is clearly an emergent property of the system that could not be predicted solely on basis of the linear comparison of gene lists.

Informative frameworks and models The construction of metabolic and regulatory blueprints provides a framework to study the consequences of alterations in the genotype and to gain insight into the phenotype-genotype relationship. Ultimately, this analysis defines the entire metabolic space of the possible flux distributions and metabolic interactions within the network. A direct comparison of this “phenotypic space” for both bacteria will possibly help in identifying “orphan genes”, evolutionary features and genetic plasticity. Such models will be used to choose the most informative knockouts and to rationally design experiments relevant for the elucidation of the behaviour of these bacteria in polluted environments or within the scope of their relationships with an infected host.

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01.5 Microbial Communication

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PROJECT MEMBERS | Dr. Helena Sztajer | Dr. Brigitte Kunze | Dr. Wei Wang | Andre Lemme | Xiaoli Xue | Ina Buchholz | Jürgen Tomasch

Bacteria produce small diffusible signal molecules called autoinducers. They regulate the expression of fundamental physiological capabilities in a concentration dependent way. For example, *Vibrio* cells start to luminesce and to produce toxins only if their numbers are above a certain value, the so-called quorum. Therefore, this type of cell-to-cell communication is called quorum sensing. It controls the expression of virulence factors and the formation of biofilms in many pathogens. Understanding and interfering with microbial communication therefore opens up new opportunities for fighting infectious diseases and developing potential alternatives to antibiotics.

Quorum sensing and biofilm formation in the caries bacterium *Streptococcus mutans* *Streptococcus mutans* is an important constituent of dental biofilms and one of the major causes of caries, or tooth decay. Its virulence traits, including biofilm formation, are controlled by quorum sensing. We have developed a whole genome microarray for *S. mutans*, which contains all 1,950 genes of this bacterium. By transcriptome profiling we have identified a large number of genes for the first time, which are controlled by the bacterial universal signalling molecule, autoinducer-2. The function of many of these genes is unknown. We are now cloning and expressing them to study the encoded proteins, which might represent new targets to inhibit biofilm formation in *S. mutans* and thus help to control caries.

Quorum sensing in the ecologically important marine *Roseobacter* lineage The *Roseobacter* lineage is a phylogenetically coherent, physiologically heterogeneous group of *Alphaproteobacteria*, comprising up to 25% of marine microbial communities. It plays an important role for the global carbon and sulphur cycles, which both have a profound impact on the climate. By studying representative isolates in culture, whole genome sequencing, and the analysis of marine metagenome libraries we are unravelling the environmental biology of this important marine group. We have discovered a great diversity of novel quorum sensing signalling molecules, the regulatory role of which is the subject of future functional genomics investigations.

Search for inhibitors of quorum sensing Using bioassays, based on quorum sensing signalling mechanisms, we have identified compounds that inhibit microbial communication. They have been isolated from various biological sources, *e.g.* the volatile compounds emitted by bacteria, spiders, and reptiles, and the culture supernatants of Myxobacteria. They are also produced by *Alphaproteobacteria* living on the surface of marine ascidiae, spongae and bryozoans. Future work is directed at purifying new quorum sensing inhibitors from culture supernatants and characterising their mechanism of action.

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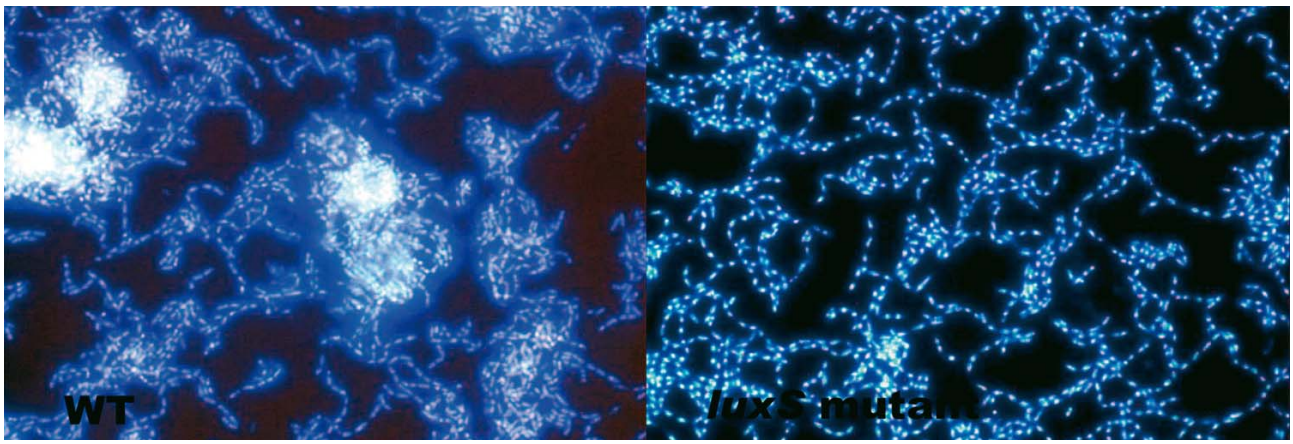


Differences in biofilm formation of Streptococcus mutans *wild type strain (wt) (left) and knock out mutant defective in AI-2 driven quorum sensing system (luxS mutant) (right)* *Photo: HZI, Sztajer*

01.6 Molecular Mechanisms of Intracellular Trafficking, Survival and Persistence of Streptococci

PROJECT LEADER | **Priv.-Doz. Dr. Manfred Rohde | Department of Microbial Pathogenicity | mro@helmholtz-hzi.de**

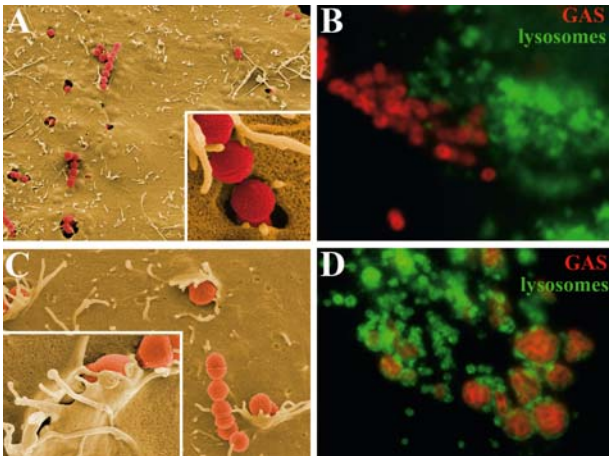
PROJECT MEMBERS | Claudia Preuß

Streptococcus pyogenes, the group A Streptococcus (GAS), is the major cause of human streptococcal infections that range from uncomplicated to severe and life-threatening infections such as necrotising fasciitis. Streptococci are also able to cause recurrent infections such as erysipel and tonsillitis. This phenomenon has been described as carrier stage of streptococcal infection and it is believed that streptococci have a safe ecological niche, most probably inside eucaryotic cells, that allows GAS not only to persist but also to resist prolonged antibiotic treatment. The carrier stage of streptococci has been more or less neglected in the past. Only scant information is available on the mechanisms and the factors involved.

Invasion and survival mechanism of SfbI-expressing Group A streptococci Fibronectin binding proteins of streptococci have been demonstrated to play an important role in adherence and invasion of streptococci. The streptococcal fibronectin-binding protein (SfbI) from group A streptococci for example is involved in the intracellular survival of streptococci by formation of a novel compartment termed caveosome. SfbI utilises fibronectin as a bridging molecule to bind to $\alpha_5\beta_1$ integrins. Due to multiple binding of fibronectin to a single SfbI protein molecule integrin, clustering is induced resulting in a signalling cascade which leads to aggregation of caveolae around adherent streptococci. Subsequently, a large invagination is formed through which streptococci invade the host cell (see Fig.1, A). By co-opting this caveolae-mediated pathway SfbI-carrying streptococci bypass the degradation mechanism of the host cell since no fusion with lysosomes is visible (see Fig.1, B).

Invasion mechanism of the SfbI-mutant strain In contrast to SfbI-expressing streptococci, the SfbI mutant strain exhibits a morphologically different invasion mechanism which is characterised by the formation of membrane ruffles (see Fig.1, C). Inside the host cell streptococci then follow the classical endocytic pathway. This means that phagosomes containing streptococci subsequently fuse with lysosomes to form phagolysosomes (see Fig.1, D). As a consequence for the intracellular streptococci this means that streptococci are now confronted with the degrading machinery of the host cell and have to circumvent this possible degradation for their own survival and persistence.

GfbA, the fibronectin-binding protein A of Group G streptococci (GGS) GfbA-expressing Group G streptococci exhibit a different invasion mechanism compared to SfbI-expressing GAS strains, despite the fact that similar amounts of fibronectin are bound compared to the SfbI-carrying strains. GfbA-mediated invasion leads to the formation of membrane ruffles in up to 90% of all invading streptococci and subsequent uptake into the host cell. Only rarely can the formation of invaginations be observed. As for the SfbI mutant strain, GfbA-expressing streptococci also follow the classical endocytic pathway. Thus, fusion with lysosomes to form phagolysosomes is detectable. Heterologous surface expression of GfbA in the non-pathogenic S. gordonii demonstrated that GfbA alone is responsible for the morphological distinct invasion mechanism compared to the SfbI-mediated mechanism. Sequencing of the GfbA gene demonstrated that only the C-terminal part shows a high similarity with SfbI.



Different invasion mechanisms of streptococci
A) SfbI-expressing streptococci invade host cells via the formation of large invaginations and subsequentely avoid intracellular fusion with lysosomes; B) streptococci are labelled in red whereas lysosomes are Lamp-1 labelled in green; C) in contrast to SfbI-mediated invasion the SfbI mutant triggers the formation of membrane ruffles for invasion and (D) fuses with intracellular lysosomes to form a phagolysosome.

Photo: HZI, Rohde

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Pathogenesis

TOPIC SPEAKER | **Prof. Dr. Werner Müller | Department of Experimental Immunology | werner.muller@manchester.ac.uk**

Pathogens invade their host and cause disease. This simple, general feature of pathogens, however, is associated with great variability in terms of disease and of the type of pathogen, which could be a virus, bacteria or parasite. A pathogen induced disease can cause discomfort for a few hours, but it can become deadly within a few days and can also, once inside our body, live with us for the rest of our life.

At the Helmholtz Centre for Infection Research, several research groups are working in the area of pathogenesis. As one can already see from the introduction, pathogenesis covers a wide range of potential diseases that can cause many different impairments to our health. The underlying thrust of our research is to understand the mechanisms of pathogenesis. Pathways may be used by more than one pathogen although each individual pathogen may use its own specific way to invade our body. Examinination of these pathways in the past has revealed that they usually fulfil normal functions in our body, but are then hijacked by pathogens so that these are able to invade. In addition, it turned out that the affected pathways were not only used by pathogens but when altered by other causes, could lead to disease such as cancer or autoimmunity.

We approach the analysis of pathogenesis at various levels, starting with analysis of alterations on the protein network using proteomics, a task performed by the research group of Lothar Jänsch. We analyse the signalling cascades induced by pathogens in cell lines that are genetically altered by either the specific knock-down of genes using RNAi technology, or by inactivating single genes by gene targeting. The groups of Theresia Stradal and Klemens Rottner are following these approaches. Both groups rely on state-of-the-art microscopy and are able to follow the invasion of pathogens at the single cell level *in vitro*.

While the groups above use mainly *in vitro* techniques, the groups of Eva Medina, Werner Müller and Klaus Schughart rely on *in vivo* models using various mouse mutants and inbred strains of mice as a tool to dissect the action of pathogens in the complete organism. Such an *in vivo* approach is very important because *in vitro* approaches can only analyse small components of the complete and complex action of pathogens.

The Medina group is searching for the genes that control sepsis in mice. For this, they analyse various mouse strains that are either susceptible, or resistant, to a bacterial infection to identify the gene regions that control sepsis. In the long term, these genes may allow medical doctors to assess individual patients and group them as high or low risk patients, which would have consequences for the therapy chosen.

The group of Müller performed analysis of infections in genetically modified mouse mutants that lack certain genes involved in the host defence against infections. The genes analysed affect the functions of macrophages or T lymphocytes. The infection models used involve bacterial and parasite infections. These infection models are performed in a special infrastructure, the so-called infection challenge platform. This infrastructure was established with the help of the NGFN. The Department of Experimental Immunology is now headed by Jochen Hühn , his group is working on aspects of the “development and functional properties of Foxp3^{+}-expressing regulatory T cells” (see New Projectgroups 04).

The groups of Schughart and Medina uses recombinant inbred strains to study infections. These strains possess much greater genetic variability and they are, therefore, much closer to the human population in which each person has a unique set of genes – except in identical twins. The analysis of the recombinant inbred strains aims to define quantitative genetic traits; that is, gene loci which influence pathways to a variable degree and in combination with several other genes. While the analysis of recombinant inbred strains is more difficult compared to the analysis of inbred strains, the answers we get will better reflect the situation in the human population because they take genetic variability into account. Because the availability of so-called second generation recombinant inbred strains allows researchers to test almost a million combinations of defined complex genomes, the overall task is demanding but also highly informative.

Finally, the group of Christiane Ritter analyzes structural and mechanistical aspects of functional amyloids. These fibrous protein aggregates are associated with devastating protein misfolding diseases or beneficial cellular functions. For the analyses they successfully apply a combination of quenched hydrogen exchange measured by NMR, other spectroscopic techniques and mutagenesis strategies to derive the fold of amyloid fibrils.

02.1 Molecular Mechanisms of Host-Cell / Pathogen Interactions

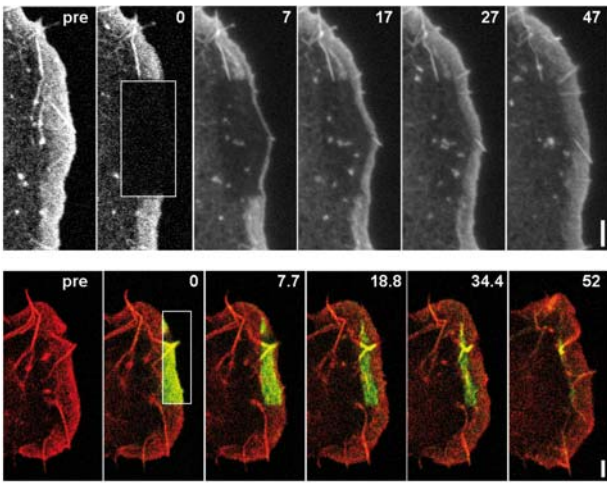
PROJECT LEADER | **Dr. Klemens Rottner** | **Research Group Cytoskeleton Dynamics** | **kro@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Frank Lai | Dr. Markus Ladwein | Tanja Bosse | Dr. Malgorzata Szczodrak | Jennifer Block | Margit Schloen

This project aims at characterising the precise molecular mechanisms driving actin reorganisation during different types of motility processes and the interaction of different pathogens with their hosts.

Actin polymerisation is catalysed by protein complexes enhancing actin filament nucleation, such as the Arp2/3-complex or formins. Prominent important actin regulators include activators of Arp2/3-complex, e.g. WASP and WAVE family members, which can operate downstream of the Rho-GTPases Cdc42 and Rac1. We have recently characterised the roles of these molecules in InlB-mediated host cell entry of *Listeria monocytogenes*, which usurps a host cell signalling pathway downstream of the receptor tyrosine kinase c-Met. Genetic removal of Cdc42 and combined inhibitor studies established both Cdc42 and the phospholipid kinase PI3-Kinase to co-operate in Rac1 activation, the interaction of which with WAVE-complex is essential for InlB-induced *Listeria* invasion. In contrast, the Cdc42-effector and direct interaction partner N-WASP, which drives various types of host-cell pathogen interactions, such as *Shigella* motility or pedestal formation induced by pathogenic *E. coli*, was not involved in Cdc42 functions downstream of c-Met signalling, highlighting the selective role for Cdc42 in driving Rac activation in InlB-induced *Listeria* invasion.

We have also begun to study the precise spatial and temporal features of actin filament nucleation in lamellipodia. Using advanced imaging techniques such as FRAP (fluorescent recovery after photobleaching) and photoactivation (see Figure), we were recently able to establish that actin assembly and nucleation, as well as Arp2/3-complex activation is restricted to the tip region of the lamellipodium, which constitutes the interface between the pushing actin filament network and the plasma membrane. In contrast, we failed to accumulate convincing evidence in support of driving lamellipodial actin filament nucleation for both the actin severing protein ADF/cofilin and the type II Arp2/3-complex activator cortactin. We also got engaged in studies highlighting a remarkable degree of flexibility and reorganisation of the lamellipodial actin network, as evidenced by the wide distribution of angles that actin filaments subtend to the protruding front.



Representative examples for FRAP (top, bar: 3μm) or photoactivation (green, bottom, bar: 2μm) of actin in lamellipodia of motile B16-F1 melanoma cells. Red in bottom panel corresponds to RFP-tagged actin expressed as control. Note the exclusive recovery of the actin network from the front. Time is in seconds (adapted from Lai et al., EMBO Journal ***27(7), 982-992).***

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02.2 Analysis of the Protein Networks of early Host-Pathogen Interactions

PROJECT LEADER | **Dr. Lothar Jänsch | Research Group Cellular Proteom Research | lja@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Uwe Kärst | Dr. Josef Wissing | Dr. Tobias Reinl | Dr. Roman Fischer | Claudia Hundertmark | Sebastian König | Zofia Magnowska | Susanne Freund

The focus of the RG Cellular Proteomics is the analysis of fundamental signal transduction events that contribute significantly to early steps in infection and pathogenicity processes of humans, ranging from bacterial invasion models to activation studies of involved immune cells coordinating the adaptive immune response of the host. Cytological, biochemical, mass spectrometric and bioinformatic working modules were developed and will be combined. The objective: a quantitative analysis of the expression, localisation, interaction and in particular posttranslational modification (PTM) of proteins in primary and immortalised human cells.

Methods Immobilised drug molecules are optimised for the identification of cellular „target proteins“ and the proteomic study of signal networks. Amongst other things, the combination with chromatography and MS procedures enables the quantitative analysis of phosphorylation of protein kinases and their substrate proteins involved in the signal transmission. The statistical evaluation of quantitative peptide data on the basis of iTRAQ™ constitutes the definition of involved signalling networks in primary human cells.

Phosphorylation-dependent signal paths in the invasion process of *Listeria monocytogenes* The bacterium *L. monocytogenes* causes severe illness in immune-compromised patients as well as prenatal infections. The virulence factors InlA and InlB interact with the adhesion protein E-Cadherin (InlA) and the receptor tyrosine kinase c-Met (InlB) to induce the invasion of the host cell. The signal transmission pathways are controlled via kinase-catalysed protein phosphorylation. The identification and quantification of InlA-dependent phosphorylated substrate proteins enabled the accurate determination of a functional network of protein kinases in the E-Cadherin signal pathway. The InlB-activated Met signal pathway was analysed using the protein kinases and InlB-influenced phosphorylation sites identified. In addition to protein kinases already identified, it proved possible to identify a further nine that had previously not been associated with either the listerial invasion or the fundamental Met signal pathway. The significance of the newly identified proteins in both signal pathways for listerial invasion will be further valiated (*e.g. via* RNAi).

T cell antigen receptor-conveyed signal transduction T lymphocytes are essential for the regulation of the immune system. The cellular processes and effector functions of T cells are closely linked to their activation status, and the activating signal pathways are of particular immunological interest. Quantitative phosphokinome analyses of various regulatory T cells should now identify new components of the CD3/CD28-dependent signal pathways. Moreover, we are also comparing CD3/CD28-induced signal networks of effectory and regulatory T cells. Expression and phosphorylation status of around 100 kinases have already been determined. Novel signal components and phosphorylation sites as well as altered phosphorylation patterns have been identified in regulatory T cells.

Kirsten Minkhart performing analyses with the stereoscope
Photo: HZI, Krämer

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02.3 Signalling to Acting Dynamics

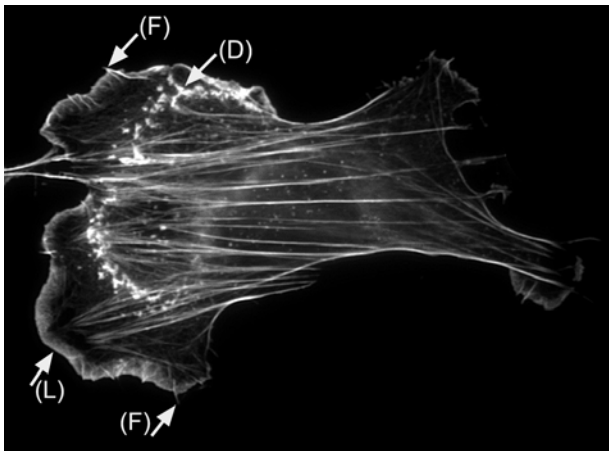
PROJECT LEADER | Dr. Theresia E.B. Stradal | Research Group Signalling and Motility | ths@helmholtz-hzi.de

PROJECT MEMBERS | Jan Hänisch | Kai Städing | Stefanie Weiß | Kathrin Schloen | Kai Schlüter

Numerous cellular functions and processes depend on dynamic changes of the actin cytoskeleton, including all types of cell motility. A large number of actin binding proteins have been described regulating the reorganisations of the actin cytoskeleton. However, the exact signalling pathways and principles for the tight control of spatially and temporally regulated actin assembly remain largely enigmatic.

One major player driving the *de novo* nucleation of actin filaments is the Arp2/3 complex, which is activated by so called nucleation promoting factors (NPFs). The most prominent family of NPFs is the WASP/Scar family of proteins acting downstream of the small GTPases that are key in the translation of extracellular stimuli to actin rearrangements. The structures in the focus of our research are actin based projections at the cell periphery, namely Rac-induced lamellipodia and ruffles or Cdc42-induced filopodia.

The discovery that similar dynamic changes of the actin cytoskeleton play an essential role in host-pathogen interactions has an enormous impact on the field.



Migrating fibroblast displaying the different actin structures formed as it moves. (L) Lamellipodium, (D) dorsal circular ruffle, (F) filopodium.

Lamellipodia and WAVE WAVE proteins, prominent members of the WASP/Scar family, are essential for Rac-mediated lamellipodia formation. However, the pathway leading to WAVE-activation is indirect, since the small GTPase Rac1 is not able to directly bind to WAVE, which led us to search for the missing links. We were able to establish the ability of four proteins, Sra-1 (specifically Rac associated protein 1), Nap1 (Nck associated protein 1), Brick1 and the Abl interacting protein family (Abi proteins) to link Rac to WAVE proteins. The resulting protein assembly, the ubiquitous WAVE complex, is now well established as being essential for the formation of lamellipodia downstream of Rac. Current research focuses on the exact mode of activation and recruitment at the onset of protrusion.

Filopodia, beyond WAVE- and Arp2/3 complex We found that the WAVE-complex was, in contrast to lamellipodia formation, not required for the transduction of Cdc42 signals to filopodia formation, a finding that contrasted with earlier models of protrusion regulation. To clarify whether or not Arp2/3 complex is required for this process, we suppressed its expression by RNAi and found that, again in contrast to lamellipodia, filopodium formation was not affected. Therefore, identification of actin polymerisation machineries other than Arp2/3-complex is currently pursued. We were able to demonstrate the significant ability of the formin-protein mDia2/DRF3 to nucleate and elongate actin into filopodia like structures. However, this protein cannot explain all types of filopodia formed through the GTPase Cdc42, which is why we have now started to systematically analyse proteins with potentially similar activities.

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02.4 The Innate Immune Response to *Streptococcus pyogenes* in an Experimental Infection Model

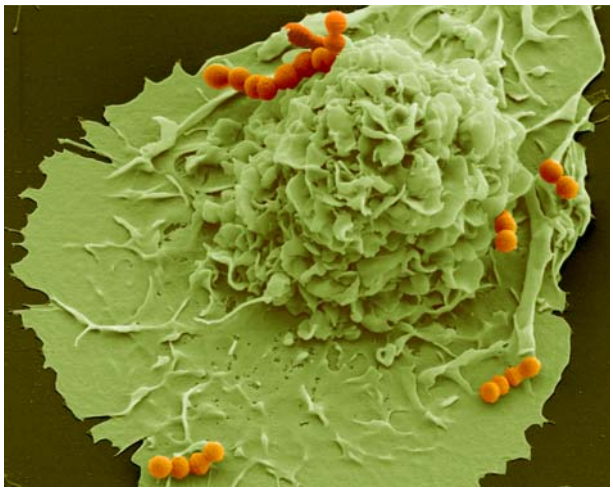
PROJECT LEADER | **Priv.-Doz. Dr. Eva Medina | Research Group Infection Immunology | eme@helmholtz-hzi.de**

PROJECT MEMBERS | Oliver Goldmann | Jens Abel | Christina Ziegler | Daniela Bruhn

Streptococcus pyogenes is an important human pathogen that causes a variety of diseases affecting the skin or the upper respiratory tract. The identification of immune components required for host defences against this pathogen constitutes an important area of research. We have shown the importance of resident macrophages for controlling *S. pyogenes* infection. Here we have investigated the complex response of murine macrophages to *S. pyogenes* at the level of gene expression.

More than 400 genes were identified as being differentially regulated. Many of the up-regulated genes encode molecules involved in the immune response and in inflammation, transcription, signalling, apoptosis, the cell cycle, electron transport and cell adhesion. Of particular interest was the up-regulation of proinflammatory cytokines, typical of the classically activated macrophages (M1 phenotype). For example, tumour necrosis factor alpha, interleukin 1 (IL-1), and IL-6, as well as the up-regulation of anti-inflammatory mediators, such as IL-1 decoy receptor and IL-10, associated with alternative macrophage activation (M2 phenotype). Furthermore, the gene encoding inducible nitric oxide synthase (iNOS) was not induced in infected macrophages. Instead, the gene encoding arginase, a competitor for the iNOS substrate arginine involved in the alternative activation pathway, was up-regulated in *S. pyogenes*-infected cells.

Thus, the microarray-based gene expression analysis demonstrated that *S. pyogenes* induces an atypical activation program in macrophages, with some but not all features of the classical or alternative activation phenotypes. The microarray data also suggested that the bactericidal activity of macrophages against *S. pyogenes* is mediated by phagocyte oxidase, as *p47phox* was up-regulated in infected cells. Indeed, the *in vivo* and *in vitro* killing of *S. pyogenes* was markedly diminished in the absence of functional phagocyte oxidase (p47*phox*$^{hoch-/-}$) but not in the absence of iNOS (iNOS$^{hoch-/-}$). An understanding of how macrophages respond to *S. pyogenes* at the molecular level may facilitate the development of new therapeutic paradigms.



Streptococcus pyogenes *infecting a dendritic cell.*
Photo: HZI, Rohde

Dendritic cells are likely to be among the cells first encountered by *S. pyogenes* in the respiratory mucosa or skin. Therefore, we have investigated the role played by dendritic cells during infection with *S. pyogenes* by use of CD11c–diphtheria toxin (DT) receptor (DTR) transgenic mice, in which CD11chigh cells (conventional DCs) can be transiently depleted *in vivo* by treatment with low doses of DT. Using the CD11c-DTR transgenic mouse model, we have demonstrated that the ablation of dendritic cells results in the exacerbation of *S. pyogenes* infection, indicating that dendritic cells play a protective role during infection with this pathogen. In addition, the depletion of dendritic cells resulted in complete abrogation of interleukin IL–12 production, a cytokine shown to provide significant protection against lethal infection with *S. pyogenes* in a mouse model of skin infection. Taken together, our study clearly indicates that dendritic cells are a cell population central to the host defences against infection with *S. pyogenes.*

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02.5 Systems Genetics of Infection and Immunity

PROJECT LEADER | **Prof. Dr. Klaus Schughart | Department of Experimental Mouse Genetics | kls@helmholtz-hzi.de**

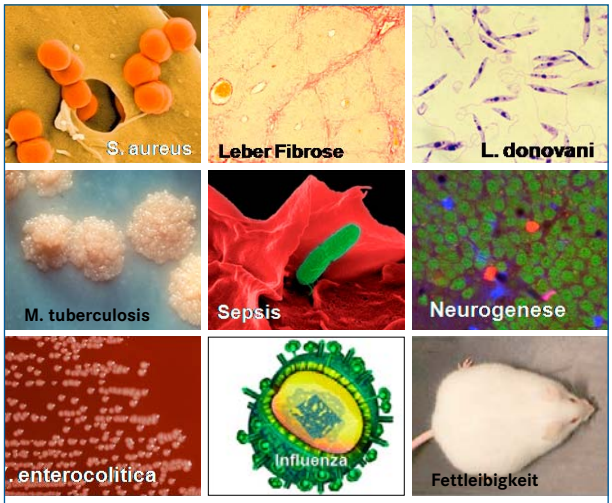
PROJECT MEMBERS | Dr. Bastian Pasche | Dr. Manuela Heßmann | Dr. Nuno Viegas | Dr. Heike Kollmus | Barkha Bhatnagar | Paulina Blazéjewska | Harong Chen | Haiya Wu | Dr. Ursula Frischmann

Our research activities aim to identify complex genetic networks that determine the susceptibility to infections or regulate the immune system. Comprehensive phenotypic studies are performed in mouse genetic reference populations representing sets of inbred mouse strains which differ in their genetic background. In this way, many phenotypic traits as well as gene expression patterns can be associated with genetic variations, and thereby the genomic regions that control phenotypic traits or regulatory interactions can be identified. This approach is called "Systems Genetics." We are applying a systems genetics approach in mice to understand the basic molecular mechanisms in two disease areas that are highly relevant for human health: infection of the mammalian host with influenza A virus and the regulation of the immune system.

Host susceptibility to infections with influenza virus
Every year, infections with the influenza A virus cause about 500 million severe cases of disease worldwide and 500,000 cases in Germany. About 8,000 to 30,000 people die each year from influenza infections in Germany. We have infected several different mouse inbred strains and identified strong differences in their response to influenza A virus H1N1 and H7N7. Three inbred mouse strain are highly susceptible to infection, whereas most other strains can clear the virus. Presently, detailed comparisons of the pathology, course of disease, immune response and whole genome expression analysis of the lungs is being performed between these mouse strains. In addition, we are analysing factors of host susceptibility to infections with influenza virus in different mouse recombinant inbred strains and interspecific recombinant congenic strains. These studies will allow us to identify genomic regions contributing to susceptibility and resistance and help to unravel the underlying gene regulatory networks.

From single gene interactions to regulatory networks
When encountering an infection, the immune system must activate its defence mechanism in a very controlled manner. In order to orchestrate this response in such a way that the invader is killed but the host tissues are left intact; a complex interaction of effector T cells and regulatory T cells (Treg) is needed. We are analysing, at a genome-wide level, the expression profiles in Treg and naïve T cells from different recombinant inbred mouse strains and associate gene expression levels with genotypes. At present, we have identified several expression Quantitative Trait Loci (eQTL) that may be involved in the regulation of groups of genes and thereby maintain the differentiation state of Treg cells. The role and molecular function of candidate genes in these eQTLs is currently being investigated.

A world-wide network for systems genetics and infection research Our activities are highly integrated into national and international research networks: The German Network for Systems Genetics (GeNeSys) is co-ordinated by our department. We are a member of the FluResearchNet, a German network for influenza research, the National Genome Research Network (NGFN), the EU Co-ordinate Action CASIMIR, the EU ESFRI consortium Infrafrontier and the PTR programme of the Institute Pasteur/HZI. In addition, this work is supported by fellowships from the Helmholtz-Chinese-Scholarship Council and the Georg-Christoph-Lichtenberg Foundation.



*Ten partners from universities and research institutes in Germany and the Netherlands have established a virtual institute – **GeNeSys** – where mice form the same genetic reference population, the BXD set of recombinant inbred strains, will be analyzed for various phenotypic traits.*

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02.6 Biology of the Immune Defence

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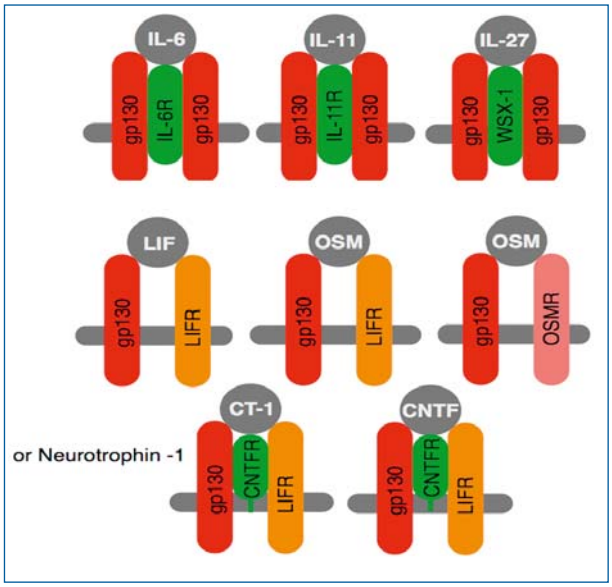
PROJECT MEMBERS | Dr. Angela Schippers | Dr. Ursula Frischmann | Nicolas Fasnacht | Marina Greweling | Annika Kochut | Karina Nawrath | Fabio Pisano | Mandy Reichenbach

Dissecting the cytokine network Cytokines are the key elements of the regulatory networks of the immune system. Cytokines are produced by cells of the immune system and influence the development, differentiation and function of the immune system. The Department of Experimental Immunology dissects this regulatory network via the conditional gene targeting approach in mice. This method allows the cell type specific inactivation of genes in the mice by the use of the P1 phage derived recombinase system Cre/Loxp.

Interleukin-10 and regulatory T lymphocytes Interleukin-10 is an important anti-inflammatory cytokine that controls pro-inflammatory immune responses in the body. Inactivation of Interleukin-10 leads to inflammatory bowel disease in mice, and recently it has been shown that certain Interleukin-10 gene alleles increase the susceptibility to Morbus Crohn disease (a form of inflammatory bowel disease) in humans. By conditional gene inactivation we have shown previously that Interleukin-10 has to be produced by T lymphocytes in order to prevent the development of inflammatory bowel disease.



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T lymphocytes can be subdivided into many subsets. One T cell subset is named regulatory T lymphocyte. It is characterised by the expression of certain specific cell surface markers and by the expression of a key transcription factor named FoxP3. In collaboration with Alexander Rudensky (Seattle), mice were generated in which only the regulatory T lymphocyte subset is unable to produce Interleukin-10. Such mutants will develop inflammatory bowel disease, just like the complete IL-10 deficient and the T cell specific IL-10 deficient mouse mutants. This very important experiment now clearly links the regulatory function of the regulatory T lymphocyte to the cytokine Interleukin-10.

Gp130 and regulatory T lymphocytes Gp130 is the key-signalling component of a group of cytokines called the Interleukin-6 cytokine family. This cytokine family not only acts within the immune system but also has a big impact on almost all organ systems of the body including heart, brain and liver. Within the immune system, one member, namely Interleukin-6, is involved in the differentiation of normal T cells into a new T lymphocyte subset called the Th17 lymphocyte. If we specifically inactivate the gp130 gene in T lymphocyte, we can prevent the generation of Th17 cells but instead increase the number of regulatory T lymphocytes. This shift in the T lymphocyte subsets now changes the way the immune system is able to respond to infection. When we infect for example Interleukin-10 deficient mice with a parasite, these mice are not able to fight the infection, partially because they generate a very active Th17 lymphocyte subset leading to a very strong immune response.

Gp130, the liver and atherosclerosis Gp130, also initially identified as a molecule expressed on immune cells, is expressed on all cells of the body and can interact with many members of the Interleukin-6 cytokine family. Again, using cell type specific gene inactivation, we are also able to delete this molecule on cells outside of the immune system. In collaboration with Bernhard Schieffer (Hannover), the gp130 molecule was inactivated in hepatocytes, the major cell type of the liver. The consequence of this is that the hepatocytes are no longer able to generate an acute phase response, a reaction to an inflammatory response in the body. If such mutants are now kept on an unhealthily high-fat diet and in a genetic background prone to atherosclerosis, such mutants no longer develop the atherosclerosis. By inactivating the gp130 molecule in cells of the liver, we prevented an inflammatory response in the endothelium of the heart artery, keeping the mice healthy. When one then looks in the genome of many individuals of the human population one can indeed find a subset of patients that carry a particular allele of the gp130 gene which increases their risk of atherosclerosis.

02.7 Structural and Mechanistical Analysis of Functional Amyloids

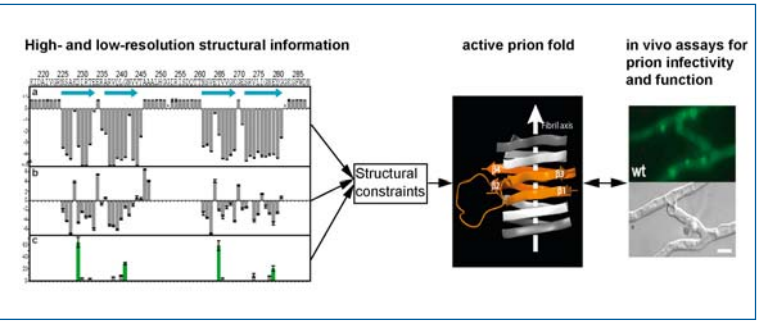
PROJECT LEADER | **Dr. Christiane Ritter | Helmholtz University Junior Research Group Macromolecular Infections | cri07@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Alexander Eberth | Agnes Zimmer | Madhu Nagaraj

Amyloids are ordered, fibrous protein aggregates associated both with devastating protein misfolding diseases and with beneficial cellular functions. In recent years, amyloid-forming proteins have been identified in a number of bacterial and fungal pathogens, where they adopt an amyloid fold in their native state and carry out a diverse set of functions. Of particular interest for infection research is the formation of amyloid "coats" on the cell surfaces of bacteria and fungi, because they can facilitate microbial survival in a wide range of environments, promote adhesion to surfaces and interactions between microbial cells or with host proteins. Functional amyloids exhibit the same structural characteristics as amyloids associated with diseases such as Alzheimer's, Parkinson's or prion diseases. However, they are not toxic and their functions can be studied directly in their native organism. They are thus promising new systems for the study of general aspects of amyloid formation. The major focus of our group lies on the determination of the structural and mechanistical basis of these functions for selected bacterial and fungal amyloids.

Tools for the structure determination of amyloid fibrils

One major bottleneck in amyloid research is the sparseness of high-resolution structural information on naturally occurring amyloids, because the size and non-crystalline nature of the fibrils drastically restrict the use of established structural techniques. We have therefore introduced a new, generally applicable approach that derives the fold of amyloid fibrils from a combination of quenched hydrogen exchange measured by nuclear magnetic resonance (NMR), solid state NMR, other spectroscopic techniques and mutagenesis strategies. With this approach we have previously determined the infectious fold of the fungal prion protein HET-s, and the structure of Alzheimer's A beta(1-42) fibrils.



Fold determination of HET-s(218-289) fibrils. Structural constraints were derived from quenched hydrogen exchange measured by NMR (a), from the ^{13}C chemical shift deviations from random coil values measured by solid state NMR (b), and from fluorophor accessibility studies of single cysteine mutants (c).

Curli: an amyloid coat that increases bacterial virulence

Curli is the major proteinaceous component of the extracellular matrix produced by Enterobacteriaceae such as *E. coli* and *Salmonella typhimurium*. Curli fibrils are involved in the adhesion to biotic and abiotic surfaces and the promotion of biofilm formation. They also interact with several host proteins, resulting in increased tissue penetration, inflammation and sepsis. Curli biogenesis is also interesting mechanistically, because it involves the nucleation of the major curli component, CsgA, by the homologous protein CsgB. In order to understand the features responsible for the different functionalities of CsgA and CsgB, we analyse the structures, aggregation kinetics and thermodynamic stabilities of the fibrils formed by both proteins.

HET-s: a functional prion found in filamentous fungi

Only a subset of amyloids are self-propagating prions. To understand the molecular determinants that govern the infectivity of an amyloid, we investigate the biophysical properties of the functional prion protein HET-s from the filamentous fungus Podospora anserina, and of a recently identified homolog from Fusarium graminearum. The prion form of HET-s functions in a fungal non-self recognition system. A structure-function relationship for HET-s amyloid fibrils has already been established. Our current data suggest that the functional HET-s prion forms a robust, evolutionarily optimised fold. This is in striking contrast to disease-related amyloids, for which often even single point mutations can result in dramatically different fibril morphologies.

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Inflammation and Immunity

TOPIC SPEAKER | **Prof. Dr. Hansjörg Hauser | Division of Molecular Biotechnology | hha@helmholtz-hzi.de**

Innate defence activation and inflammation belong to the first reactions of the immune system upon infection. These events, in turn, are essential to initiate the specific and often long-lasting adaptive immunity. The reactions are initiated by the affected cells themselves and are then orchestrated by a variety of leukocytes and lymphocytes. The mediators responsible consist of low molecular weight components like prostaglandins or small proteins like cytokines as well as molecules that are responsible for cell:cell interactions. Finally, cells of the immune system like T cells and their mediators or antibody producing B cells are responsible for the clearance of a pathogen. Consequently, the topic “Inflammation and Immunity” deals with the processes which lead to the immediate defence reactions and long-lasting protection. It also deals with negative outcomes of such reactions as toxic shock and autoimmunity, and an as yet not understood influence in oncogenesis and tumour surveillance.

Within the theme “Pathogen-induced host reactions” the structure-activity relationships of pathogen receptors and the role of lipid rafts as an entry or exit point for pathogens is studied. The analysis of TLR2,6 and NALP3 by X-ray crystallography aims at a better understanding of the ligand-receptor interaction. Biological follow-up events under study comprise intracellular signalling induced by pathogens, PAMPs or during the inflammatory response. A major subject in this topic concerns the understanding of induction and signalling by type I interferons. Online reporters that allow the monitoring of IFN-ß induction, the induction of IFN-stimulated genes and a reporter that monitors the positive feed-back loop of IFN secretion have been constructed by homologous recombination and BAC recombineering. The latest results indicate that IFN-ß is not only induced by TLR4, but also by TLR2. It was also found that IFN-ß is produced at very low levels in non-infected mice. Mice lacking this constitutive IFN production are inefficient in T cell stimulation and show higher angiogenesis rates in tumours. The location and dynamics of events during virus- and biofilm-induced IFN production is under study. A future goal concerns the construction of a time-resolved model of the IFN-network.

Another key hypothesis we follow is that inflammatory signalling mediators and mechanisms may be used as a target for the prevention and therapy of inflammatory and infectious diseases. Therefore, relevant signal transduction pathways for infection and inflammation like TNF-alpha, IL-1- and Toll-like receptors are investigated in cell culture and animal model systems. As a result, we will further assess the role of the central inflammatory signalling mediator TAK1 in inflammatory mouse models such as rheumatoid arthritis. In particular, first studies indicate the beneficial role of a systemic TAK1 targeting strategy by the RNAi technology to alleviate rheumatoid arthritis symptoms in a collagen-induced arthritis model (CIA). In addition, first results document a successful intrabody-mediated strategy to interfere with Toll-like receptor-mediated signalling pathways for the treatment of inflammatory diseases.

Three projects deal with the interrelationship between infection, oncogenesis and tumour surveillance.

1) Bacteria that are injected into tumour-bearing mice accumulate specifically in the tumour and lead to a partial tumour shrinkage. This reaction could be enhanced by depletion of granulocytes that are attracted by the bacterial invasion. When a complete tumour remission was induced the establishment of an anti-tumour immune response could be found.

2) A new aspect is brought in by Lars Zender, who studies the senescence surveillance in a chronic hepatitis model through inflammatory processes. Early results with this mouse model indicate that the cellular senescence programme together with the innate immune system limit tumour growth. Follow-up studies concern the mechanisms of how NK cells and macrophage recognise and kill senescent cells, define genetic lesions that allow to bypass cellular senescence, and study the biological significance of “senescence surveillance” for suppression of hepatocarcinogenesis.

3) The transcription factor IRF-1 that is normally induced by infections and follow-up inflammatory cytokines. When induced in tumours, IRF-1 induces an unusually rapid immune response and elimination of the tumour. The response also includes protection of these mice from tumorigenesis by the same tumours.

Within the theme “immunoregulatory networks” the role of T cells and mesenchymal cells in infection and autoimmunity play a key role. One activity aims at dissecting the basic mechanisms underlying the induction and regulation of mucosal T cell responses in the context of autoimmune diseases. Studies to investigate maintenance or breakdown of peripheral T cell tolerance during bacterial or viral infection have been initiated. The function of T regulatory cells, decision makers for tolerance, anergy, autoimmunity or defence is included in these studies. This work is mostly carried out in murine models, but human cells are studied in parallel.

The topic harbours the development of technologies that are specifically used for the projects but are also of generic nature. These include the development of nonviral episomal vectors, bacteria as carrier for DNA vaccination and gene therapy, the development of novel cell lines as in vitro infection models, ablation of gene expression by intrabodies, protein transduction, and imaging techniques to monitor intracellular and intercellular events. Novel transgenic animal models for the study of immune events such as tissue specific antigen induction as models for chronic infections or autoimmunity are in the focus of such projects.

Systems biology approaches concern the development and implementation of relevant mathematic and bioinformatic methods for modelling of regulatory networks. In the last year several grant applications supporting this new subject were successful. In this way a networking within the Helmholtz society with the neighbouring Magdeburg FORSYS centre and within the European Community is initiated. The projects aim at application of these tools for studying aspects of interferon regulation and Treg cell biology as well as the understanding of *Pseudomonas aeruginosa* and *putida* in the context of relevant environmental and host-related constraints. These studies will be used to eventually develop mathematical descriptions, modelling and simulation of the major events involved in the infection and immune response. The ultimate goal is to develop new intervention strategies.

03.1 Structural Analysis of the Innate Immune System

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In the Research Group Molecular Host-Pathogen Interactions (MHPI) of the Division of Structural Biology (SB), we are concentrating on the molecular details of general human defence mechanisms against invading pathogens (innate immunity), as well as the molecular strategies of pathogens in infecting humans.

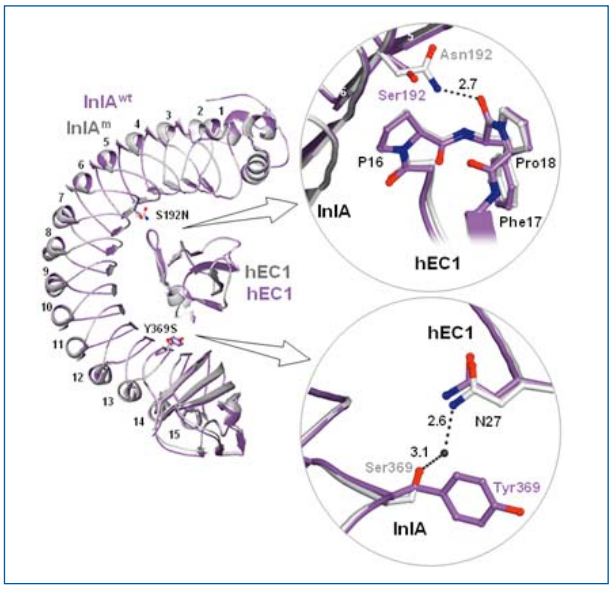
A new mouse model to study human listeriosis The bacterium *Listeria monocytogenes* is spread by contaminated food. It is able to infect humans by breaching the intestinal barrier. How it does this exactly and the route it uses were previously not clear, as infections in humans are normally only diagnosed when the infection has spread throughout the body and the infection could not be simulated in a small animal model.

We had previously solved the crystal structure of internalin (InlA), the main protein of *L. monocytogenes* involved in forcing its way into epithelial cells that line the intestinal wall, on its own and in complex with its human receptor E-cadherin. This analysis confirmed that, amongst other residues, Pro16 of human E-cadherin is crucial for the mutual recognition of these two proteins. In murine E-cadherin this residue is replaced by glutamate, which is physically larger and negatively charged compared to the smaller and hydrophobic proline. As a result, InlA cannot bind murine E-cadherin inhibiting the first step of a listerial infection in mice.

Analysing the complex of InlA and human E-cadherin in detail, we identified individual amino acid residues in InlA that did not optimally interact with E-cadherin. We replaced these residues by potentially more suitable amino acids and investigated the effect on the binding affinity between the two proteins. Essentially, all single-point mutations were found to increase binding affinity substantially. Combining the point mutations proved a little more complicated but we identified one combination denoted S192N/Y369S, the binding affinity of which is around 5000-fold higher than that of the wild-type protein.

Interestingly, this modified InlA-variant is now able to recognise and bind murine E-cadherin. Clearly the additional two interactions overcome the repulsive force of glutamate16 of murine E-cadherin. By incorporating the point mutations into the genome of *L. monocytogenes,* we created a mutant strain, which in theory should be able to recreate the first step of listerial infections in humans in the murine system.

Through appropriate infection studies in mice, we were able to demonstrate that our mutant strain of *L. monocytogenes* does indeed cause listeriosis-like symptoms when administered by the oral route – simulating the situation in humans. For the first time, we could identify the bacterial route of infection, starting with the invasion of the intestinal villi, through deeper intestinal tissues to the lymph nodes, spleen, and liver.



Modifying internalin (InlA) to increase its affinity towards human and hence also murine E-cadherin (hEC1).

In summary, our investigation stretches from the atomic level (analysing the role of individual amino acids in the interaction of InlA and E-cadherin), via individual molecules (modifying InlA) and single celled organisms (genetically modifying *L. monocytogenes*) to the level of interacting organisms (listerial infections of mice). In the process, we have created a small animal model to systematically analyse the early steps of the intestinal invasion of *L. monocytogenes*. Our model is currently being used to deepen our understanding of the invasion mechanisms of this human pathogen and to investigate how the human immune system responds to invading bacteria.

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03.2 IFN-Dependent Host-Responses upon Infection using Transgenic Reporter Mouse Models

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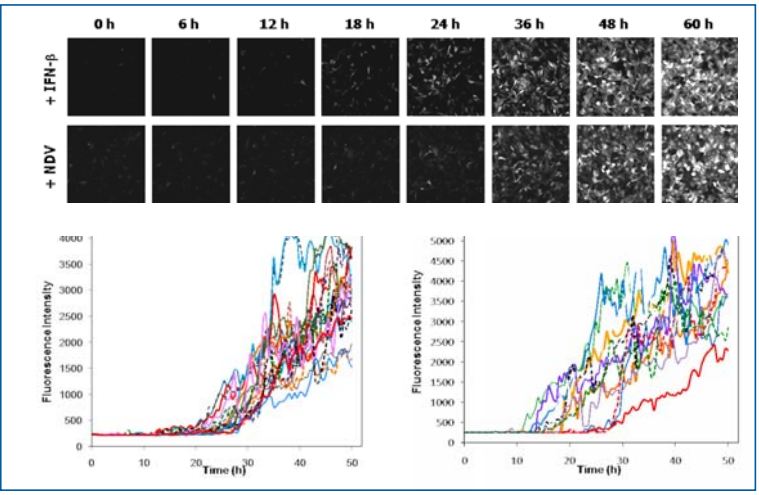
The innate immune system provides the first line of defence against viral, but also bacterial and fungal infections. Following infection, specific receptors initiate an intracellular signalling network that lead to the production of type I interferon (IFN) and proinflammatory cytokines, which mediate the innate antiviral immune response. Upon binding of type I IFN the activated receptor complex mediates tyrosine phosphorylation of the signalling molecules STAT1 and STAT2, resulting in dimer formation and their subsequent accumulation in the nucleus. IFNs can act on cells either directly or indirectly through innate and adaptive immune responses.

To analyse the IFN signalling network in single cells we combined key markers representing the successive steps of IFN induction and response with reporter genes. We made use of Bacterial Artificial Chromosomes (BAC) and have replaced the coding regions of different genes by genes encoding fluorescent or bioluminescent reporters. By this strategy BAC constructs were generated in which the whole upstream region of the gene remains intact. Using this technology, we have established reporter systems for online monitoring of signal transduction and gene induction of type I IFN response. Subjecting these cells to time-resolved fluorescence microscopy we have achieved quantitative information about the kinetics of IFN activity in single cells (Figure). Further, we will use them to characterise different phases of the infection process and to distinguish between auto- and paracrine activities of IFN.



NIH3T3 cells containing the recombinant BAC Mx2-tdTomato were treated with IFN-β or infected with NDV. Increase of red fluorescence was monitored by time lapse microscopy. An unbiased set of cells was selected for quantification of fluorescence intensity. Data of single cells are displayed in different colors.

To determine IFN activity in vivo we have established a BAC transgenic mouse model containing the interferon-inducible Mx2 gene locus and luciferase as a marker gene. By using a bioluminescent imaging system, macroscopic analysis of interferon activity in the whole organism was performed and the in vivo kinetic of IFN-dependent induction of luciferase expression in the living mouse was visualised. Our work will allow advancing questions of the spatio-temporal dynamics of the interferon response and tracing the cellular targets of these reactions within the whole organism upon viral infection. Moreover, novel reporter cell lines for type I and type II interferons were established. These cells are now used for accurate quantification of type I interferons and also for high-throughput screenings of libraries containing *e.g.* natural products to discover new agonists, enhancers and also antagonists of the interferon system. In an initial screening the family of Vioprolides were found to interfere with the cellular response to type I IFN measured by Mx2-driven luciferase gene induction.

The IFN network constitutes a powerful innate defence system that efficiently controls virus infection in mammalian cells, but the integration of single processes that influence the overall network has not yet been achieved. The fine-tuning of IFN induction and response is a result of several feedback loops. The induced products are responsible for new phenotypes and influence the next level of IFN signalling. The paracrine coupling between cells is an essential aspect that leads to spatial pattern formation. Our approach aimed to dissect the spatio-temporal dynamics of type I IFN using genetic techniques that enable highly accurate life-cell imaging of IFN signalling *in vitro* and *in vivo* and quantitative image analysis. Thereby, a mathematical modelling of the underlying reaction-diffusion processes will be possible.

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03.3 Epigenetic Principles of Gene Regulation

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PROJECT MEMBERS | Dr. Manfred Wirth | Dr. André Oumard | Sandra Broll | Dr. Junhua Qiao | Sören Turan | Stefanie Binius | Lijing Sun | Dr. Melita Vidakovic | Dr. Angela Gluch

Our studies focus on the functional organisation and differential regulation of loci relevant for host-pathogen interactions and inflammation, *i.e.* the type 1 interferon gene clusters and the PARP-1 gene in humans/mice (3).

Recent work has identified human IFN-α2 (the homologue to murine IFN-α4), as the second immediate-early interferon gene besides IFN-β. In their upstream regions both genes share unique SIDD-signatures consisting of three remote, highly destabilised SIDD sites to which we could assign regulatory potential. All sites carry functional YY1/YY2 consensi at their flanks; in the absence of SIDD structures consensus motifs are non-functional. Other functional IFN-α genes carry distinct, but related signatures.

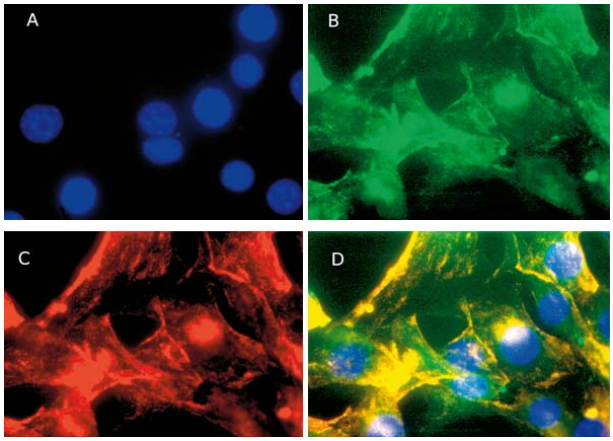
PARP-1 is well known for its recognition of ss- and dsDNA breaks. More recent functions concern the control of chromatin structure and transcriptional potential (3). Given the facts that i-PARP-1 inhibitors have a proven potential to counteract excessive inflammatory responses and ii-regulatory circuits exist to adjust a basal level of PARP-1 activity, we have investigated the molecular details. These studies unravelled an autoregulatory circuit: by binding to a novel consensus motif (AGGCC) a S/MAR next to its promoter, PARP-1 down-regulates its expression. Depletion of cellular pools at the nuclear matrix re-activates PARP-1 transcription.

In addition to their structural role, S/MARs mediate enhancer- and replication functions. The IFN-β associated upstream S/MAR is now routinely applied for the development of novel vector systems, most notably self-replicating nonviral minicircles (MCs; 1), for which the element contributes to both origin of replication (ORI) and maintenance functions. S/MAR-optimisations enabled a size reduction of MCs to 3.2 kb, providing these with a cloning capacity of >10kb. Several MCs can be established side-by-side, permitting the adjustable expression of multiple-subunit proteins.

Owing to their particular properties and to the fact that MCs can be withdrawn at will, they represent entirely novel tools for gene therapy, biomedicine and basic research. Their development enabled our participation in the excellence cluster “REBIRTH” and the SFB738 “innovative transplants”, both in co-operation with the MHH.

For two decades we have been developing biological test procedures for a variety of biophysical and biological activities of bordering elements. These projects have led to novel approaches like Flp-RMCE, which permits the targeted integration of expression cassettes at pre-determined genomic loci and the study of their interaction with a defined genomic environment. J. Qiao has developed a novel variant that selects for highly expressed RMCE-competent sites which enable high exchange efficiencies in the absence of selection. The procedure is unique in that it is not hampered by random (co-)integration or the co-introduction of selection markers/plasmid sequences, which would trigger epigenetic silencing. Based on seven novel and fully functional FRT-mutats S. Turan has succeeded in establishing a dedicated RMCE multiplexing routine to establish multiple genomic addresses that can be individually targeted . These techniques are of increasing relevance for the rational construction of ES/iPS cells and transgenic animals and thereby another contribution to the REBIRTH program.

Guided by MWI, we investigate both lentiviral persistence and the susceptibility to LV-infection with emphasis on the understanding of LV-integration, protein transport and virus assembly mechanisms. These studies serve the identification of novel therapeutic targets as well as the improvement of vector and vaccine production. A promising multifunctional component is caveolin-1, for which we identified roles in Gag transport and positioning as well as in the resistance to SIV-1 infection and a contribution to influenza virus replication.



Cav-1 positions related to newly synthesized MLV Gag at lipid rafts of the plasma membrane. Colocalization (D; yellow in overlay of A-C) of MLV-Gag (B; green) and Caveolin-1 (C; red) in NIH3T3 cells at the plasma membrane. *Photo: HZI*

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03.4 Cellular Models for Infection

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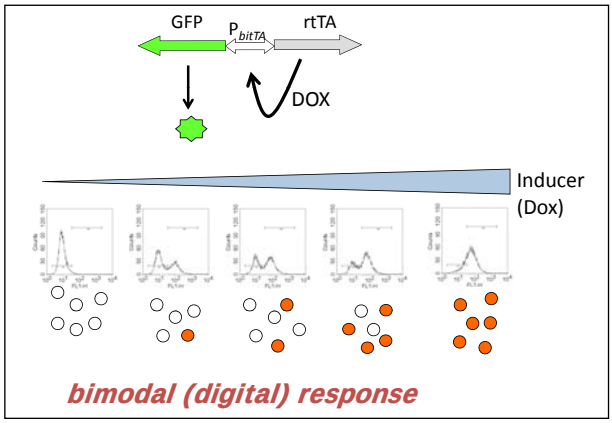
Investigation of complex questions in infection and immunity requires equivalent model systems with controllable features. We are designing such models by combining previously developed strategies for precise genetic modification of cells and mice with methods for tightly controlled transgene expression.

Stochastic expression from autoregulated feedback modules To mimic naturally occurring patterns of gene regulation we designed synthetic modules that allow the control of transgene expression by low molecular weight compounds such as Dox. Modules for graded and stochastic expression were developed. Stochastic expression was realised employing a recently developed autoregulated positive feedback module that is controlled by Dox. We could show that in a population of genetically identical cells the concentration of Dox in the medium determines the probability of transgene expression rather than the level of expression. These modules were mathematically described employing stochastic tools.



Stochastic expression from synthetic expression cassettes: A Dox dependent synthetic module was employed to establish an autoregulated positive feedback loop. This module was introduced into NIH3T3 cells. Upon increasing the concentration of the inducer (Dox), individual cell clones stochastically switch on GFP expression which can be monitored by single cell analysis (flow cytometry). Thus, this autoregulated positive feedback loop gives rise to a bimodal expression phenotype.

Controlled cell expansion Immortalisation of cells is used to provide sufficient numbers of homogeneous cell populations for various questions. However, such immortalised cell lines only partially reflect the properties of their primary progenitors, which is attributed to the permanent expression of the immortalising genes. We exploited the above described autoregulated expression modules for controlled expression of immortalising genes. Expansion of mouse and human primary cells of different cell types was achieved upon lentiviral transduction of expression modules controlling expression of various immortalising genes. Proliferation of these cells strictly depends on the presence of Dox, while absence of Dox leads to proliferation arrest. Fibroblasts, endothelial cells and Gingiva cells were successfully expanded. Importantly, the conditional immortalisation procedure preserved relevant tissue specific properties: for the expanded endothelial cells expression of CD31 and CD34 surface markers, the ability to take up acetylated LDL and tube formation could be confirmed. *Ex vivo* expanded cells and primary endothelial precursor cells are now investigated for *de novo* vessel formation upon injection of these cells either as circulating cells or as pre-differentiated spheroids.

Models for acute and chronic inflammation New models for acute and chronic inflammation in mice are developed. Blood and lymph vessel formation is being investigated. Moreover, lymphocytes releasing signal molecules for *de novo* vessel formation and their interaction with endothelial cells are being studied.

Interaction of controlled expanded cells with microbial biofilms We employed Dox controlled expanded cells to investigate interactions of oral microbial biofilms with the cells. Gene expression analysis revealed that the microbial biofilm had only minor effects on the gene expression pattern. These results indicated that oral microbial biofilms may have a limited inflammatory capacity *in vitro*.

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03.5 Mucosal Immunity and Inflammation

PROJECT LEADER | **Dr. Dunja Bruder | Research Group Immune Regulation | dbr@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Marcus Gereke | Milena Tosiek | Julia Schiller | Andreas Jeron | Sabine Stegemann

The Research Group Immune Regulation focuses on basic mechanisms underlying the induction and regulation of T cell mediated autoimmunity. For that purpose we have established transgenic mouse models for acute and chronic lung disease, colitis and type 1 diabetes which allow for the characterisation of complex disorders *in vivo*. Our long term goal is to improve our understanding in the complex cellular and molecular processes underlying autoimmunity. We intend to develop strategies for therapeutic modulation of the immune system to correct failure in the immunological balance between tolerance and autoimmunity and to restore immunological self tolerance. A further important aspect that we address is the impact of infections on the outcome of autoimmune diseases.

To study $CD4^+$ T cell reactivity to lung self antigen we generated a transgenic mouse model that is based on the expression of the model antigen influenza hemagglutinin (HA) in alveolar type II epithelial cells (AECII). Antigen recognition in the lung mucosa results in chronic obstructive pulmonary disease which is accompanied by the induction of peripheral tolerance mechanisms. Recent studies revealed that antigen expressing AECII are critically involved both in the induction and regulation of autoreactive $CD4^+$ T cell responses in the lung. We could show that AECII from the inflamed lung secrete factors that inhibit $CD4^+$ T cell proliferation *in vitro* and, in a TGF-ß dependent mechanism, support the differentiation of naïve $CD4^+$ T cells into $Foxp3^+$ Treg. Our results demonstrate that the immune modulatory function of AECII cells for the outcome of pulmonary disorders has been underestimated so far and that these cells represent an important link between innate and adaptive immunity in pulmonary disorders.

In a similar mouse model for autoimmunity in the lung we could show that auto-reactive $CD8^+$ T cells initially ignore their specific antigen in the lung mucosa. However, this immunological tolerance breaks down when mice were infected with influenza A, applied with LPS to mimic bacterial infection, $CD4^+$ T cell help was provided or immunosuppressive $Foxp3^+$ $CD4^+$ T cells were removed. These conditions result in massive lung-specific $CD8^+$ T cell activation and immunopathology in the lung. Our results provide important insights into the specific requirements leading to loss of immunological self tolerance in autoimmune prone individuals.

Comparing mice with and without chronic lung disease with respect to their response to influenza infection, we found that the course of infection is clearly different in diseased versus healthy mice. This is of particular interest in light of the fact that respiratory infections represent a major cause for disease exacerbation in patients suffering from chronic pulmonary disorders like COPD and asthma. We could demonstrate that infections have a strong impact on peripheral tolerance in the lung and that effective adaptive immunity to fight viral infection dominates over immune regulation and protection from autoimmunity.

A further topic deals with chronic infection and Treg. We have sorted Tregs from mice chronically infected with *B. bronchiseptica* and *H. hepaticus* and performed whole genome expression profiling with these cells. Our aim is to identify pathogen-specific Treg markers. Knowledge of such markers would allow for the targeted interference with T cell responses during persistent infections, *i.e.* to deplete or block pathogen-specific Treg to restore efficient pathogen clearance. A further project in this context is the development of a therapeutic vaccination against hepatitis C virus (HCV) infection. HCV infections often become chronic and are the major cause for hepatocellular carcinoma. In close collaboration with Thomas Pietschmann, Twincore, we are currently developing a therapeutic vaccination against HCV that is based on *in vivo* targeting of HCV antigen to dendritic cells.

Dr. Milena Tosiek analysing immune cells using a flow cytometer FACSCanto *Photo: HZI, Bierstedt*

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03.6 Immune Effectors: Molecules, Cells and Mechanisms

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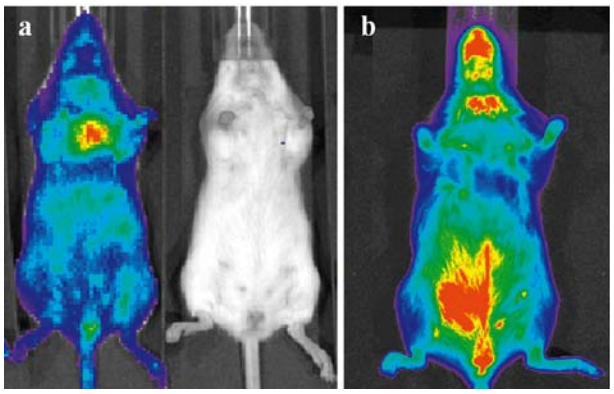
Our immune system comprises several lines of defence. One of the first of these to be encountered is the so-called Type 1 interferons, with their key representatives IFN-α and IFN-β. These were discovered as a result of their anti-viral properties. However, in the meantime it has also become evident that they are also induced with many other infections, as well as playing an important role in normal physiological processes. We have generated a reporter mouse in order to better understand this highly complex system. The primary characteristic of this mouse is that it carries the reporter enzyme luciferase under the control of the IFN-β promoter. This makes it possible to observe the induction of IFN-β in both tissue and cell homogenates, as well as via non-invasive *in vivo* imaging. In this, advantage is taken of the fact that a luminescent tissue is formed on the skin of the mouse, which can in turn be detected with a sensitive camera (Fig. 1). With this mouse we are able to show that the IFN-β gene can also be present in some tissues in an activated state without infection. The fact that the IFN system is activated via a feed-forward loop probably enables the entire system to be rapidly activated when required. Surprisingly, the highest constitutive expression was found in the thymus.



Non-invasive in vivo *imaging of IFN-β reporter mice. a) An untreated reporter mouse expressing luciferase under the promoter of IFN-β was injected with the substrate luciferin, anaesthetised and the spontaneous light emission measured with the IVIS 200 device. Distinctive here is the high signal in the area of the thorax, emitted by the thymus. On the right is a control mouse, which is expressing no luciferase. b) Expression of IFN-β was induced via the administering of polyI:C to a reporter mouse. After six hours luciferin was injected and the mouse measured in the IVIS 200. A clearly higher luminescence can be seen in comparison to the spontaneous expression. The primary expression appears to take place in the liver and intestinal area. Sensitivity was reduced by a factor of 100 in comparison to a).*

The constitutive expression of IFN-β also appears to have a key physiological significance. For example, dendritic cells of IFN-β KO mice were unable to efficiently stimulate T-cells. We were able to trace this to a defect in the expression of the heat shock proteins Hsp70.1 and Hsp70.3, which are regulated by IFN-β. IFN also plays a major role in defence against tumours. They are employed therapeutically in the clinic for specific types of cancer. Interestingly, B16 melanoma grew considerably faster in IFN-β KO mice than in normal mice. We were able to trace this back to the improved formation of blood vessels in the tumour in the absence of IFN-β. In the process we were able to show that IFN-β inhibits the expression of various blood vessel forming factors. IFN consequently represents a significant component of the surveillance system of the body against cancer.

Many bacteria, such as the *Salmonella typhimurium* that we used, possess the astonishing property of being able to concentrate in solid tumours. This means that they may be used to express therapeutic molecules directly into the tumour, in the process largely avoiding damage to the healthy tissue. In order to increase the efficiency of the tumour colonisation we undertook a detailed investigation of the migration of the bacteria into a solid murine tumour. This revealed that the bacteria presumably do not migrate actively into the tumour, as immobile mutants and mutants in which the chemotactical system has been inactivated were also able to colonise tumours efficiently. Rather, it was apparent that the bacteria trigger the secretion of tumour necrosis factor α leading to the increased flow of blood into the tumour. This means that the bacteria are flushed into the tumour passively. At the point of entry of the blood flow the tumour cells die, resulting in the creation of a large necrosis in which the bacteria are able to multiply and disseminate. Both the bacteria and presumably also the dying cells draw in neutrophil granulocytes which form a barrier around the necrotic areas, preventing the further spread of the bacteria in the still-living parts of the tumour. Removal of these barriers leads to a significant reinforcement of the therapeutic potential of the bacteria. These findings represent an important discovery in the progression towards a clinical application of this tumour therapy.

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03.7 Bioinformatics of Cellular Networks

PROJECT LEADER | **Prof. Dr. An-Ping Zeng | Research Group Systems Biology | aze@tuhh.de**

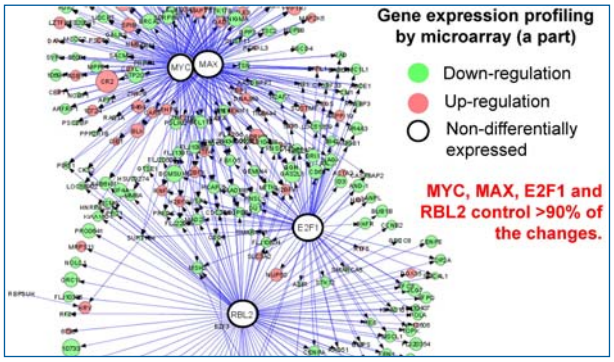
PROJECT MEMBERS | Feng He | Dr. Yuanhua Liu | Dr. Michael Stelze | Dr. Jibin Sun | Dr. Ping Zheng

Modelling and analysis of genome-scale metabolic network Bioreaction databases are a prerequisite for reconstruction and analysis of metabolic networks (MN). We upgraded our previous bioreaction database by almost doubling the number of reactions together with extensive revision regarding reversibility, reactant pairs, currency metabolites and spontaneous reactions. This upgrade significantly improved the quality of MN reconstruction, demonstrated with three model organisms *Escherichia coli, Aspergillus niger* and *Homo sapiens*.

Genome-scale MN is normally very large and complex. Previous studies have shown that it is organised in a hierarchical and modular manner. In particular, a core-periphery modular structure has been proposed for MN. We proposed a parameter called "core coefficient" to quantitatively evaluate the core-periphery structure of MN which is based on the concept of closeness centrality of metabolites and a newly defined parameter: network capacity. The method was applied to study genome-scale metabolic networks of five representative organisms which include *Aeropyrum pernix, Bacillus subtilis, E. coli, Saccharomyces cerevisiae* and *H. sapiens*.

Modelling and analysis of regulatory network A previously proposed method of reverse engineering of a gene network was applied to study oxidative stress of *Pseudomonas aeruginosa* under iron limited conditions. For this purpose, time-series dynamic data of genome-scale gene expression under well controlled physiological conditions were experimentally obtained and analysed to identify key regulators involved in the oxidative stress response.

In co-operation with Dr. M. Wirth we studied the gene expression profiles of a human CD4hoch+ T-cell line infected with the simian immunodeficiency virus (SIV) to elucidate the regulatory network leading to resistance of CD4hoch+ T-cells to SIV-induced death. For this purpose, a new network- and knowledge-based approach was proposed to identify pathways or sub-networks involved in resistance to SIV-induced cell death. In particular, this new approach uncovers significantly affected interactive molecular chains which affect the activities of non-differentially-expressed regulators, leading to identification of the so-called 'hidden' key regulators (Figure). The study indicated that resistance to SIV-1-induced death is a complex multi-component process involving sub-networks acting on different early steps in the viral replication.



Identification of non-differentially expressed genes as "hidden" key regulators by using a new network- and knowledge-based approach as demonstrated for human CD4⁺ T cells infected with the simian immunodeficiency virus.

Stochastic modelling of dynamics of synthetic gene networks The development of synthetic gene regulatory networks or circuits is the subject of increasing interest for basic research and therapeutic applications. The control of gene expression is essential for the application of synthetic gene circuits. The group of Dr. D. Wirth established a positive autoregulated feedback circuit based on the tet-system. Unexpectedly, in individual clones with the same gene regulatory circuit randomly integrated within the genome highly heterogeneous induction efficiency was observed. Also, a long period of induction was required to achieve full induction. To explain and to improve the efficiency and predictability of the autoregulatory system, we developed a stochastic model to describe the induction dynamics of cells with the synthetic gene circuit. In particular, modelling suggested chromosomal position effects to be an important parameter.

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Sun,J., Liu,X., Rinas,U. & Zeng,A.-P. (2007) Metabolic peculiarities of *Aspergillus niger* disclosed by comparative metabolic genomics. *Genome Biology* **8**, R182.

Prevention and Therapy

TOPIC SPEAKER | **Prof. Dr. Dr. Carlos A. Guzmán | Department of Vaccinology and Applied Microbiology | cag@helmholtz-hzi.de**

One third of all deaths occurring each year worldwide are directly caused by infectious agents. Microorganisms are also responsible for at least 15% of new cancers and they are involved in the pathogenesis of many chronic non-infectious diseases. The major public health problem represented by infections is rendered even more dramatic by the global emergence of multiple drug-resistant strains. It is, therefore, critical to establish new approaches for combating microbial pathogens. Thus, the main objective of this topic is to develop new tools and strategies to prevent, diagnose and treat infectious diseases.

The “Antigen Delivery Systems and Vaccines” project is focused on the development of tools and strategies to optimise the delivery of vaccine antigens, particularly via the mucosal route, and their subsequent exploitation for the generation of candidates against specific diseases. New candidate adjuvants were developed by improving existing molecules or discovering new entities. A pegylated derivative of alpha-galactosylceramide was generated which is water-soluble and exerts stronger adjuvant activities than the parental compound. Additional work showed that the bacterial second messenger bis-(3’,5’)-cyclic dimeric guanosine monophosphate exhibits potent adjuvant activity when given by systemic or mucosal route. A regulatory control circuit activated by acetyl salicylic acid (ASA) was also implemented in attenuated Salmonella which enables tightly regulated *in vivo* expression of target genes after bacterial infection. When Salmonella carrying an expression module coding for the 5-fluorocytosine (FC)-converting enzyme cytosine deaminase were given to mice bearing tumours, induction with ASA before 5-FC administration resulted in a significant reduction of tumour growth.

The “Therapeutic Cellular Vaccines” project is geared to developing strategies to break the immune escape mechanisms operating in persistent infections and cancer. To this end, tools to study the interactions between effector, regulatory and target cells were developed, such as 3D high-resolution imaging techniques, as well as murine models to investigate T cells activation, tolerance and autoimmunity. Antigen presenting cells were modified using adenoviral vectors encoding antigens and immunomodulatory molecules to improve their antigen presentation capacities. To facilitate translation of basic research into cell therapies, flexible and robust cGMP-compliant processes for the production of adenovirally modified dendritic cells were established, using a closed integrated bag system.

The “Intracellular Trafficking of Phagosomes and Immunity” project focuses on the mechanisms whereby *Mycobacterium tuberculosis* arrests phagosome maturation and avoids killing by macrophages. Towards this goal, the intracellular transport of non-pathogenic and pathogenic mycobacteria in macrophages is studied to identify proteins involved in vesicular trafficking during mycobacterium infection. These proteins are promising candidates for involvement in the lysosomal-mediated killing process, as well as in the molecular events linking innate and adaptive immune responses. The long-term goal of this project is to identify new targets for development of prophylactic or therapeutic interventions.

In the “Molecular Mechanisms of Hepatitis C Virus Infection and Replication” project virus-host interactions crucial for Hepatitis C Virus (HCV) replication are investigated. These efforts should help to define new drug targets and to implement screening assays for the identification of molecules preventing these crucial interactions. A particular focus of the work is the characterisation of

determinants defining the narrow species-tropism of HCV. Cell-based high-throughput screening systems are being developed to identify HCV-specific inhibitors using HZI compound libraries. The assays will address the complete viral replication cycle, encompassing virus entry, RNA replication and production of viral progeny.

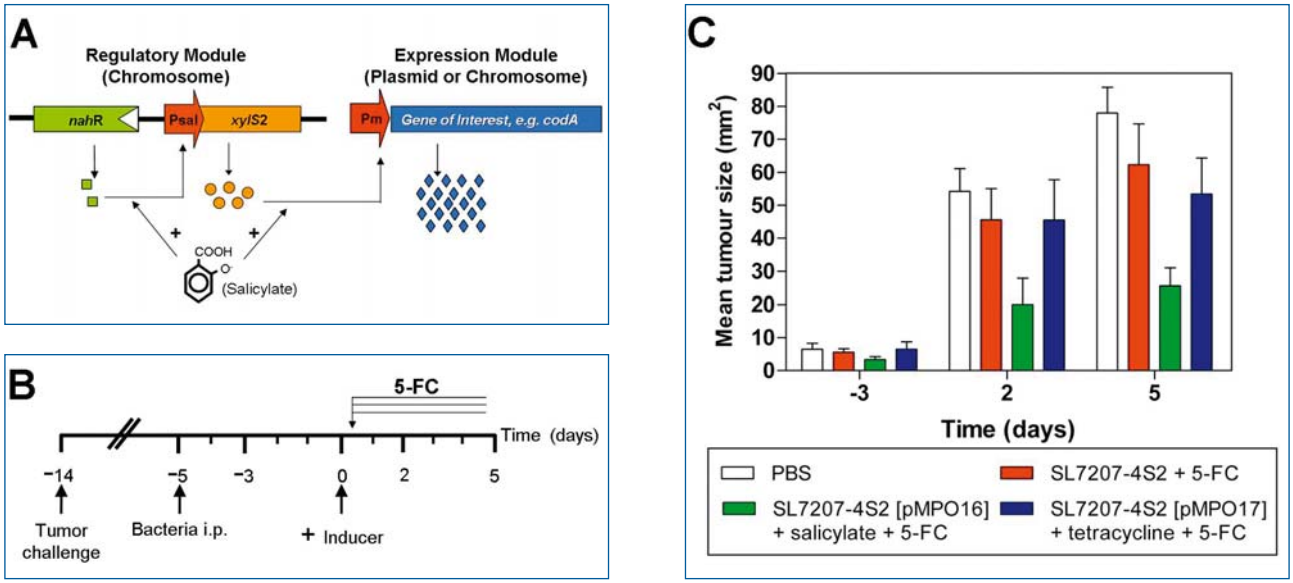
The "Molecular Diagnostics of Microbial Pathogens" project concentrates on environmental bacteria and viruses that cause infectious diseases and the processes that control these pathogens in natural and man-made environments. The focus lies on drinking water supply systems (DWSS) from source to tap. The project addresses three main questions: (i) How do the seasonal cycles influence the drinking water bacterial microflora, (ii) Which part of this microflora is still alive after chlorination, and (iii) Which specific groups of bacterial pathogens in a DWSS occur and how are they affected by seasonal cycle and chlorination. On the other hand, a Multi-Loci Variable Number of Tandem Repeats Analysis (MLVA) method was developed. This high resolution genotyping technique enables high-throughput analysis of clinical and environmental isolates of *V. parahaemolyticus*, thereby allowing discrimination of all major clones, including the pandemic ones.

The research activities in the anti-infective discovery area are focused on the identification and structure/function analysis of new active compounds, as well as in the elucidation of their mechanisms of action. To this end, microbial extracts and combinatorial chemical libraries are employed to search for small molecules with anti-infective activity. These activities form the "Chemical Pipeline" of the HZI. In the "Microbial Diversity and Natural Products Discovery" project novel groups of myxobacteria were evaluated for their biosynthetic potential. The annotation of the *Sorangium cellulosum* genome allowed the identification of new gene clusters encoding secondary metabolites, including silent ones. Mutagenesis of polyketide and peptide synthases allowed the identification of key genes involved in the biosynthetic pathway of secondary metabolites. Screenings were also initiated to find compounds able to interfere with quorum sensing and biofilm formation. The projects "Medicinal Chemistry of Anti-infectives" and "Development of Novel Antibiotics from Natural Sources" are focused on natural product synthesis and analogue design. These projects cover the chemical syntheses of novel antibiotics, such as corallopyronin, chlorotonil and chondramide. To this end, an interdisciplinary approach at the interface between organic synthesis, biochemistry and structural biology is pursued to analyse the interdependence of conformation and biological function. The pharmacodynamic and pharmakokinetic properties of lead structures are then optimised through the total synthesis of analogues and derivatives.

The central objective of the project "Chemical Biology of Infectious Diseases" is the elucidation of molecular mechanisms of infectious processes, using low molecular weight chemicals as tools. With the development of dedicated assays and screening technologies, bioactive compounds are selected from chemical repertoires and analysed. Results from these analyses shall lead to the discovery of new antibiotics, chemotherapeutics and immune modulators. Protein-protein interactions are essential steps in signalling pathways and protein complex assemblies but are difficult targets for drug development. Therefore, peptide SPOT-array technology was exploited to identify peptide fragments derived from one partner of a protein-protein interaction pair for binding to the other partner and vice versa. The peptide binders found can then be utilised as surrogate ligands in high-throughput competition assays to search for small molecules able to compete with the peptide for binding. This approach was successfully exploited

to identify binders to the trimeric influenza virus RNA polymerase which, after delivery into infected cells, were able to inhibit virus proliferation. A cell-based fluorescence reporter assay was also used to screen for compounds able to increase the intracellular level of cell cycle regulator p27. A cyclic peptide was identified which is a highly selective proteasome inhibitor and exhibits potent antitumor activity.

Candida albicans is one of the most important pathogens associated with nosocomial fungal infections, particularly in immuno-compromised patients. Therefore, in the "Identification of Molecular Targets of Anti-infectives" project histidine kinases (HK) of *C. albicans* were selected as potential drug targets. Chemical and genetic interaction studies with single gene deletion mutants showed that among the HK only CaNik1 was essential for the activity of the fungicidal natural products ambruticin VS-3 and jerangolide. A deletion mutant of the HK CHK1 also showed reduced virulence. Based on these results, CaNik1 and CHK1 will be exploited as candidate drug targets for future studies.



Tightly regulated expression of Salmonella genes by using a circuit based on the regulatory module nahR/PsaI::xylS2. (A) Schematic representation of the regulatory circuit. (B) Experimental design for assessing salicylate-mediated in vivo expression of the 5-fluoro-cytosine (5-FC) converting enzyme cytosine deaminase (CD) within tumor cells. (C) Tumor growth in untreated mice (PBS), and in animals receiving plasmidless SL7207-4S2 or bacteria carrying vectors encoding CD under control of either salicylate (pMPO16) or the tetracycline (pMPO17) induced expression systems. (For more details see under 04.6, page 86)

04.1 Microbial Diversity and Natural Product Discovery

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In autumn 2007 the launch of an epothilone for the treatment of breast cancer focused attention once again on the significance of the natural products of myxobacteria in health research. At the same time, it also indicated that fermentative production with myxobacteria can be industrially useful and economically viable. An expression of the biosynthetic gene cluster in heterologous hosts would be an alternative option, with work also underway in this area. For the molecular biology of the myxobacteria the sequencing and annotation of a *Sorangium* genome was a milestone, representing the analysis of the largest known bacterial genome at that time. The annotation revealed that many more genes exist for the synthesis of secondary metabolites than was previously assumed. The investigation of the biodiversity through the isolation of unknown myxobacteria is a further focus for the future.

Biodiversity Microorganisms related to myxobacteria can be identified by comparison of 16S rDNA with entries in databases. Sequences from the metagenome of terrestrial and marine sites form new branches in a common family tree. Is new potential for the production of secondary metabolites developed when such bacteria are isolated? Intensive efforts are underway to address this question. For example, in recent years more and more „myxobacteria“ have been isolated that do not fit into the traditional system. Initial chemical investigations of the metabolites confirm this expectation: new antibiotics have been isolated and their structures elucidated. Further antibacterially effective metabolites are in process.

Molecular biology Peptide synthetases and polyketide synthases (PKS) are the most important enzyme complexes for the synthesis of secondary metabolites of myxobacteria. To find the gene clusters responsible for this we attempted to interrupt the biosynthesis in a targeted manner. For thuggacin such mutants were created via transposon mutagenesis, whereas the leupyrrin synthesis could be inhibited via homologous „knockouts“. In addition to the expressed polyketide synthases, myxobacteria also have so-called „dormant gene clusters“ of the secondary metabolite synthesis. We are in the process to express these PKS cxlusters to produce new secondary metabolites.

Screening models While in the past the search for anti-infectives was limited to inhibitors of growth, the focus now is upon the search for metabolites that interact with pathogenicity of pathogenic organisms. The key in this are “two component signal transduction systems”. These complex, global regulation systems interfere with multi-drug resistance and quorum sensing, and, ultimately, with formation of biofilms and virulence. Metabolites that bind to highly conserved areas of the enzyme complexes could be natural products with a broad spectrum of effectiveness. It proved possible to isolate two metabolites of interest here from gliding bacteria and elucidate their structures.

Chemistry In the work with the elansolid producers a new elansolid was isolated that transforms itself irreversibly into the known elansolid A. The structural elucidation revealed the same constitution for both elansolids. As both substances can be converted into identical compounds by opening of the ring system this represents an unusual case of a natural compounds that exists in two conformations. The structural details are investigated with the aid of NMR spectroscopy and modelling of the structures.

Birte Trunkwalter and Prof. Rolf Müller observing myxobacteria under the microscope. *Photo: HZI, Gramann*

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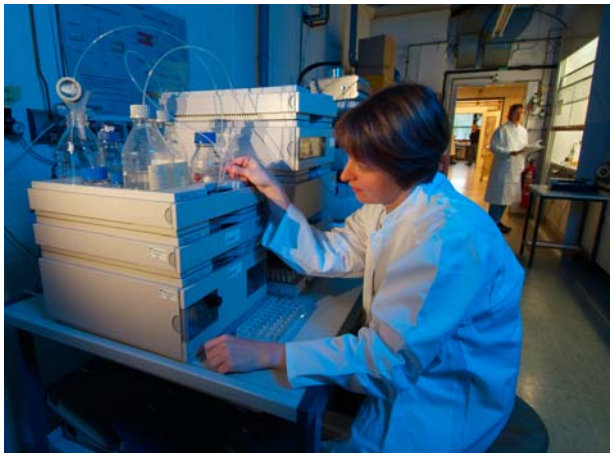
04.2 Medicinal Chemistry of Antiinfectives

PROJECT LEADER | **Prof. Dr. Markus Kalesse | Research Group Medicinal Chemistry of Antiinfectives | mka05@helmholtz-hzi.de | markus.kalesse@oci.uni-hannover.de**

PROJECT MEMBERS | Dr. Jutta Niggemann | Dr. Nicole Horstmann | Inga Degenhardt | Nicola Rautzenberg

This project covers the chemical syntheses of novel antibiotics. The biological profile of lead structures will be optimised through the synthesis of analogues and derivatives. Together with its biological experiments this approach will provide a detailed picture of SAR data. The identification of pharmacophoric groups will be achieved at the same time and allows a rational design of optimised compounds as tools for cell biology. Additionally, new synthesis strategies of automation such as the SPOT- or the PASS-Flow synthesis will be included.

Synthesis of corallopyronin, chlorotonil and chondramide. In order to optimise the pharmacodynamic and pharmacokinetic parameters of new lead structures and to understand the mode of action on a molecular level, a synthetic approach to these structures is the prerequisite of our approach. The natural products corallopyronin, chlorotonil and chondramide are promising examples of potential antibiotics and anti-tumour compounds. For all three compounds, initial biological data indicated their potential use as drugs. Nevertheless, structural optimisations are still required in order to fully exploit their medicinal potential. Therefore, it is necessary to provide access to structurally modified analogues through a total synthesis approach.



Larissa Jundt isolating active compounds through HPLC

Photo: HZI, Bierstedt

For the condramides, first derivatives were already provided and have been published together with their SAR data. These results clearly show that the polyketide segment is important for adjusting the active conformation in the peptidic part of the molecule. Further derivatives will be synthesised in order to complete the overall picture of SAR data.

A similar picture is available for corallopyronin. Here, first data indicating the mode of action is available and analogues indicate the pharmacophoric group. Based on these results, synthesis will provide derivatives with optimised *in vivo* activities.

Chlorotonil has its problems with the poor water solubility; derivatives should increase the hydrophobic character while keeping the pharmacodynamic properties in place. An additional project covers the synthesis of chivosazole. The antibiotic chivosazole contains 10 chiral centres. After our structure elucidation of these sterocentres we began to confirm the structure through a total synthesis. Additionally, this can provide analogues for detailed SAR studies. Since we completed the synthesis of the northern segment last year, we will focus on the synthesis of the southern segment and coupling both parts in the endgame of the synthesis.

Janssen,D., Abert,D., Jansen,R., Müller,R. & Kalesse,M. (2007) Chivosazole A – Elucidation of the full absolute and relative configuration. *Angewandte Chemie* **119**, 4985-4988.

Eggert,U., Diestel,R., Sasse,F., Jansen,R., Kunze,B. & Kalesse,M. (2008) Chondramide C: Synthesis, Configurational Assignment and First SAR Studies. *Angewandte Chemie International Edition* **47**, 6478-6482.

Rahn,N. & Kalesse,M. (2008) The First Total Synthesis of Chlorotonil. *Angewandte Chemie International Edition* **47**, 597-599.

04.3 Development of Novel Antiinfectives from Natural Sources

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PROJECT MEMBERS | Wiebke Ahlbrecht | Fatih Arikan | Dr. Nicole Horstmann | Dr. Herbert Irschik | Dr. Rolf Jansen | Jun Li | Pengfei Li | Sven Rudolph | Dr. Florenz Sasse | Heinrich Steinmetz

The exquisite and varied architectures of natural products provide a rich pallet for discovery in antiinfective research, whether these are used to probe biological mechanisms or to provide the basis for pharmaceutical drug discovery, natural products continue to command attention. Research in this project is centred around various aspects of natural product chemistry and antiinfective research, ranging from natural product isolation and the development of new synthetic methods to novel strategies in natural product synthesis and analogue design.

Polyketides are a particularly interesting class of natural products with an extremely broad range of biological activities and pharmacological properties. Polyketide antibiotics, antifungals, cytostatics, antiparasitics and natural insecticides are in commercial use. Myxobacteria are a rich source of novel types of polyketides. Prominent examples include the archazolids, highly potent inhibitors of vacuolar type ATPases (V-ATPases), etnangien, a macrolide antibiotic which inhibits RNA polymerase and rhizopodin, which interacts with actin and disrupts the actin cytoskeleton by binding specifically to a few critical sites of G-actin. An interdisciplinary approach at the interface between organic synthesis, biochemistry and structural biology has been initiated to analyse in detail the interdependence of conformation and biological function of these potent natural antibiotics. Based on an innovative approach, we have recently determined the full stereochemistry and ground state conformation of these polyketides, through high field NMR studies in combination with restrained molecular dynamics simulations. Besides more conventional techniques such as J-based configuration analysis (of both CH- and HH-dipolar couplings) and NOE experiments in combination with molecular modelling, analysis of residual dipolar couplings as well as gene-based methods have also been applied. This understanding of the 3D structure enables a directed total synthesis and the design of analogues and opens the way for a rational design of simplified analogues and an understanding of SAR data. Furthermore, convergent and, in particular, modular strategies for the synthesis of these complex polyketides have been developed. This involved novel methods for the stereoselective assembly of the characteristic arrays of methyl and hydroxyl-bearing stereogenic centres. Recently, application of these methods culminated in the first total synthesis of the archazolid. In addition, the design of equipotent analogues with improved biological profiles and/or simplified core structure has been established, with these expected to enhance the further development of these promising macrolide antibiotics.

Tatjana Arnold controlling the quality of a purified substance
Photo: HZI, Bierstedt

Arikan,F., Li,J. & Menche,D. (2008) Diastereodivergent Aldol Reactions of β-Alkoxy Ethyl Ketones: Modular Access to (1,4)-syn and -anti Polypropionates. *Organic Letters* **10**, 3521-3524.

Menche,D., Arikan,F., Perlova,O., Horstmann,N., Ahlbrecht,W., Wenzel,S.C., Jansen,R., Irschik,H. & Müller,R. (2008) Stereochemical Determination and Complex Biosynthetic Assembly of Etnangien, a Highly Potent RNA-Polymerase Inhibitor from the Myxobacterium *SorangiumCellulosum. Journal of the American Chemical Society* **130(43)**, 14234-14243.

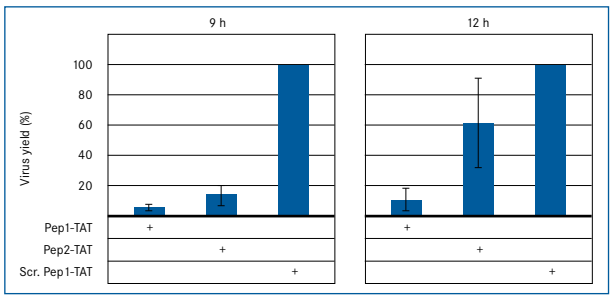
04.4 Chemical Biology of Infectious Diseases

PROJECT LEADER | **Dr. Ronald Frank | Department of Chemical Biology | rfr@helmholtz-hzi.de**

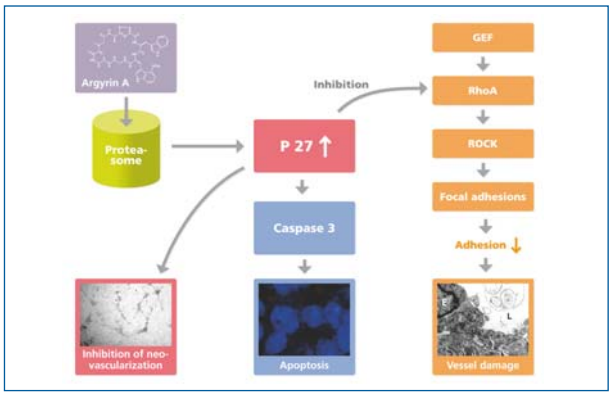
PROJECT MEMBERS | Jihat Al-Qudsi | Ulrike Beutling | Randi Diestel | Dr. Raimo Franke | Dr. Bernd Hofer | Fardous Kaneer | Denis Koska | Michael Mrosek | Dr. Irina Nickeleit | Dr. Mahtab Nourbakhsh | Dr. Marc Reboll | Dr. Florenz Sasse | Galina Sergeev | Dennis Schwab | Dr. Dr. Werner Tegge | Dr. Peter Washausen | Marina Wöhl

The central objective of this project is the elucidation of molecular mechanisms of infection processes, using low molecular weight chemicals as tools. With the development of dedicated assays and screening technologies, bioactive compounds are selected from chemical repertoires and analysed. Results from these analyses are intended to lead to the discovery of new antibiotics, chemotherapeutics and immune modulators. Knowledge about their mechanisms of action will provide novel targets for therapeutic intervention. As part of the "Chemical Pipeline" we have implemented a HTS (high throughput screening) infrastructure to enable the systematic search for novel anti-infective compounds. Our compound repository of currently around 90,000 samples also includes a unique collection of natural products isolated from myxobacteria (see Rolf Müller, 04.1). This infrastructure is also open to external collaborators and other applications through the German ChemBioNet (www.chembionet.de).

Inhibitors of protein-protein interactions Protein-protein interactions are essential steps in signalling pathways and protein complex assemblies but are difficult targets for drug development. They are mediated through shallow and large interaction surfaces which are not well suited to binding small molecules. We therefore exploit our peptide SPOT-array technology to first identify peptide fragments derived from one partner of a protein-protein interaction pair for binding to the other partner and vice versa. The peptide binders found can then be utilised as surrogate ligands in high-throughput competition assays to search for small molecules able to compete with the peptide for binding. We have successfully employed this approach to the subunit assembly of the trimeric influenza virus RNA polymerase. When delivered into infected cells, the peptide binders are able to inhibit virus proliferation. Within the EU integrative project FLUINHIBIT, a peptide-based competition assay in an ELISA format was established and first hit compounds were identified.



Reduction of influenza virus A proliferation in infected MDCK cells after treatment with synthetic peptides derived from the PB1 subunit of the viral polymerase. The PB1-peptides were fused to a short TAT peptide that mediates cellular uptake. A peptide with the same amino acid composition but different sequence than Pep1 served as negative control.



Phenotypic effects observed with cancer cells after inhibition of the proteasome by treatment with Argyrin A. The key cell cycle regulator p27 is no longer degraded by the proteasome, leading to reduced growth of solid tumors.

A myxobacterial cyclic peptide metabolite is a highly selective proteasome inhibitor and potent antitumor lead In collaboration with the research group of N. Malek at the Medical School Hannover, a cell-based fluorescence reporter assay was utilised to screen for compounds that are able to increase the intracellular level of the cell cycle regulator p27. This screen revealed among other small organic molecules a unique cyclic heptapeptide structure with unusual amino acid building blocks (HZI150006, Argyrin A) as a potent positive effector molecule. Mode of action studies confirmed a highly specific inhibition of all three catalytic activities of the eukaryotic proteasome, which results in a reduced degradation of p27. Intracellular increase of p27 effects growth arrest or apoptosis in many tumour cell lines. HZI150006 is reducing growth of solid tumours in *in vivo* xenotransplanted mouse models at a much lower dose and toxicity compared to the approved proteasome inhibitor Bortezomib. Further development of HZI150006 is carried out in the "TransMed-Lab project" funded by a BioProfile grant.

Ghanem,A., Mayer,D., Chase,G., Tegge,W., Frank,R., Kochs,G., García-Sastre,A. & Schwemmle,M. (2007) Peptide-mediated interference with the influenza A virus polymerase. *Journal of Virology* **14,** 7801-7804.

Nickeleit,I., Zender,S., Sasse,F., Geffers,R., Brandes,G., Sörensen,I., Steinmetz,H., Kubicka,., Carlomagno,T., Menche,D., Buer,J., Gossler,A., Manns,M.P., Kalesse,M., Frank R. & Malek,N.P. Argyrin A – a p27kip1 stabilizing drug with potent anti-proliferative and anti-angiogenic activity. *Cancer Cell* **14,** 23-35.

04.5 Identification of Molecular Targets of Antiinfectives

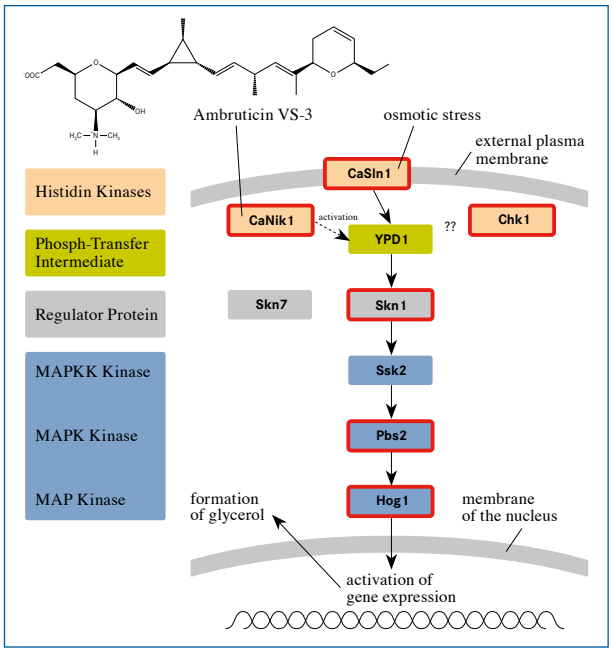
PROJECT LEADER | Prof. Dr. Ursula Bilitewski | Research Group Biological Systems Analysis | ubi@helmholtz-hzi.de

PROJECT MEMBERS | Janine Wesolowski | Nina Klippel | Dörthe Sokolis | Shuna Cui | Rabeay Hassan | Anna Buschart | Anja Köhler

Increasingly, infections are caused by resistant, commensal or persistent organisms. Commensal and persistent organisms colonise the host for even long periods of time without any obvious symptoms. Under predisposing conditions in the host, such as a compromised immune system due to a severe disease, an infection can develop from these organisms. As this complicates the therapy of the major disease, appropriate antiinfectives are needed. Screening for new aniinfectives usually does not consider the development of an infection from the commensal or persistent state of the pathogen, i.e. the interaction with host cells. Therefore, we aim to acquire a detailed understanding of this system, as this may lead to the identification of new targets for antiinfectives. We focussed the project on the commensal yeast *Candida albicans* and macrophages and epithelial cells as host cells.

Histidine kinases of C. albicans as drug target candidates Bacterial histidine kinases (HKs) are known as sensor proteins for environmental conditions activating the respective adaptation reactions. In recent years these proteins have also been identified in lower eukaryotes, such as fungi, whereas they are absent in mammalian cells. In *C. albicans* there are three HKs, of which CaSln1 is homologue to the only HK of *S. cerevisiae,* where it is involved in the sensing of and defence against osmotic stress. The second *C. albicans* HK, CaNik1, is homologue to histidine kinases in fungal plant pathogens. By chemical – genetic interaction studies with single gene deletion mutants we could show that among the HKs only CaNik1 is essential for the activity of the fungicidal natural products ambruticin VS-3 and jerangolide. Additionally, the complete osmotic stress defence pathway is required. Cloning the gene of CaNik1 and transformation of *S. cerevisiae* transferred the sensitivity for these fungicides to *S. cerevisiae*, confirming the relevance of this protein as a fungicide target. The deletion mutant of the third HK, CHK1, had shown a reduced virulence. In our investigations, we observed a significantly improved phagocytosis efficiency of macrophages and neutrophils for this mutant in particular and could correlate these effects to changes in the β-glucan structure of the cell wall. Based on these results we will continue the exploitation of the HKs CaNik1 and CHK1 as drug target candidates.

Signal transduction pathways in macrophages and epithelial cells Both epithelial cells and macrophages are the first barrier of defence of the host against invading pathogens. As a consequence of the recognition of pathogens by cell-membrane bound receptors, they activate the innate immune response and cells of the adaptive immune system by phagocytosis of the pathogens and secretion of cytokines and nitric oxide (NO). We analyse the structure of the intracellular signal transduction networks via their perturbation by specific inhibitors. The pattern of secreted cytokines, the NO concentration, the activation of an NF-κB dependent reporter and Western Blot analysis of selected phosphorylated proteins are used as analytical endpoints. These studies shall lead to the identification of critical nodes which are involved in the maintenance of the commensal state of the pathogen and may be inactivated during infection.



Signal transduction cascade, which is activated in Candida albicans *by either osmotic stress or fungicides, such as ambruticin VS-3. The cascade originates at the histidine kinases CaSln1 and CaNik1, respectively. The structure of the cascade downstream of CaNik1, which is targeted by ambruticin VS-3, was elucidated by a chemical-genetic interaction approach based on ambruticin VS-3-treatment of a number of deletion mutants and the determination of glycerol in the culture supernatatnt. The pathway-relevant deletion mutants are indicated in the scheme and were the CaNik1-, CaSln1-, Chk1-, Ssk1-, Pbs2- and Hog1-mutants.*

J. Behnsen, P. Narang, M. Hasenberg, F. Gunzer, U. Bilitewski, N. Klippel, M. Rohde, M. Brock, A.A. Brakhage, M. Gunzer, The environmental dimensionality controls the interaction of phagocytes with the pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*, PLOS Pathogens, **3** (2) (2007) e13

N. Klippel, U. Bilitewski, Phagocytosis assay based on living *Candida albicans* for the detection of effects of chemicals on macrophage function, Anal. Lett., **40** (2007) 1400 – 1411

J. Wesolowski, R.Y.A. Hassan, S. Hodde, C. Bardroff, U. Bilitewski, Sensing of oxygen in microtiterplates: a novel tool for screening drugs against pathogenic yeasts, Anal. Bioanal. Chem. **391** (2008) 1731 - 1737

04.6 Antigen Delivery Systems and Vaccines

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The main aim of this project is the development of tools and strategies to optimise the delivery of vaccine antigens, particularly by the mucosal route, and their subsequent exploitation for the generation of candidates against specific diseases.

Gene expression induced by aspirin Systems enabling tightly regulated expression of prokaryotic genes are critical for studying gene function *in vivo* and exploiting recombinant bacteria for targeted expression of therapeutic molecules. To this end, we integrated a regulatory control circuit activated by acetyl salicylic acid (ASA) in attenuated Salmonella (Fig. see page 80). This enables tightly regulated *in vivo* expression of the target gene after bacterial infection in response to salicylate, resulting in 20-150 fold induction ratios *ex vivo*. The regulatory circuit was also efficiently induced by ASA when bacteria resided in eukaryotic cells, both *in vitro* and *in vivo*. To validate the circuit, we administered Salmonella carrying an expression module coding for the 5-fluorocytosine (FC)-converting enzyme cytosine deaminase (CD) in the chromosome or a plasmid to mice bearing tumours. Induction with ASA before 5-FC administration resulted in a significant reduction of tumour growth with respect to both controls and mice receiving bacteria in which CD expression was controlled by a tetracycline-induced system. These results demonstrate the usefulness of the control circuit to selectively switch on gene expression during bacterial infection. This method should facilitate functional studies to elucidate the role played by bacterial genes during the infection process, as well as the implementation of bacteria-based therapies.

Optimising antigen delivery by mucosal route The use of adjuvants represents a successful approach for the stimulation of immune responses following mucosal vaccination. Previous work led to the development of the TLR2 agonist MALP-2 and derivatives as mucosal adjuvants. However, a single molecule with well-defined immune modulatory properties is insufficient to address all needs in vaccinology. Thus, our discovery programme was expanded to identify new candidate adjuvants by modifying existing molecules or searching for new entities. A pegylated derivative of alpha-galactosylceramide (alphaGalCerMPEG) was generated which is water-soluble and retains both the specificity for CD1d and the *in vitro* stimulatory properties on dendritic cells. Intranasal vaccination showed that alphaGalCerMPEG exerts stronger adjuvant activities than the parental compound. High titers of antigen-specific antibodies were detected in serum, together with Th2 and sIgA responses, both at local and remote mucosal effector sites. Additional work showed that the bacterial second messenger bis-(3',5')-cyclic dime ric guanosine monophosphate (cdiGMP), also exhibits potent adjuvant activity. Mice receiving β-galactoside (β-Gal) with cdiGMP showed 512-fold higher anti-β-Gal IgG titers in sera than controls, as well as sIgA in both lung and vagina. The profiles of secreted cytokines suggest the induction of a dominant Th1 response. *In vivo* CTL responses were also observed in C57Bl6 mice immunised with ovalbumin and cdiGMP. The obtained results indicate that these two new molecules are promising tools for the development of mucosal vaccines.

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Ebensen,T., Link,C., Riese,P., Schulze,K., Morr,M., & Guzmán,C.A. (2007) A pegylated derivative of alpha-galactosylceramide exhibits improved biological properties. *Journal of Immunology* **179**, 2065-2073.

Ebensen,T., Schulze,K., Riese,P., Morr,M., & Guzman,C.A. (2007). The bacterial second messenger c-diGMP exhibits promising activity as a mucosal adjuvant.. *Clinical and Vaccine Immunology* **14**, 952-958.

Fiorentini,S., Riboldi,E., Facchetti,F., Avolio,M., Becker,P.D., Guzman,C.A., Sozzani,S., & Caruso,A. (2008). HIV-1 matrix protein p17 induces human plasmacytoid dendritic cells to acquire a migratory immature cell phenotype. *Proceedings National Academy of Sciences USA* **105,** 3867-3872.

04.7 Therapeutic Cellular Vaccines

PROJECT LEADER | **Dr. Werner Lindenmaier | Department of Gene Regulation and Differentiation | wli@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Kurt E. J. Dittmar | Dr. Wilhelm Meyring | Dr. Oliver Schön | Dr. Nadia Zghoul | Claudia Preuß | Ellen Kuppe

In addition to the control of acute infections the immune system is responsible to recognise and eliminate tumour cells and persistently infected cells. Persistent pathogens and tumour cells, however, have developed mechanisms to escape immune surveillance. The aim of this project is to overcome the escape mechanisms and to develop cell based technologies for immunotherapies of tumours and persistent infections. An important prerequisite for this approach is a better understanding of the interactions between effector cells and regulatory cells. This knowledge is necessary to avoid immune escape and to control side effects and immunopathology. When the efficiency of the concepts has been proven in animal models, methods for cGMP compliant generation of therapeutic cells have to be developed.

Visualisation/investigation of cellular interactions The highly complex and dynamic interactions of different cell types responsible for activation and down regulation of an immune response mainly take place in specialised lymphoid organs (e.g. lymph node, spleen, gut associated lymphoid tissue). Specific staining protocols and software tools were developed for simultaneous, three-dimensional high resolution imaging. To analyse dynamic interactions of cells in normal or diseased tissues confocal laser screening microscopy was used. High resolution images of tissue structures were obtained by electron microscopy (coll. M. Rohde, HZI).

Functional analysis in murine model systems To analyse the influence of different therapeutic cells a transgenic murine model was established which allows the investigation of T cell activation, tolerance and autoimmunity. The influence of vaccination with adenovirally modified dendritic cells and adoptive transfer of transgene specific T cells on the induction of an immune response was investigated. Vaccination with DC expressing the transgene could only break tolerance when antigen specific T cells were present.

Tools for the generation of therapeutic human cells
A central aim of the project is the development of optimised protocols for the generation of therapeutic human cells. We have established a flexible, reproducible and cGMP compliant process for the production of adenovirally modified dendritic cells.

Coating of the inner surface of a bag by dielectric plasma discharge at atmospheric pressure, courtesy of M. Thomas, FhG-IST. Top: bag before coating, bottom: light emission due to plasma discharge during coating. Photo: HZI

This process is based on a bag cultivation system which is closed from cell isolation by leukapheresis, via differentiation, adenoviral gene transfer, maturation up to the final formulation. Until now, this bag system could only be used for suspension culture. In co-operation with the Fraunhofer Institute for Surface Engineering and Thin Films, Braunschweig, and additional partners (http://www.vdivde-it.de/innonet/) we were able to change the properties of the bag surface by dielectric barrier discharge in a way which allows bag cultivation of adhesion dependent cells, *e. g.* immunoregulatory mesenchymal stem cells. Such modifications of the bag system will enable the closed bag system to be employed in a number of different applications in immunotherapy and regenerative medicine.

Ma,B., Zimmermann,T., Rohde,M., Winkelbach,S., He,F., Lindenmaier,W., & Dittmar,K.E.J.*. (2007) Use of autostitch for automatic stitching of microscope images. *Micron* **38**, 492-499.

Ma,B., Lin,Z., Winkelbach,S., Lindenmaier,W., & Dittmar,K.E.*. (2008) Automatic registration of serial sections of mouse lymph node by using Image-Reg. *Micron* **39**, 387-396.

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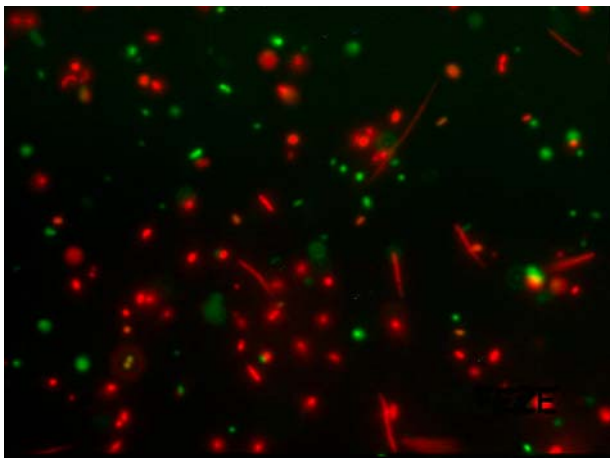
04.8 Molecular Diagnostics of Microbial Pathogens

PROJECT LEADER | **Priv.-Doz. Dr. Manfred Höfle | Department of Vaccinology and Applied Microbiology | mho@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Ingrid Brettar | Dr. Erika Harth Chu | Joesphin Draheim | Karsten Henne | Leila Kalisch

The molecular diagnostics of microbial pathogens is a rather recent focus within the topic "Prevention and Therapy". This new focus concentrates on environmental bacteria and viruses that cause infectious diseases and the processes that control these pathogens in natural and man-made environments. Man-made environments, such as sewage treatment plants, drinking water supply systems (DWSS) or hospitals, are often important reservoirs of infectious bacteria. Currently, we concentrate on drinking water supply systems from source to tap. We focused on three main questions: 1) How does the seasonal cycle influence the drinking water bacterial microflora, 2) Which part of this microflora is still alive after chlorination, and 3) Which specific groups of bacterial pathogens in a DWSS occur and how are they affected by seasonal cycle and chlorination.

Waterborne bacterial pathogens The main reason for our limited knowledge of bacteria in drinking water is their unreliable detection by culture-based conventional techniques. Almost all monitoring systems currently used in the public health sector are based on these conventional techniques. On the other hand, most pathogenic bacteria in the environment are in a so-called viable but non-culturable state (VBNC) and cannot be recovered with conventional procedures, although they are still infectious. Over the last decade, direct analysis of DNA extracted from environmental samples has been used to circumvent these limitations and to detect bacteria using molecular methods without cultivation. Therefore, we followed the seasonal cycles in the DWSS of the city of Braunschweig using a nucleic acid (DNA/RNA) based approach over a two year period. This approach used SSCP community fingerprints and demonstrated that there are some seasonal changes in the overall community composition of the drinking water microflora. Some of the species occurred only in summer or winter, but many of the bacterial key species, consisting mostly of uncultured bacteria, remained constant. The assessment of the dead/live status of the drinking water microflora using fluorescent dyes indicated that about 50% of the total cells are still alive after chlorination (see Figure). SSCP fingerprints of the live bacteria revealed that this fraction of bacteria stayed rather constant during the course of the year. The detection of members of two pathogenic bacterial genera, namely *Legionella* and *Helicobacter*, were successfully detected in various parts of the drinking water supply systems.



Epifluorescence microscopy picture of the drinking water microflora after dead/life staining (Molecular probes). Green are living bacterial cells, red cells are damaged or dead bacterial cells. *Photo: HZI*

Foodborne bacterial pathogens In addition to water, food is another important infection route. *Vibrio parahaemolyticus* is a foodborne pathogen that is primarily obtained from uncooked mussels. It causes severe diarrheal diseases in countries bordering the Pacific Ocean, such as Japan, Chile and Peru. We have developed a Multi-Loci Variable Number of Tandem Repeats Analysis (MLVA) method that enables the High-Through-Put analysis of clinical and environmental isolates of *V. parahaemolyticus.* The method was validated with environmental and clinical *V. parahaemolyticus* strains. It could be demonstrated that this high resolution genotyping technique allowed discrimination of all major clones including the pandemic ones. This newly developed tool for the molecular diagnostics of pathogens will enable the rapid detection of specific *V. parahaemolyticus* clones and is a valuable contribution to the prevention of outbreaks of foodborne diseases with pandemic potential.

Brettar,I., Guzmán,C.A. & Höfle, M.G. (2007) Human pathogenic micro-organisms in the marine environment - an ecological perspective. CIESM Reports No. 31, Marine Sciences and public health - some major issues. Geneva, September 2006, p. 59-68. (http://www.ciesm.org/online/monographs/Geneva.html)

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04.9 Molecular Mechanisms of Hepatitis C Virus Infection and Replication

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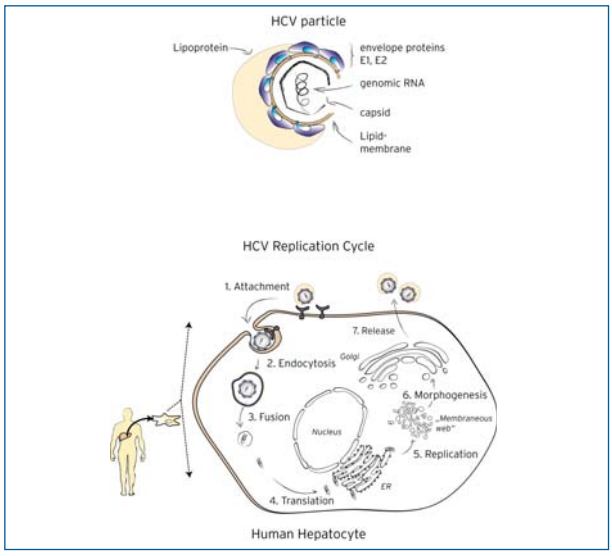
The hepatitis C virus (HCV) is a highly variable RNA virus that causes chronic infection associated with severe liver disease. With about 130 million individuals chronically infected worldwide and approx. 500,000 chronic carriers in Germany, HCV infection is a major cause of liver disease worldwide. At present there is no preventive or therapeutic vaccine available and standard of care IFN-based antiviral treatment is limited by insufficient response rates and severe side effects. Clinical trials of HCV enzyme inhibitors revealed that drug-resistance mutations compromise the efficacy of these antiviral compounds. Therefore, novel substances with antiviral activity, different mode of action, and cross-genotype efficacy are urgently needed to implement highly active combination treatment regiments for control of virus replication and escape.

Characterisation of virus-host interactions First, by dissecting the requirements for HCV replication and infection in human cells, we set out to identify key molecular interactions between viral proteins and host factors. These efforts should help to define new drug targets and to implement screening assay for the identification of molecules preventing these crucial interactions.



Schematic representation of the virus particle and the HCV replication cycle. Upon interaction of HCV particles with key entry receptors on the cell surface, virions are internalized and escape the endocytic vacuole by a low pH-induced fusion mechanism. Uncoating of the nucleocapsid liberates the viral RNA into the cytoplasm. Viral proteins establish membrane-bound replication complexes which amplify the HCV genome. Progeny virions are assembled at intracellular membranes and leave the cell.

Like all viruses, HCV is a strict intracellular parasite that can only replicate in permissive host cells. Due to its simple genetic composition and limited coding capacity, HCV relies heavily on host cell proteins and molecular machines for its replication. On the one hand, a delicately tuned interplay between viral proteins and host cell factors ensures virus propagation in permissive cells. On the other hand, lack of essential host factors may restrict the spread of viruses to other tissues or species thus defining viral tropism. A particular focus of our work is devoted to the characterisation of determinants defining the narrow species-tropism of HCV. We use this approach to define crucial mechanisms of the virus-host interaction and also to contribute to the development of convenient small animal models for analysis of virus replication and pathogenesis (further Fig. see page 127)

Identification of novel antiviral compounds
Secondly, in collaboration with the Department of Chemical Biology we develop cell-based high-throughput screening systems to identify HCV-specific inhibitors utilising the compound library of the HZI. Importantly, the assay will be implemented to interrogate the complete viral replication cycle encompassing virus entry, RNA replication and production of novel viral progeny. Due to this setup, in principle inhibitors which block a viral or cellular target and which may interfere with any phase of the viral life cycle may be identified. We have established a dual reporter gene assay which permits measurement of HCV replication, infection and assembly in cultured hepatoma cells in parallel to cell viability. Due to this format, specific inhibition of HCV can be distinguished from indirect blockade of virus propagation due to cytotoxicity. Leads identified during the screening will be characterised with regard to the mode of inhibition and the cellular or viral target addressed. Knowledge of the mechanism of action should facilitate future development of candidate molecules for clinical application and in addition may further our understanding of the requirements for HCV replication.

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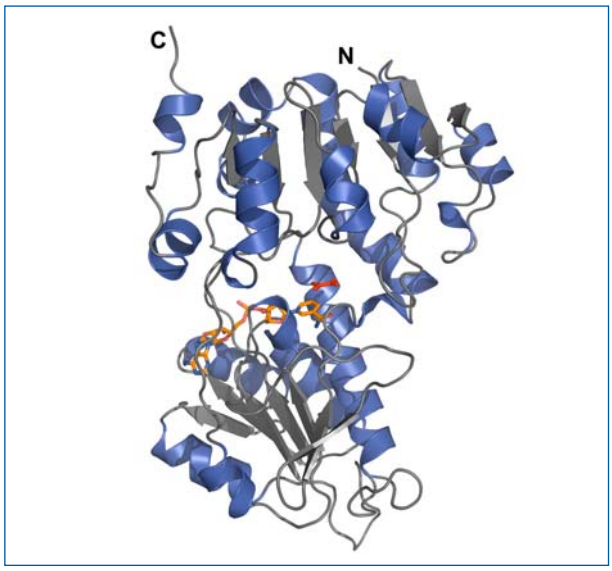
GENOME AND HEALTH RESEARCH

PROGRAMME SPEAKER | **Prof. Dr. Dirk Heinz | Division of Structural Biology | dirk.heinz@helmholtz-hzi.de**

Pathogenesis often results from the complex interplay between genetic and environmental factors. Besides inherited genetic defects and dispositions, factors such as age, lifestyle and environmental stress predominantly contribute to disease processes. Genome-wide studies and in-depth analyses of genome information are thus important elements in studying genotype-phenotype relationships with both prognostic and diagnostic aspects in health care.

In addition, the role of individual genes within the cell and their interactions in cell complexes and cellular networks, for example tissues, as well as their epigenetic, translational and post-translational regulation, still remain to be elucidated. Comparative genome research comprises model-driven experimental approaches that are complemented with information-driven computational and theory-based data interpretation. These techniques are applied to the deep annotation of complete bacterial genomes and the comparison of clinical isolates of the human pathogen Mycobacterium tuberculosis.

Furthermore, the specific interactions between gene products, *i.e.* proteins, and their ligands are investigated using synthetic chemistry. Here the design and generation of synthetic mimetics closely representing discontinuous binding sites leads to novel inhibitors of *e.g.* host-virus interactions.



Structure of a top priority target for persistant tuberculosis.
(see page 92)

01 Inhibitors of Protein-Ligand Interactions

PROJECT LEADER | **Prof. Dr. Jutta Eichler | Research Group Conformational Protein-Ligand Interactions | jutta.eichler@medchem.uni-erlangen.de**

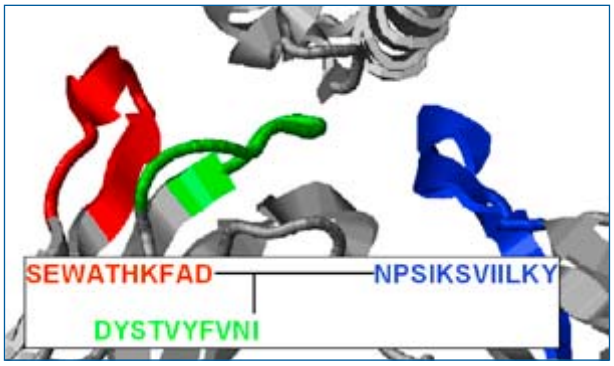
PROJECT MEMBERS | Dr. Kalle Möbius | Enge Sudarman

The overall objective of this project is to design and generate synthetic mimetics of conformationally defined protein binding sites. Such molecules are excellent tools for the exploration and, ultimately, control of biological function through interference with the underlying binding events.

Specific interactions of proteins with their ligand are the molecular basis of essentially all biological processes. The exploration of these interactions at the molecular and atomic level is an important step towards the modulation of protein function through controlled interference with underlying binding events. The design and generation of molecules that are capable of mimicking conformationally defined binding and/or functional sites of natural proteins, represents a promising strategy for the exploration and understanding of protein structure and function. In addition to their basic significance, such binding site mimetics are also useful tools for a range of biomedical applications, in particular the inhibition of protein-ligand interactions.

The functional and binding sites of proteins are often not localised in continuous stretches of the amino acid sequence, but rather in sequentially distant fragments of the molecule, which are brought into spatial proximity by protein folding. Synthetic molecules aimed at mimicking such discontinuous protein binding sites should therefore also be conformationally constrained and/or sequentially discontinuous.

This concept is based on using assembled and scaffolded peptides, in which the fragments making up a discontinuous protein binding site are presented in a non-linear, discontinuous fashion. The goal of our projects is to design and generate synthetic mimetics of the binding sites of a range of biomedically relevant proteins, including interaction domains, as well as the binding sites of viral proteins (HIV-1 gp120 and SARS-CoV S1) for their cellular receptors, and to use these molecules as inhibitors of pathogen-host interactions, as well as synthetic immunogens to raise virus neutralising antibodies.



Synthetic mimicry of the binding site of the cytokine receptor gp130 for viral interleukin-6 (vIL-6). Such mimetic molecules are able to inhibit the interaction between gp130 and vIL-6, as well as the vIL-6-induced proliferation of cytokine-dependent gp130-expressing cells.

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Taussig,M.J., et al (including Eichler,J., Frank,R.) Proteome Binders: planning a European resource of affinity reagents for analysis of the human proteome. *Nature Methods* **4**, 13-17.

02 Generation and Exploitation of DNA Sequence Data

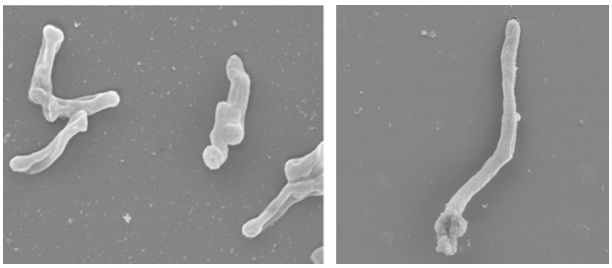
PROJECT LEADER | **Dr. Helmut Blöcker | Department of Genome Analysis | hbl@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Sabin Bhuju | Dr. Michael Böcher | Dr. Igor Deyneko | Ayssar Elamin | Michael Jarek | Yulia Kalybaeva | Dr. Rosa Martínez | Dr. Gabriele Nordsiek | Maren Scharfe | Dr. Oliver Schön | Prof. Dr. Mahavir Singh | Dr. Matthias Stehr

Sequence analysis projects More than ever, the sequencing and analysis of primary DNA is one of the principal approaches/basic techniques in modern fundamental biological research. Our work includes the comparative sequence analysis of clinical isolates from pathogenic organisms such as *M. tuberculosis* with special emphasis on genes involved in virulence, persistence, antibiotic resistance and host preference.

We are now wrapping up the complete sequencing and functional analysis of chimpanzee chromosome X (international consortium). This study led in particular to a lower transition/transversion ratio (3.88) than previously estimated (4.31). We analysed selected regions from the horse, pig and cattle genomes, most of which are suspected to be disease-related. We analysed bacterial communities in the gut of mice and the influence of nutrition on the composition of gut flora (metagenomics). Deep annotation of a number of bacterial genomes is complete. The implementation of two so-called next generation DNA sequencers (Illumina/Solexa) enabled us to considerably expand the genomic analyses, quantitatively as well as qualitatively. Thus, to allow for RNAi screening with complex libraries, especially negative selection screening, we established deep sequencing for deconvoluting shRNA representation in complex shRNA libraries (with L. Zender's group).

MycoGenomes Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) complex members is a common human diseases, causing 3,000,000 deaths per year worldwide. Supported by an EU project "NEW TB DRUGS", we characterised several targets for diagnosis of persistent and Mtb multi-drug-resistant (MDR) strains. Alanine dehydrogenase (AlaDH) is a potential target for new anti-TB drugs. In the project the 3D structure of the AlaDH was solved, which is the basis for rational drug design. The prodrug pyrazinamide (PZA) is one of the most important drugs for anti-TB shortcourse chemotherapy because it is also bactericidal to semidormant *M. tuberculosis.* In order to predict PZA resistance of Mtb strains we analysed mutations in the PZase genes from more than 100 clinical strains and identified several new mutations leading to a loss of PZase activity. Within the framework of the EU project "Fastest TB" we search for new antigens, suitable for TB diagnosis. We have detected new antigens, which are currently being assayed. Further problems of TB research and vaccine development for poverty-related diseases (PRD) are tackled in the frame of the EU funded projects FAST-XDR-DETECT and Transvac, the latter is the European platform for vaccine development for HIV, TB and malaria.



Overexpression of 85A altered the morphology of Mycobacterium smegmatis cells (left) enlargement and branching of M.smegmatis with changing surface shape, also after induction the cell exhibits expression systems. Wild type M.smegmatis mc2 155 was used as control (right), all the transformants and wild type were harvested and observed by scanning electron microscope (SEM) *Photo: HZI, Rohde*

Novel bioinformatics technology We are exploring applications of signal theory (as established in image analysis and speech recognition) for the function-oriented analysis of biomolecules. Our intent is to reveal similarities, homologies and analogies based on considerations of their physico-chemical properties and to confirm these findings by wet lab data. The software "FeatureScan" is publicly available (http://genome.helmholtz-hzi/featurescan). Our system is able to spot property-dependent similarities where letter code-based systems fail.

In the analysis of clones from the genome of *Salmonella typhimurium* (with the group of S. Weiß) we identified promoters and respective genes, up-regulated in colon cancer cells. A significant bias in gene functional classes was observed. In-depth analysis is now in progress. In the course of the analysis of human genomes we searched for very small potential peptides. We assume that the function of a sub-fraction of such small units may consist of the regulation of protein modifications or as adaptive building blocks.

Further Fig. see page 90

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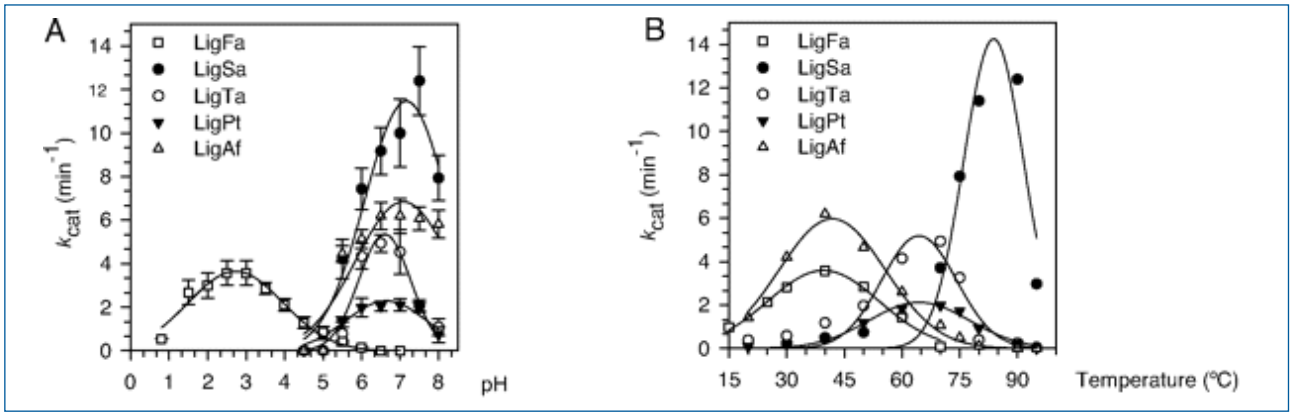
GENES, ENVIRONMENT AND HEALTH

PROGRAMME SPEAKER | **Dr. Wolf-Rainer Abraham** | **Research Group Chemical Microbiology** | **wab@helmholtz-hzi.de**

Microorganisms are omnipresent and the fact that they tolerate far more extreme environmental conditions than higher organisms means that their habitats also define the biosphere of our planet. With their activities microorganisms exercise a major influence on global processes such as the carbon cycle and global warming, as well as on local factors such as plant and animal diseases. In addition, they also supply indispensable nutrients for plants and animals. Microorganisms have a range of different effects, positive and negative, on man and his activities: they are responsible for the majority of illnesses and deaths, others provide medicines for the treatment of disease and yet more make a decisive contribution to cleaning the environment of organic waste products. Biotechnology utilises microorganisms and their products in many fields. However, if we are to be able to influence the activity of microorganisms in order to benefit more from the positive aspects and restrict the negative aspects as far as possible, then we need to know just how they exist and function in their habitat and how their activities are controlled.

In classical microbiology pure cultures are investigated that grow under laboratory conditions. In nature, however, microorganisms multiply in complicated, complex, dynamic communities, the members of which are interdependent on one another and use the available resources jointly in complex manners. This interdependency and the interaction with other living organisms and inanimate components of the environment determine the activity of a community. We have no general knowledge of such interdependencies as yet.

With this research programme we are pursuing the goal of comprehending the biocoenosis of microorganisms as a functional unit and clarifying the decisive interdependencies in the control of their activities. We aim to develop and validate intervention options that can lead to a reinforcement of biotechnological procedures and, by investigating the variety of different forms, discover new products and metabolic processes of the microorganisms. The research programme is characterised by work at different levels – gene, organism, biocoenosis, *in vitro*, chemostatic, natural habitat – and interdisciplinary activities such as microbial ecology, physiology, evolution, biochemistry, analytical chemistry, genetics/genome research, bioinformatics and modelling. The findings obtained in this will in principle be applied to a large part of all microorganism communities, however, our research is concentrated on those communities that can either trigger disease in humans or metabolise environmental toxins. A key objective of this programme consists of contributing to the sustainable development of our society.



Activity vs. pH assays of LigFa and other studied ligases. (Fig. 2 of K. N. Timmis' contribution, see next page)

01 Functional Genomics and Niche Specificity

PROJECT LEADER | **Prof. Dr. Kenneth N. Timmis** | **Research Group Environmental Microbiology** | **kti@helmholtz-hzi.de**

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Ferroplasma acidiphilum is an acidophilic ferrous-iron-oxidising microbe, an archaeon, remarkable in its phylogenetic position, physiological features, geochemical role, biotechnological significance, as a subject for the study of mechanisms of acid resistance, and, probably most interestingly, its evolutionary origins. With a pH optimum for growth of pH1.7, *F. acidiphilum* belongs to the most acidophilic microbes known to date.

Enzymes from *F. acidiphilum* have low pH activity optima *in vitro*. Through enzymatic screening of a genomic expression library of *F. acidiphilum*, we identified three α-glucosidases whose activities had extraordinarily low pH optima: 1.5–3.5. Detailed analysis of a membrane-bound α-glucosidase, revealed no significant similarity to any known glycoside hydrolase, a new mechanism for sugar glycosylation and transglycosylation, and iron to be necessary for the integrity and activity of the enzyme.

The cellular machinery of *F. acidiphilum* is iron-protein-dominated. In fact, all of the enzymes we retrieved through the initial activity screening turned out to be iron-dependent enzymes. To determine whether or not other proteins of *Ferroplasma* are iron-containing, we identified proteins and determined their metal content. Of 78 unique luminal-stained proteins, all contained iron and 10% contained other metals. Of these 78 metalloproteins, 50 belonged to classes never previously shown to contain iron. A subsequent genome-wide analysis revealed that 86% of *F. acidiphilum* proteins are iron-metalloproteins. Many of these are housekeeping proteins that do not ordinarily contain iron and, in most cases, no other metal ion. Quantification of the metal content of individual polypeptides showed that the iron:protein stochiometry is consistent with the notion that iron is bound in *F. acidiphilum* proteins in a specific way, co-ordinated by iron ligands in defined domains and is functional. Iron is crucial for maintenance of the three-dimensional structure, and hence activity, of *Ferroplasma* proteins, a function we designated the “iron rivet”.

Proteins of phylogenetic and habitat neighbours of *Ferroplasma* exhibited far fewer and only typical metalloproteins. *F. acidiphilum* has therefore a unique iron-protein-dominated cellular machinery and biochemical phylogeny.

DNA ligase from *Ferroplasma* has the lowest pH optimum of any ligase. Purified DNA ligase (LigFa) of *Ferroplasma* is deep purple in colour (Fig. 1), contains two ferric iron centres and has the lowest pH activity optimum of any known ligase (2.5-3.0), unique characteristics for any organism thus far. As the pH of the enzyme solution is increased, there is a concomitant loss of activity (Fig. 2, s. page 93), loss of iron and loss of the purple colour (Fig. 1). Reduction of the ferric atoms to ferrous results in an 80% decrease in catalytic activity and DNA substrate binding, and displacement of the pH activity optimum (5.0) towards neutrality. Thus, iron content and low pH optimum are coupled functions in this protein involved in organising and stabilising its 3-D structure.

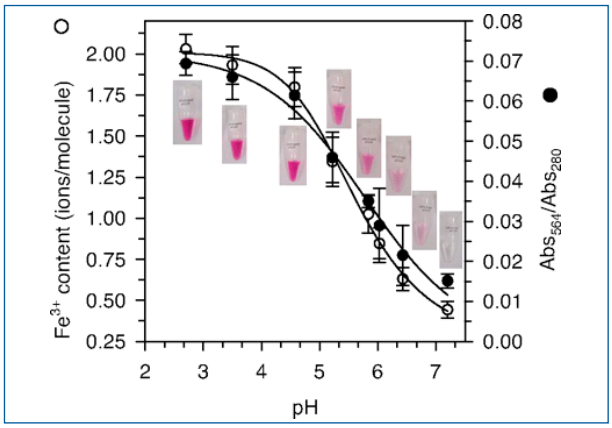

Fig. 1. Iron (III) content, purple color and low pH activity optimum of LigFa are coupled characteristics.

The extraordinary features of LigFa and some other *Ferroplasma* proteins currently under investigation, and the thus far unique iron-protein-dominated metabolic machinery of *F. acidiphilum*, promise potential for new biotechnological applications, new aspects of mechanisms of acid tolerance of organisms and, most intriguingly, new insights into the evolution of early life and its probable origins on iron-sulfur-rich surfaces.

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02 Metabolic Diversity

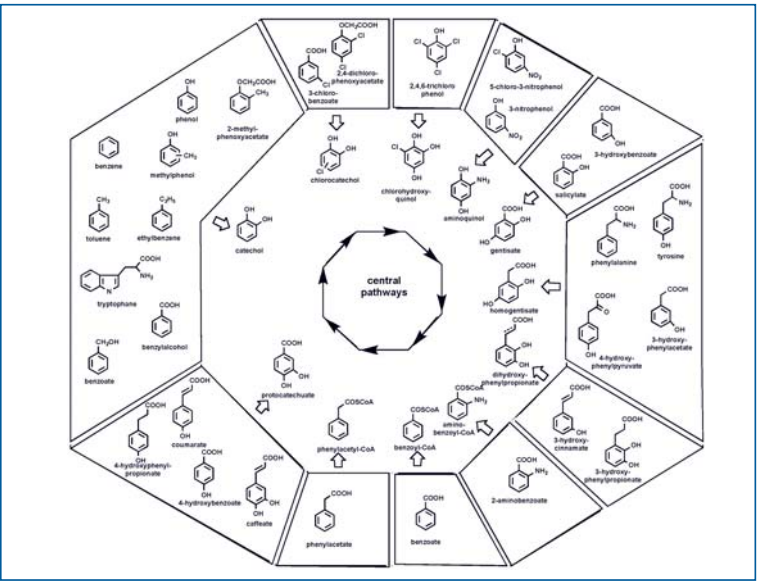
PROJECT LEADER | **Priv.-Doz. Dr. Dietmar Pieper | Research Group Biodegradation | dpi@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Howard Junca | Dr. Ramiro Vilchez | Macarena Marin | Dr. Hannes Nahrstedt | Dr. Melissa Wos

Project metabolic diversity The goal of the project is the understanding of the functioning of microbial communities under in-situ conditions focusing on human associated communities and interaction with pathogens. It is based on knowledge gained and methods developed in efforts to characterise communities in contaminated environments.

From strains to communities Substituted furanones are a group of natural products which comprise quorum sensing molecules, inhibitors and various secondary plant metabolites but which are also central metabolites in aromatic degradation. We have now established the function of the first member of an uncharacterised protein family as being responsible for transformation of furanones such as *trans*-dienelactone and substituted muconolactones in *Pseudomonas reinekei* MT1 and proven the enzyme to be a metal-dependent hydrolase.

The availability of complete genomes permits rational exploitation of the capabilities of microorganisms. We performed the metabolic reconstruction of aromatics degradation by *Cupriavidus necator* JMP134, linking the catabolic abilities predicted in silico with the range of compounds that support growth and gained a better understanding on the metabolic net of single baterial species. Similar work was performed on *Bordetella petrii*.



Overview of the degradation capacity of aromatic compounds by C. necator *JMP134 . Aromatic compounds (boxed) are funneled through a variety of peripheral reactions (represented by arrows) into central intermediates, which are then processed by a central pathway to TCA cycle intermediates.*

Gene arrays dedicated to achieving fast monitoring of catabolic gene diversity and abundance and thus the catabolic landscape have successfully been developed. Specific groups of dioxygenases could be detected in contaminated sites under study and their presence validated by catabolic gene fingerprinting as well as by a metagenomic approach. Genetic analysis revealed a highly upgraded picture of catabolic gene diversity in contaminated environments, as subfamilies assumed to be important from culture-based studies were not abundant. Analysis of catabolic properties of gene products indicates complementary metabolic properties and carbon sharing by the gene hosts and thus gave insights into catabolic nets in complex communities.

Human associated communities Biliary stents are catheters placed to overcome obstructions of the biliary duct, which typically become occluded by biofilms and have to be replaced. Our work revealed a more complete survey of the identities of bacterial species that form such biofilms, their co-colonisation patterns and the natural variation in species composition between different patients, hospitals and location along the stent.

The anterior nares are the major reservoir in humans of *Staphylococcus aureus* and transmission of *S. aureus* occurs by direct contact to a colonised carrier. However, very little was known about the composition of the nasal microbiota and possible community interactions. We have now elucidated the community composition of various individuals.

Additionally, we aim to understand how genetic factors or nutrition can influence the composition of bacterial communities in the gut and how these changes in bacterial communities affect the host response to infections. In an effort to approximate the human situation, we have analysed the effectivity of human microbiota establishment in gnotobiotic mice and rats. Comparisons of overall community structures revealed significant host differences but also allow the optimising of further analyses to specific groups of microorganisms in specific genetic backgrounds and under specific environmental influences.

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03 Biofilm Communities in Environment and Health

PROJECT LEADER | **Dr. Wolf-Rainer Abraham | Research Group Chemical Microbiology | wab@helmholtz-hzi.de**

PROJECT MEMBERS | Andreia B. Estrela | Ahmed Gebreil | Marcela G. Heck | Dr. Heinrich Lünsdorf | Dr. Alexandre J. Macedo | Dr. Sonja Pawelczyk | Esther Surges

This project aims to understand microbial communities as functional units and to discover new methods of controlling them by exploring microbial diversity and interaction. With our research work we aim to combine the knowledge of microbial communities in the environment with the investigation of clinical biofilm communities.

Carbon flux in bacterial communities ... The knowledge of what bacteria are found in a microbial community is usually not enough to enable the comprehension of that community. A key step towards answering the question of who has what task within the community is the investigation of the use of the substrates by the various bacteria types. To this end, we have optimised a process that works with the stable, *i.e.* non-radioactive, carbon isotope ^{13}C. This procedure involves ^{13}C-labeled substrates being added to the community and the investigation of the incorporation of the marking in biomolecules, in this case fatty acids. Fatty acids were chosen because they are often different in bacteria and can be used to differentiate between individual bacteria taxa. However, this is not always the case and we have therefore developed a model in which the individual bacteria species are coloured via specific antibodies, following the separation of the coloured bacteria from the non-coloured ones in a cell sorter. In this process we were able to render the analysis of the isotope marking so sensitive that we are able to investigate 100 million bacteria cells with regard to their incorporation of the ^{13}C isotope. The sorting process is significantly faster than in previously applied methods and we are subsequently not only able to determine the rate of incorporation of the substrate into the fatty acids but also the speed at which incorporation takes place. In toxin-reducing microbial communities we were able to indicate that intermediate products that are poisonous for one type of bacteria are utilised and consequently detoxified by others. Interestingly, this process revealed clear differences in the speed and strength of incorporation of the marking in the various bacteria types, which may explain tolerance of the community of high concentrations of pollutants.

On the surface of a stream near Waldau polluted with tar products a white biofilm has formed. This is a community of many different bacteria and is working to break down the pollutants *Photo: HZI, Abraham*

... and in complex bacterial communities The knowledge acquired was then applied to studies of the interactions in complex microbial communities. Here, we continued our studies on the fate of methane in rice fields and in the Siberian tundra. We analysed samples of the Max Planck Institute for Terrestrial Microbiology in Marburg for the incorporation of the stable isotope ^{13}C from methane and were able to contribute to the understanding of the flux of this gas detrimental for the climate in the environment. Reforestation of grassland is viewed as one way of reducing the increased CO_2 content of the atmosphere by fixation into biomass. Within the same co-operation we could show that the microbial communities involved in methane degradation do not lose activity in afforested areas. Nevertheless, three times less methane is degraded compared to the grassland and we could show that the reason for this is the reduction in the amount of methane degrading bacteria. The carbon budget in total, however, is positive in afforestation because the increase in carbon fixation is much higher than the reduction in the methane degradation. CO_2 fixation was also the topic in a very different habitat, the Baltic Sea. In co-operation with the University of Rostock we were able to illustrate that in the deep Baltic a significant fixation of CO_2 occurs at the transition from aerobic to anaerobic water. The determination of the ^{13}C-content of fatty acids of bacteria along this gradient leads to the estimation that about ¼ of the carbon is derived from this CO_2 fixation. To our knowledge, this is the first time that such an effect could be shown directly in environmental samples.

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04 Communities of Pathogenic Bacteria

PROJECT LEADER | **Dr. Wolf-Rainer Abraham | Research Group Chemical Microbiology | wab@helmholtz-hzi.de**

PROJECT MEMBERS | Andreia B. Estrela | Marcela G. Heck | Dr. Heinrich Lünsdorf | Dr. Alexandre J. Macedo | Dr. Sonja Pawelczyk | Esther Surges

In the environment bacteria are not usually found as pure strains, instead living and working in communities. In this project we address the question of how such bacteria behave in the environment and how they interact with non or facultatively pathogenic bacteria in the human body.

Pathogenic bacteria in the environment Bacteria that lead to infections in humans depart the human body at some point and are then exposed to a completely different environment. How do they survive? Together with the University of São Paulo we investigated this question on the Rio Tieté. The river flows through the 25-million-inhabitant city of São Paulo, taking on a large amount of untreated waste water, which also includes faeces. The river is extremely polluted in the urban area of São Paulo and contains many human pathogenic bacteria. 100 km downstream from São Paulo the number of human pathogenic bacteria decreases strongly. Interestingly, the degree of antibiotic resistance of all of the bacteria found in the river also decreases significantly over this distance.

Increasingly, microbiology indicates that there is no principle difference between bacteria that lead to infection and those that live in the environment. We have been able to illustrate this with previously unknown bacteria from a Swedish blood sample. We have identified it as a new species within the genus *Phenylobacterium*. To date, this genus has been known solely for its role in breaking down pollutants and from environmental samples. However, our *Phenylobacterium haemophilum* is closely related to similar bacteria types that have been isolated in freshwater samples in North America, indicating the close relationship of species from highly different habitats.

Diversity of biofilm communities on cardiac pacemakers According to the comprehension of biofilm infections thus far, infections are caused in patients when biofilms form on implants. We investigated the question of whether biofilm communities can also be identified on implants where the patients display no signs of infection. In collaboration with the Hannover Medical School cardiac pacemakers removed from patients due to battery exhaustion were examined for microbial biofilms. In the meantime we have examined over 150 of these devices. After an average of five years in the patient we were able to identify bacterial DNA in 47.2%. Interestingly, in the majority of cases it was not one species of bacteria that was observed but almost always - as with the environmental samples - bacterial communities. When the 16S rRNA gene sequences of these bacteria are investigated it becomes apparent that most of these bacteria are atypical for clinical device infections. Only in 3.7% of patients staphylococcae were found, which in turn triggered symptoms of infection in the investigated patients during the examination period. It is apparent that there are biofilm communities in humans that trigger no recognisable symptoms but that may offer potential reservoirs for the colonisation of pathogenic germs which may in turn lead to infections.

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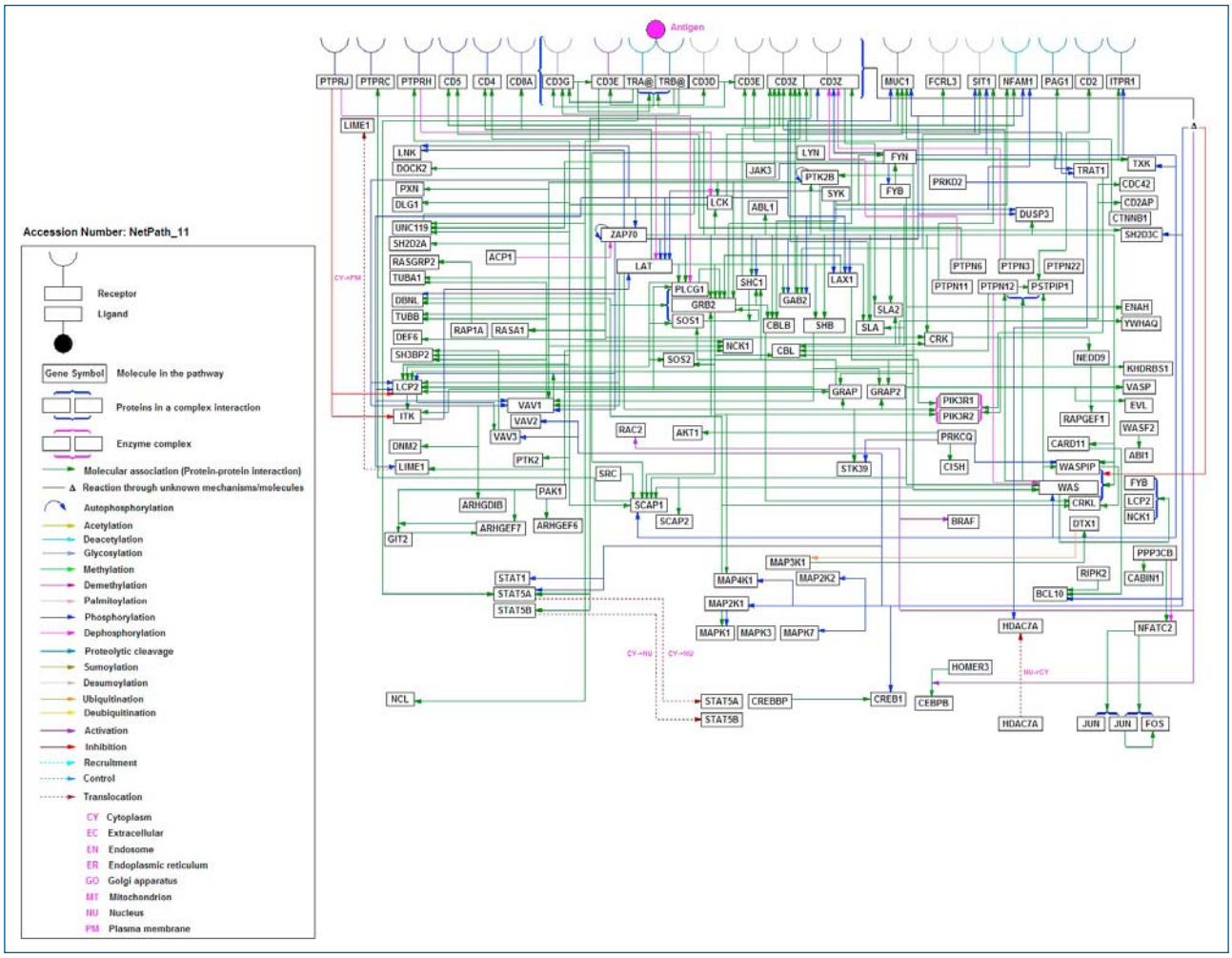
Pichlmaier,M., Marwitz,V., Kühn,C., Niehaus,M., Klein,G., Bara,C., Haverich,A. &Abraham,W.-R. (2008) High prevalence of asymptomatic bacterial colonisation of rhythm management devices. *EUROPACE* **10,** 1067-1072.

The Tieté River in São Paulo, Brazil, receives the waste waters from more than one Million people of the City of São Paulo. Therefore, the river contains a lot of quite pathogen bacteria. These have different sensitivities against antibiotica than those from the Elbe River. But may be that also the different use of antibiotica in both countries plays a role. *Photo: HZI, Abraham*

Technological Platforms

A number of platform technologies essential for research and development carried out at the Helmholtz Centre for Infection Research are made available to the scientific projects as centralised facilities. In the context of national and international research programmes, these platforms provide services not only to internal projects, but also to scientific collaborators from other Helmholtz research centres, universities, other public research institutes, and industry. On the following pages the most important platforms are described in detail.



T cell receptor signalling pathway. (Fig. 2 of R. Geffers' contribution, see page 101)

01 Central Animal Facility

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The Central Animal Facility provides state-of-the-art laboratory animal care and service for the scientific needs of the HZI in compliance with the German and European animal welfare guidelines. This facility consists of 15 animal rooms with a total capacity of 4000 cages which equals 10,000 mice. These rooms are located in 3 buildings (K, T and D) which comprise an animal holding area of 390 m2. We are housing exclusively mice in individually ventilated cage systems in 3 specific-pathogen-free (SPF)-barrier units, one of which is registered as animal biosafety level 2 (ABSL2) unit for infection experiments. Currently we have a daily animal census of about 10,000 mice and keep about 350 different strains and genetically engineered lines. The health status, which is monitored on a quarterly basis with a sentinel programme, fully complies with the FELASA recommendations. The staff includes 1 lab animal veterinarian, 1 facility manager, 1 lab animal technologist, 15 animal care technicians and 4 cage washing personnel.

The following services are offered:

- Basic animal care
- Breeding services and colony management (according to the instructions of the scientists)
- Assistance with experimental procedures (compound administration, sampling of blood and tissue, immunisation)
- Health monitoring
- Animal procurement
- Organisation of national and international mouse shipments including quarantine and rederivation of imported lines
- In vitro fertilisation for rescue, speed expansion and rederivation of mouse lines
- Cryopreservation of mouse embryos and banking of germplasm (currently 58 lines)
- Training programme for animal care technicians (9 apprentices)
  Animal welfare services for about 40 animal experimental protocols
- Consultation and training in laboratory animal science
- Transgenic services such as DNA-pronucleus and ES-cell injections (following the establishment of a transgenic Core Unit in 2009)

In 2008 a new building with an animal holding area of 520 m^2 and a capacity of 8000 cages offering space for 20,000 additional mice was nearly completed. This facility will include an ABSL 3 unit with a capacity of about 1000 cages and an adjacent lab area for infection experiments. All animal holding rooms are equipped with library style rack systems providing a high stocking density. This facility will triple the mouse holding capacity and is urgently needed to overcome the current space problems, which restrict the mouse-based research activities of the HZI. Due to technical problems with the HVAC-system this building will be put into operation in the first or second quarter of 2009.

At the Twincore Hannover the remodelling of the animal facility with a capacity of 2100 cages began in 2008. Commissioning of the facility is planned for November 2009. During the construction time an interim animal facility has been set up to provide biomedical services for scientific users.

The new mouse house. *Photo: HZI, Krämer*

Many new mouse cages being prepared for their new residents.
Photo: HZI, Krämer

02 Analytical Instruments

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SCIENTIFIC COLLABORATORS | Dr. Heinrich Lünsdorf | Dr. Manfred Nimtz | Dr. Manfred Rohde

The platform provides a facility for determining the three dimensional structure of all types of natural products and has instrumentation for mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, protein sequencing, electron microscopy and confocal laser microscopy.

MS and NMR spectroscopy

For the majority of low-molecular weight natural products the total structure is elucidated in a routine manner using a combination of MS and NMR spectroscopy. The direct analysis of large, intact biomolecules such as proteins, oligonucleotides and complex carbohydrates is routinely carried out using MALDI- and ESI-MS. MS has the important advantage of providing information for very small amounts of compound. Automated MS micro-techniques are used for the identification and characterisation of proteins from 2D gels and from "gel-less" techniques for "Proteomics", through the determination of the molecular weight of their proteolytic fragments using MALDI/TOF-MS/MS and HPLC-ESI-MS/MS. The secondary and tertiary structure of peptides and proteins can be elucidated when appropriately labeled material (hoch15N and hoch13C) is available through the application of multidimensional NMR spectroscopy.

The new Bruker Ultrashield 600 Plus NMR (left), the new Zeiss Libra 120 Transmission Electron Microscope (right)

Photos: HZI

X-ray crystallography

The main emphasis in X-ray crystallography is the structural analysis of proteins. A pipette-robot and an X-ray unit with an area detector and rotating anode are available for crystallisation and data collection. The measurement of high resolution data and phase determination using anomalous dispersion is available through the use of external synchrotron facilities.

Electron microscopy

This technique is used to visualise the adherence to and the invasion into host cells of a wide range of pathogens. Preparation protocols have been customised to undertake studies using high resolution field emission scanning electron microscopy (FESEM) and have revealed distinct pathways for invading the same host cells. In addition, a methodology has been developed to immuno-localise pathogenicity factors by FESEM not only on the bacterial cell surface but also on the interface between bacterial and the host cell membrane. Several preparation schemes have been developed to visualise the binding of extracellular matrix proteins on the cell surface. These studies revealed that the method of choice for studying such interactions is Cryo-FESEM. Furthermore, electron microscopy is used for studying the quarternary structure of proteins by negative-staining techniques and is applied for high resolution elemental localisation studies.

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Xu,J., Kjer,J., Sendker,J., Wray,V., Guan,H., Edrada,R., Lin,W., Wu,J., & Proksch,P. (2009) Chromones from the endophytic fungus Pestalotiopsis sp. isolated from the chinese mangrove plant Rhizophora mucronata. *Journal of Natural Products* **72**, 662-665.

Zelena,K., Zorn,H., Nimtz,M., & Berger,R.G. (2009) Heterologous expression of the msp2 gene from Marasmius scorodonius. *Archives of Microbiology* **191**, 397-402.

03 Gene Expression Analysis

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SCIENTIFIC COLLABORATOR | Andreas Jeron

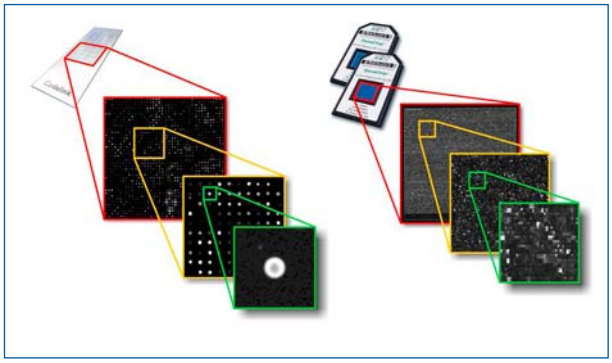
For eight years now the Array Facility has offered microarray technology that is used intensively by both research groups at the HZI campus and external research groups in the scope of scientific collaboration projects. In the past, microarrays were primarily used in gene expression analysis. In this the Array Facility provides state-of-the-art technology, such as the Affymetrix analysis platform GeneChip, as well as the latest devices for producing own microarrays. With regard to the altered field of application for array technologies for genomic and epigenomic issues, new methods have been developed in the Array Facility and established for routine. It is currently possible to measure the gene expression of 40-50,000 transcripts (genes) with one microarray. These have been joined by so-called DNA tiling arrays, with which it is possible to identify DNA recognition sequences of DNA-binding proteins. The tiling array is also suitable for the investigation of genome-wide DNA methylisation patterns, which are proven to have a decisive influence upon gene regulation or dysfunction in specific genes. SNP arrays and array CGH (microarrays for comparative genome and copy number variation analysis) are employed in particular in the diagnosis of tumours, in order to study the causes of genetic instability in tumour development.

Service The Array Facility offers expression and genome analysis as a service for research groups at the HZI, as well as their co-operation partners. In the development and creation of theme arrays the Array Facility provides advice on the selection of suitable chemicals and optimum shipping options. The manufacture of the array is supervised by the Array Facility, under the application of quality standards. In addition, optimised protocols for application are also made available.

In addition to array manufacture and sample processing, data analysis, data storage and data interpretation can also be offered on request, with these adapted to international standards, thus ensuring that the exchange of data with other microarray data is possible.

Research... In support of numerous internal and external co-operations we have conducted analyses in the fields of tumour development and typification, pathogen-host interaction (streptococcae, pseudomonads and mycobacteria) as well as for immune biology.

... and development In the course of the development and improvement of self-configurable microarrays we have developed a number of new user defined microarrays with research groups of the HZI and MHH. Further array developments are planned for the future.



Chips for the Array Facilities *Photo: HZI*

TrehanPati,N., Geffers,R., Sukriti, Hissar,S., Riese,P., Toepfer,T., Buer,J., Kumar,M., Guzman,C.A. & Sarin,S.K. (2009) Gene expression signatures of peripheral CD4+ T cells clearly discriminate between patients with acute and chronic hepatitis B infection. *Hepatology* **49**, 781-790.

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1Nickeleit,I., Zender,S., Sasse,F., Geffers,R., Brandes,G., Sörensen,I., Steinmetz,H., Kubikka,., Carlomagno,T., Menche,D., Buer,J., Gossler,A., Manns,M.P., Kalesse,M., Frank R. & Malek,N.P. Argyrin A - a p27kip1 stabilizing drug with potent anti-proliferative and anti-angiogenic activity. *Cancer Cell* **14**, 23-35.

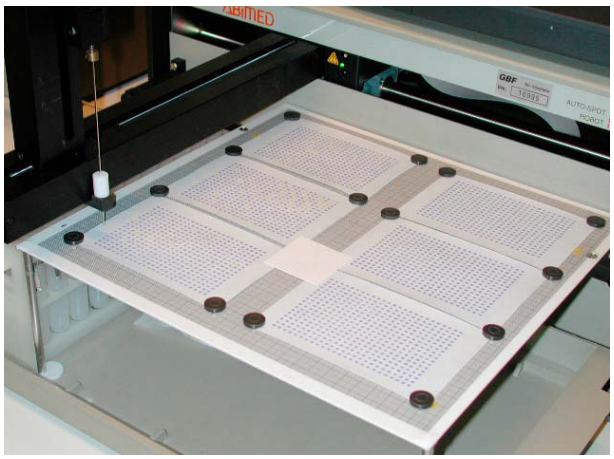
04 Peptide Systhesis

HEAD | **Dr. Dr. Werner Tegge | Department of Chemical Biology | wte@helmholtz-hzi.de**

SCIENTIFIC COLLABORATOR | Dr. Ronald Frank

Since its inauguration as a service unit in 1990, the platform generates synthetic peptides, both in soluble form and immobilised in the form of arrays for many different scientific HZI projects. State-of-the-art equipment is employed for the synthesis, characterisation and purification. By pursuing own research projects, our methodological repertoires are continuously updated and extended. In this context we have developed e.g.

- New methodologies for the generation of peptide arrays (e.g. the SPOT method);
- Methods for the preparation of phosphorylated and thiophosphorlylated peptides;
- The utilisation of new biocompatible solid supports;
- New selectively cleavable peptide linkers;
- Methods for the generation of libraries of linear and cyclic peptides;
- Assays for the utilisation of soluble and immobilised peptides in biological systems.



Method developments for parallel combinatorial chemical synthesis and screening are based on the SPOT synthesis performed on cellulose membranes. *Photo: HZI, Bierstedt*

Soluble peptides To date, over 2,700 soluble peptides with a length of two to over fifty amino acids have been generated in the platform. Soluble peptides are characterised using HPLC and mass spectrometry. If necessary, further characterisation is carried out by amino acid analysis, protein sequencing, special mass spectrometry techniques and NMR in the HZI Division of Structural Biology.

Depending on the intended usage and desired quality of the products, purifications are carried out, usually by preparative HPLC. For special applications, the platform also offers peptide modifications like fluorescence labelling, phosphorylation, biotinylation, lipid conjugation, branched peptides and cyclisations.

SPOT-arrays In the platform, immobilised peptides in the form of arrays are generated to facilitate the systematic and empirical search for peptide ligands. For the successful design of such arrays, a thorough understanding of the biological problem is essential, which is attained via close co-operation and collaboration with the users. The SPOT-arrays are generated semi-automatically and fully automatically on cellulose membranes or other polymeric supports. Each year, approximately 15,000 peptides and peptide mixtures are generated in an array format and utilised for the investigation of e.g. protein-protein interaction and enzyme-substrate recognition.

Tegge,W., Bautsch,W. & Frank,R. (2007) Synthesis of cyclic peptides and peptide libraries on a new disulfide linker. *Journal of Peptide Science* **13 (10),** 693-699.

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Dostmann,W.R.G., Taylor,M.S., Nickl,M.S., Brayden,J.E., Frank,R. & Tegge,W. (2000) Highly specific, membrane permeant peptide blockers of cGMP-dependent protein kinase I inhibit NO-induced cerebral dilation. *Proceedings of the National Academy of Sciences of the USA* **97**, 14772-14777.

Wiemann,A., Frank,R. & Tegge,W. (2000) Synthesis of Suitably Protected Hydroxymethylene Phosphonate- and ‚Phosphate Phosphonate'-Analogues of Phosphoserine and their Incorporation into Synthetic Peptides. *Tetrahedron* **56**, 1331-1337.

05 Histo-Pathology Platform

HEAD | **Prof. Dr. Klaus Schughart | Department of Experimental Mouse Genetics | kls@helmholtz-hzi.de**

SCIENTIFIC COLLABORATOR | Priv.-Doz. Dr. Reinhard von Wasielewski

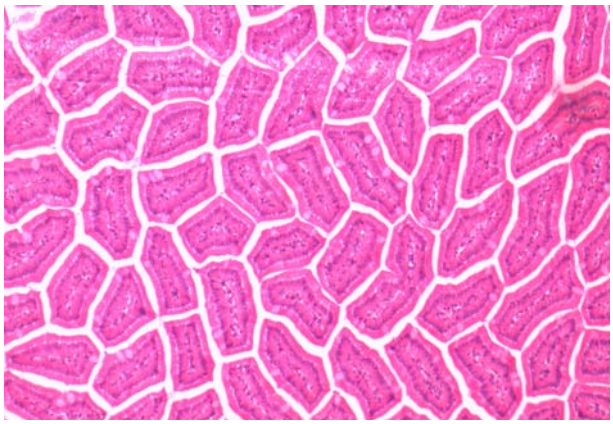
The histo-pathology platform was established as a central service unit at the HZI. It supports several projects and research groups which require histological services and pathology expertise.

Many research projects at the HZI are now performing infection challenge experiments in mice and studying mechanisms of host defence in genetically diverse mouse strains and mutant lines. Thus, the need for more histological and pathological analysis of in vivo experiments has greatly increased. The histo-pathology unit offers a central customised service and provides the entire necessary infrastructure in a single unit. Scientists from the HZI can either use its full service - from embedding, sectioning, staining, archiving of tissues, and review by a pathologist - or take advantage of its infrastructure and perform some of the tasks themselves.

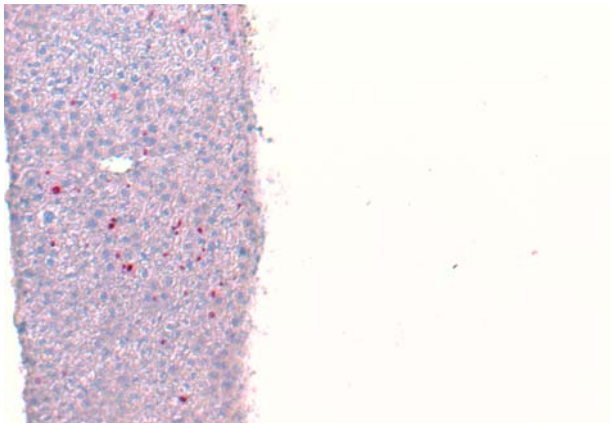
Pathological expertise and support for the planning of experiments and interpretation of results is being provided by professional pathologists who are regularly present at the HZI, or can be contacted if needed.

At present, paraffin sections and histochemical staining are offered on a routine basis. Cryostats are available and a set of immune-histochemical analyses is offered which allows the detection of immune cells during infection and inflammation in the mouse.

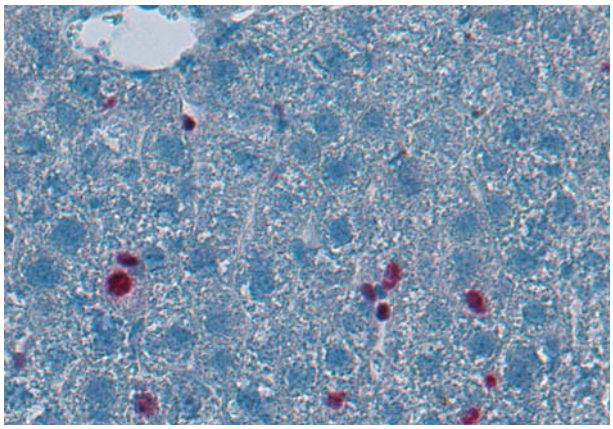
The unit maintains close collaboration with the Institute of Pathology at the University of Veterinary Medicine (TiHo), Hannover, and the Institute of Veterinary Pathology at the Free University of Berlin.



Intestine of a mouse, not infected, HE-coloured. Photo: HZI



Mouse liver (10X) Ki67-coloured. Photo: HZI



Mouse liver (50X) Ki67-coloured. Photo: HZI

06 Protein Expression

HEAD | Dr. Joop van den Heuvel | Research Group Recombinant Protein Expression | jvh@helmholtz-hzi.de

SCIENTIFIC COLLABORATORS | Dr. Konrad Büssow | Dr. Volker Jäger | Prof. Dr. Ursula Rinas

The protein expression platform is a core facility of the Structural Biology Department for the production of ultra pure protein for high resolution structural analysis. Four major expression systems have been established in the RPEX facility: *E. coli, P. pastoris*, insect cell and mammalian cell culture. This allows the production of "simple proteins" as well as proteins with complicated modifications.

The new animal cell culture facility Since 2007, a new cell culture facility has been established for protein production in insect cells and mammalian cell lines. The size of the cell culture reactors ranges from 1.6 to 6 litres of working volume in order to meet the increasing requirements for routine production of proteins on a 10-50 mg scale. For the purification of endogenous and lowly expressed recombinant multi-protein complexes a 30-litres-bioreactor has been acquired that allows the pilot-scale production of protein through the use of several hundred litres of medium. The system includes all the necessary equipment for continuous media exchange via tangential flow microfiltration or internal membrane perfusion and subsequent protein purification steps.

Services The Protein Sample Production Facility (PSPF) was established in 2007 in co-operation with the Max Delbrück Centre for Molecular Medicine (MDC) in Berlin-Buch for the large scale production and purification of protein samples for high resolution structural analysis using X-ray crystallography and NMR spectroscopy. The main goal of this Helmholtz co-operation is the support of structural biologists in Germany via circumvention of the major bottleneck of protein production. The process from cloning to purification of adequate amounts of mammalian protein is limited by the time required to establish good expression systems and production levels.

The PSPF service is offered either on a subsidised fee-for-service billing scheme or as a scientific co-operation. Currently, 50% of the capacity of the unit is used for internal HZI projects. Additionally, the facility offers external researchers training in insect and mammalian protein expression systems. This service allows the hands-on participation of the scientists during the upstream phase of protein production and isolation.

Since 2007, a total of 15 external PSPF projects have been registered. Seven of these projects have been concluded successfully. Several external scientists have been trained and have established expression of their target genes in the baculovirus expression system.

Research To date, the main focus of our research has been the development and implementation of novel eukaryotic expression systems for structural biology. We have successfully produced various fragments of the cMet receptor, different forms of HGF and the IGF1-receptor (EMBL) from mammalian cells and numerous recombinant proteins using the baculovirus expression vector system. Several in-house collaborative projects for the analysis of single reading frame genes and the multi-protein complex WAVE are in progress.

A novel technology allows transient expression of recombinant proteins in mammalian cell suspension culture in the absence of serum supplements. The system has already been shown to be highly sensitive to the use of different cultivation vessels, cell culture medium and several other process parameters. The kinetics and success of different transfection procedures for co-transfection is efficiently monitored by flow cytometry and fluorescence microscopy.

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Wiesand,U., Sorg,I., Amstutz,M., Wagner,S., van den Heuvel,J., Lührs,T., Cornelis,G.R., & Heinz,D.W.*. (2009) Structure of the type III secretion recognition protein YscU from *Yersinia enterocolitica*. *Journal of Molecular Biology* **385**, 854-866.

New Project Groups

The HZI has given young researchers with very good ideas the possibilities to do their research at the HZI facilities when their scope is within the forthcoming R&D developments of the Centre. Since 2007 several new projects groups have started their work at the HZI. During the next 5 years they will try to establish new research ways and present excellent results. The first results and further ideas of these new R&D groups are presented on the next pages.

The Gründerzentrum during the inauguration ceremony after the reconstruction of the interior part (June 2009). Various of the new project groups are working in this building. Photo: HZI, Dornbach

01 Chronic Infection and Cancer

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PROJECT MEMBERS | Daniel Dauch | Marina Pesic | Ramona Rudalska | Tae-Won-Kang

The work of our laboratory addresses the major health problem hepatocellular carcinoma (HCC), a highly lethal tumour whose neoplastic evolution is understudied compared to other major cancers. HCC represents the fifth most frequent neoplasm worldwide but due to a lack of effective treatment options, it constitutes the third leading cause of cancer death. The development of hepatocellular carcinoma (HCC) is almost invariably associated with chronic hepatitis due to infection with hepatitis B- or C virus, food contamination with aflatoxin, alcohol abuse or metabolic disorders that lead to deposition of copper or iron in the liver. Chronic inflammation of the liver leads to liver cirrhosis, which can be regarded as the *bona fide* premalignant condition for the development of HCC.

In order to find new therapeutic strategies against HCC, a detailed characterisation of the molecular events that drive hepatocarcinogenesis is urgently needed. We generated a new mouse model for HCC and established cross species oncogenomic comparison as a new algorithm for accelerated cancer gene discovery in HCC. We were able to identify and functionally validate cIAP1 and Yap as co-driver genes of the human 11q22 amplicon. More recently we demonstrated the feasibility of *in vivo* RNAi screening to identify new tumour suppressor genes in HCC. We were able to identify and functionally validate more than ten new tumour suppressor genes, most of which had never been linked to cancer before.

Mutations that drive unrestrained proliferation are requisite events in tumorigenesis. However, during evolution a variety of tumour suppressive mechanisms have evolved which counteract aberrant proliferation. While “Intrinsic tumour suppression” describes the counterbalance of aberrant proliferation by cell intrinsic programmes, the term “extrinsic tumour suppression”, also often described as “tumour surveillance”, delineates recognition and clearance of cells with oncogenic mutations by the innate or adaptive immune system.

Interestingly, high frequencies of senescent cells were found in cirrhotic livers, suggesting a role for the senescence programme as a failsafe mechanism against the transition from chronic liver damage to HCC. So far it has been the prevailing view that cellular senescence counteracts tumorigenesis by inducing a permanent cell cycle arrest. We recently used RNA interference to conditionally regulate endogenous p53 expression in a mosaic mouse model of liver carcinoma. The primary response to p53 was not apoptosis, but instead involved the induction of a cellular senescence programme that was associated with differentiation and the up-regulation of inflammatory cytokines. This programme also triggered an innate immune response that targeted the tumour cells *in vivo* and led to complete tumour remissions. Thus, our data illustrate how the cellular senescence programme can act together with the innate immune system to potently limit tumour growth. Clearly, a detailed understanding of this interplay holds the great promise to have major impact on cancer prevention and therapy.

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Zender,L., Spector,M.S., Xue,W., Flemming,P., Cordon-Cardo,C., Silke,J., Fan,S.T., Luk,J.M., Wigler,M., Hannon,G.J., Mu,D., Lucito,R., Powers,S. & Lowe,S.W. (2006) Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell* **125(7)**, 1253-1267.

02 Structural Characterisation of Pathogen Defence Factors

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PROJECT MEMBER | Sonja Wilke

Many different proteins are involved in the defence against pathogens. The elucidation of the three dimensional structure of proteins via X-ray structural analysis provides important information about the way in which these function. Structural investigations of many human proteins have not proved possible to date, due to the fact that they were unable to be produced in a pure state in sufficient quantities. This is the prerequisite in order for protein crystals to be cultivated and X-ray diffraction data recorded.

The majority of proteins for use in X-ray structural analysis are produced in bacteria. The bacteria are genetically modified to enable them to produce the respective target protein in large quantities. This procedure is both rapid and economical. However, many human proteins involved in the defence against pathogens are unable to be produced in this manner. The necessary processing stages required to transfer these proteins to their biologically active form are not implemented.

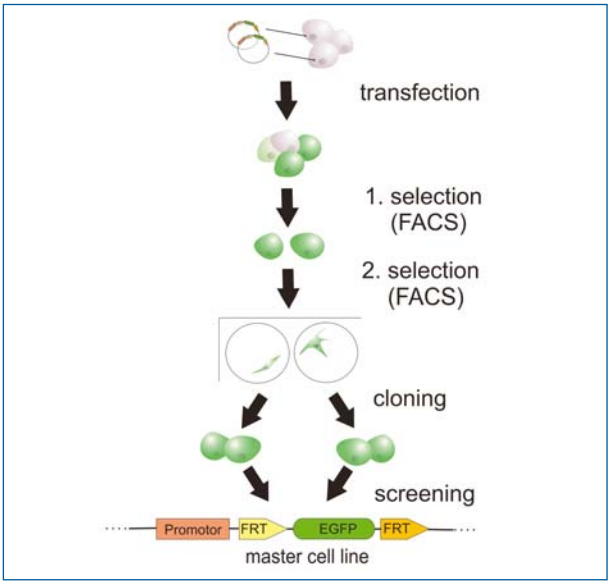
Cultivated animal cells are an alternative to bacteria in the production of proteins for X-ray structural analysis. In the majority of cases insect cells are infected with genetically modified baculoviruses. However, mammalian cell lines are also highly useful, in particular in the case of proteins discharged from the cells in their natural environment that are found in extracellular fluid or on the exterior of cells.

These discharged proteins, for example antibodies or cytokines, are often stabilized via disulfide bridges and carry carbohydrate chains on their surface. The hamster cell line CHO-Lec 3.2.8.1 is suitable for producing these for X-ray structural analysis purposes. Mutations here mean that the carbohydrate chains are small and uniform, with the consequence that the proteins produced are easily crystallized.

A disadvantage here is the long time, approximately one year, that is required for the creation of a genetically modified CHO-Lec cell line using standard methods. Using a new procedure we have now been able to significantly reduce this time. This procedure is based on a fluorescent reporter gene, GFP, which enables the cloning of genetically modified CHO-Lec cells with particularly good production properties with fluorescence activated cell sorting (FACS). Using this method, we have cloned production cell lines for the hepatocyte growth factor (HGF) in just four months. The structure of the complex of HGF with its receptor, the product of the c-Met oncogene, is of great scientific interest due to its role in the formation of cancer and due to the fact that pathogenic listeria may enter by docking on to the c-Met receptor in host cells. Our cell lines create conditions in which it is possible for HGF to be produced for crystallization experiments in the necessary quantities and form.

Recombinase-mediated cassette exchange technology (RMCE) enables a further acceleration of the process of producing cell lines. RMCE uses site-directed recombination to replace a marker gene such as GFP with another gene of choice. This process takes just a few weeks to complete. We have combined the cloning of a GFP cell line via cell sorting with RMCE and have already been able to successfully demonstrate the cassette exchange in CHO-Lec cells.

Following the establishment of improved cloning technology for CHO-Lec cells this procedure is now set to be applied in the production of proteins that play a key role in defence against pathogens, with the production of these proteins previously representing an obstacle for X-ray structural analysis.



Cloning of a stable GFP cell line through fluorescence-activated cell sorting *Graphic: HZI*

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03 Mammalian Prion Transmission Barriers

PROJECT LEADER | **Dr. Thorsten Lührs | Junior Research Group Structure-Based Infection Biology | tlu07@helmholtz-hzi.de**

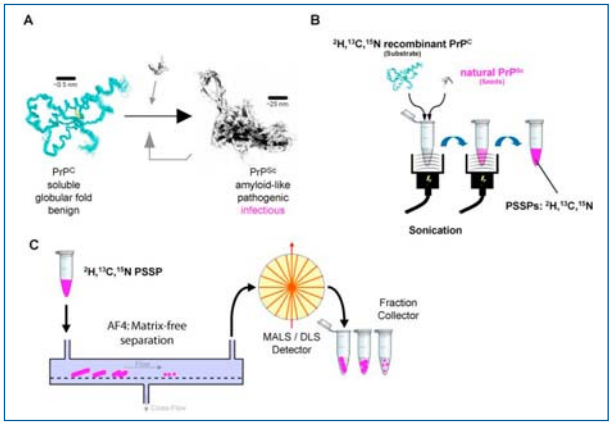
PROJECT MEMBERS | Dr. Felix Deluweit | Dr. Vandana Gupta

The structural basis of mammalian prion transmission barriers The mammalian prion protein, PrP^{C}, is able to switch its conformation from a monomeric soluble form into an aggregated transmissible prion state, PrP^{Sc}. Once a prion is introduced into a susceptible host, it triggers a PrP conversion cascade, which leads to prion disease (Fig. 1A). Interspecies transmission barriers have been observed to correlate with the amino acid sequence of PrP. On the other hand, multiple prion strains of the same PrP amino acid sequence have been identified that cause characteristic pathologies in one host species. We investigate prion 3D structures in order to understand the transmission barrier between mice and hamsters at atomic resolution, and to understand the structural basis of prion strains in relation to prion host range. In order to achieve our goals, we employ a combination of solution NMR, solid state NMR, and other biophysical techniques.

Transmission barriers Between different mammalian species, the primary transmission of prions is generally inefficient and this effect is conventionally called a 'species barrier'. The amino acid sequence of PrP is highly conserved between mammalian species: mice do not develop prion disease after challenge with hamster prions. Transgenic mice that express both hamster PrP and mouse PrP can be infected with hamster prions, and then selectively incorporate hamster PrP into prion particles. The exclusive expression of a mouse/hamster chimeric PrP, termed MH2M, makes mice susceptible to both hamster and mouse prions, and the resulting prions transmit disease to both wild-type hamsters and wild-type mice. Thus, the species barrier is in part determined by the primary amino acid sequence of the prion protein, and the substitution of only a few amino acid residues can completely alter prion transmission patterns. This notion has gained significant importance because human variant Creutzfeld Jacob Disease has been causally linked to the exposure to BSE prions of cattle.

Prion sample preparation and structural analysis A major bottleneck has been the availability of NMR-isotope labelled PrP^{Sc} specific PrP particles (PSSP), using natural PrP^{Sc} as a seed and recombinant PrP as a substrate. A key technique in achieving our research goals is the conversion of isotopically labelled (^{2}H, ^{13}C and ^{15}N) recombinant PrP into PSSPs (Fig. 1B). In order to obtain homogeneous particle preparations, we have established a powerful new technique, termed symmetric flow-field-flow fractionation (AF4), which has recently been introduced into amyloid research. This chromatography-like technique is capable of separating particles by size without the need for a stationary matrix. At the same time the absolute hydrodynamic properties of individual particle fractions are determined by multi-angle and dynamic light scattering. This technique is also routinely used to characterise the oligomerisation status of other proteins.

The core techniques for the protein structure determinations are NMR in solution and in solids, but also other biophysical techniques like fluorescence, FTIR and CD-spectroscopy. The overall approach is based on our past determination of the 3D amyloid structure of Alzheimer Aβ(1-42) fibrils and the determination of the functional fold of the [Het-s] prion. The strength of this approach lies in its robustness towards subtle structural heterogeneity of the investigated aggregates. Thus, with this a "consensus structure" can be obtained to provide essential structural insights.



Conformational conversion of prion protein (PrP). The cellular PrP is a soluble globular protein that is rather abundant in many mammalian tissues. During the prion conversion cascade PrP^{C} is sequestered into an aggregated amyloid-like form that is infectious. In contrast to conventional amyloidoses such as Alzheimer's disease, the conversion cascade can be triggered by exogeneous PrP^{Sc}. (B) In vitro *conversion of recombinant PrP^{C}. During recombinant protein misfolding cyclic amplification (rPMCA) recombinant PrP^{C} is converted into PrP^{Sc} specific PrP particles (PSSPs) under the influence of natural prions that act as seeds. This allows generation of NMR-isotope labeled PSSPs for 3D structural studies. (C) Asymmetric flow field flow fractionation (AF4). PSSPs are loaded onto the channel where they become separated according to hydrodynamic size. The precise determination of particle properties occurs in the MALS/DLS detector. Fractions are the collected for structural studies.*

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04 Development and Functional Properties of Foxp3+-Expressing Regulatory T Cells

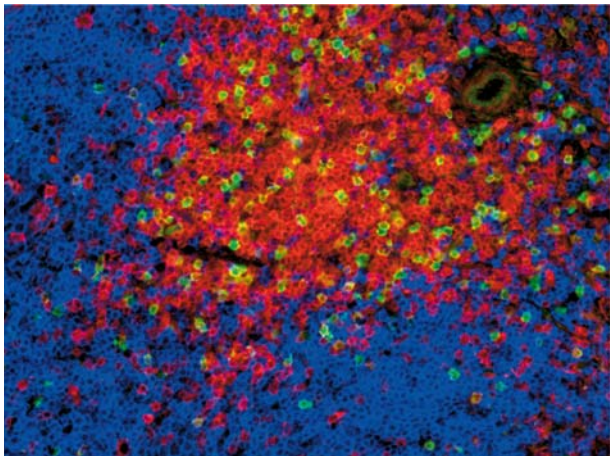
PROJECT LEADER | **Prof. Dr. Jochen Hühn | Department of Experimental Immunology | jhu08@helmholtz-hzi.de**

PROJECT MEMBERS | Sascha Cording | Dr. Stefan Flöß | Dr. Katjana Klages | Julia Polansky

Regulatory T cells (Tregs) play a key role for the control of numerous immune reactions and the maintaining of immunological tolerance. $CD4^+$ Tregs are characterized by the expression of the transcription factor Foxp3, which is central for both the development of the Tregs and their suppressive characteristics. The majority of $Foxp3^+$ Tregs is already formed during the maturation in the thymus and show features of a stable T cell line. We recently published that epigenetic modifications in the *foxp3* gene are decisive for the stability of the Foxp3 expression. One milestone of this project is to comprehend the cellular and molecular mechanisms that lead to the development of stable $Foxp3^+$ Tregs. We will focus on the cell-fate decisions that lead to the epigenetic modification of the *foxp3* locus within the thymus.

$Foxp3^+$ Tregs are not exclusively generated in the thymus, but also in the periphery, where antigen recognition under tolerogenous conditions leads to *de novo* induction of $Foxp3^+$ Tregs from conventional, naïve T cells. In particular the liver-draining lymph node is a special location with a high frequency of orally induced $Foxp3^+$ Tregs. Within this project we will investigate which characteristics of the liver-draining lymph node are responsible for the conversion of naive T cells into Tregs. In addition, we aim to use gene expression analysis to identify molecular markers for the differentiation of $Foxp3^+$ Tregs generated by the thymus and induced *de novo* by conventional T cells. We want to find out what proportion of the entire population of the $Foxp3^+$ Tregs found in the periphery consists of *de novo* induced Tregs.

In addition to their role in preventing autoimmunity, Tregs are also involved in controlling immune reactions to nutrition antigens and intestinal microflora. This contribution significantly maintain mucosal tolerance and intestinal homoeostases. It is assumed that especially *de novo* induced Tregs play a key role. We aim to investigate this hypothesis experimentally in the scope of this project. Similarly, we also will try to answer the question why $Foxp3^+$ Tregs indicate such a strong *in vivo* proliferation in the intestinal mucosa and in lymphatic organs associated with mucosa. There is much to suggest that the reduction in commensal microflora through antibiotic treatment results in a reduction in *in vivo* proliferation of the $Foxp3^+$ Tregs and that there is a direct link between the commensal microflora and the Treg homoeostases.



Foxp3-positive cells in the spleen: The cells were detected through fluorescence-coupled antibodies. B cells, T cells and Foxp 3 cells are stained blue, red and green, respectively.
Photo: Dr. Eberl, Pasteur Institute, Paris, France. The permission of Dr. Eberl is greatfully acknowledged

Furthermore, $Foxp3^+$ Tregs also have an important function in suppressing tumour-specific immune responses. With the aid of a transgenic mouse that permits the detection and selective depletion of $Foxp3^+$ Tregs we aim to investigate the creation and function of $Foxp3^+$ Tregs in the course of tumour diseases.

All in all, we expect that our investigations will result in both, a better understanding in the molecular mechanisms of Treg development and function *in vivo*. This will provide us new strategies for the induction and modulation of Tregs in the scope of a therapeutic approach.

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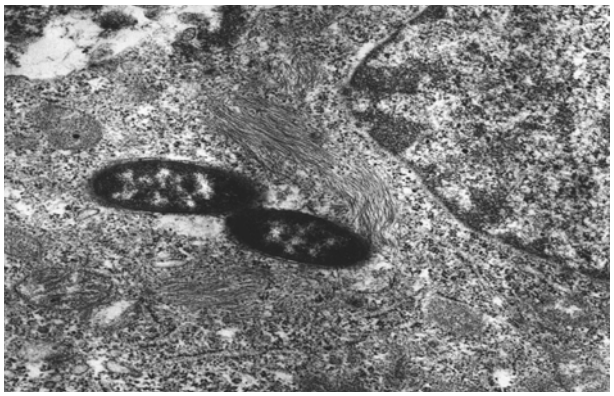
05 Regulation of Virulence Mechanism Implicated in Pathogen Host Cell Interactions

PROJECT LEADER | **Prof. Dr. Petra Dersch | Department of Molecular Infection Biology | pde08@helmholtz-hzi.de**

PROJECT MEMBERS | Katja Böhme | Katharina Herbst | Dr. AnnKathrin Heroven | Tina Kornprobst | Henriette Langhans | Tatjana Stolz | Frank Uliczka | Anna Wagner

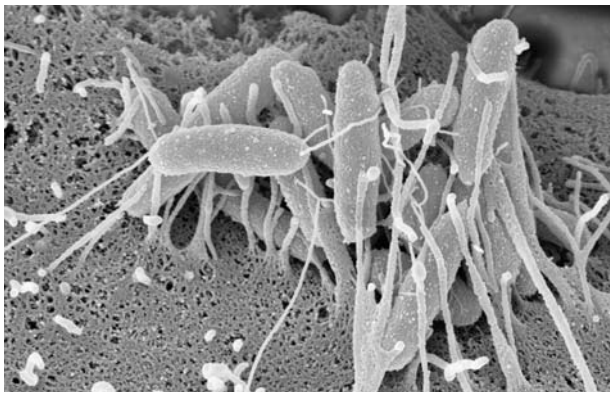
Tight interaction and internalisation into host cells is a crucial virulence strategy of many bacterial pathogens. Enteric pathogens such as enteropathogenic Yersiniae use different types of outer membrane proteins to bind to extracellular matrix components or host cell receptors to initiate uptake. This allows the microbe to translocate through the intestinal epithelial layer and promotes colonisation of subepithelial tissues and dissemination to deeper organs. In our research project we characterise the structure, function and expression of the *Yersinia* adhesion factors to understand how these enteric bacteria colonise human tissues throughout the infection. We further address the signal transduction pathways initiated within host cells after cell contact and analyse environmental factors implicated in adhesin synthesis during pathogenesis.

Molecular mechanisms involved in *Yersinia* host cell interactions We could show that the *Y. pseudotuberculosis* internalisation factors YadA and invasin, which both promote host cell entry through β_1-integrin receptors, initiate similar intracellular pathways to reorganise the host actin cytoskeleton for the formation of pseudopods, that enclose the bacteria into a membrane-bound vacuole. A detailed comparative analysis of the invasin- and YadA-promoted signal transduction pathway, using pharmacological inhibitors, knock-out cell lines and RNA interference has revealed that the non-receptor tyrosin kinases FAK and Src, the small GTPases Rac1, Cdc42 and Ras, the PI3 kinase, Akt and other crucial protein kinases and cytoskeletal proteins are required for uptake by both adhesion factors. Many cellular factors as well as the activation pattern, quantity and order of the identified signal molecules are still unknown and will be the subject of future work. We further discovered additional cell surface exposed proteins in enteropathogenic yersiniae with homology to other bacterial adhesins. The function of these components in cell adhesion and invasion as well as colonisation of different cell types and tissues from distinct hosts is currently investigated.



Internalized Yersinia pseudotuberculosis *in human epithelial cells* *Photo: HZI, Rohde*

Regulation of *Yersinia* adhesin expression A major part of our research work is directed to studying expression of the different *Yersinia* adhesins and other associated virulence factors in response to environmental parameters sensed during an infection. Invasin was found to be only expressed under condition when YadA is repressed, indicating that both adhesins are needed for colonisation of different tissues and/or at different stages during the infection. Invasin and YadA expression was found to be linked by a complex regulatory network, which includes histone-like proteins (H-NS and YmoA), transcriptional regulators (VirF, RovM and RovA), and posttranscriptional regulatory systems, implicating small regulatory RNAs and thermosensitive RNA structures (thermoswitch). The complex dynamic interplay between the different regulatory factors was further shown to be required to co-ordinate expression of virulence factors with metabolic and stress adaptation processes.



Adherent Yersinia pseudotuberculosis *on human epithelial cells* *Photo: HZI, Rohde*

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06 Intracellular Trafficking of Phagosomes and Immunity: Lessons from Mycobacteria

PROJECT LEADER | **Dr. Maximiliano G. Gutierrez | Junior Research Group Phagosome Biology | mgg08@helmholtz-hzi.de**

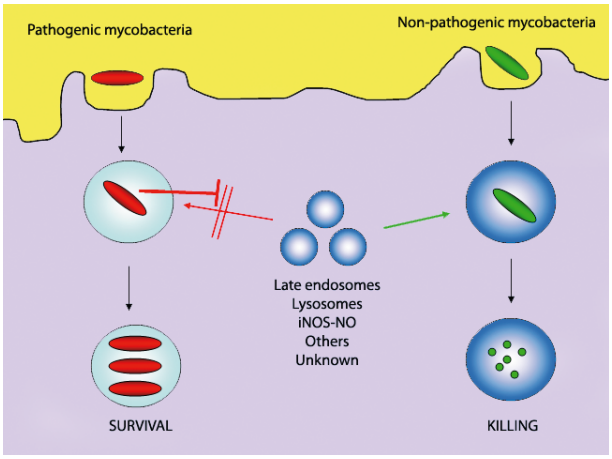
PROJECT MEMBERS | Dr. Ianina Conte | Bahram Kasmapour | Achim Gronow

Phagosomes Phagocytosis is the process by which cells engulf particles and microbial pathogens. The mechanism of the initial innate immune response of macrophages against intracellular pathogens involves phagocytosis, followed by killing. During phagocytosis, the particle becomes enclosed by a membrane to form the phagosome.

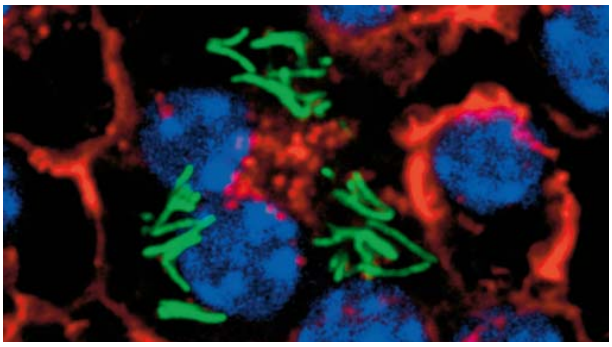
After phagosome formation, this organelle undergoes a series of dynamic fusion and fission events that modify the composition of its limiting membrane and its contents by interacting with components of the endocytic pathway. This process, referred to as phagosome maturation, provides the phagosome with degradative properties, which are central to its microbicidal function. In addition, microbial antigens are processed in phagosomes and directed to the plasma membrane associated to Major Histocompatibility Complex (MHC) class I, MHC class II and CD1 molecules, to be presented to T lymphocytes. Therefore, the phagosome can be considered as an intracellular compartment that links the innate and adaptative immune systems. Different intracellular pathogens have learnt to subvert the normal phagosome maturation pathway for their own benefit. One of the best examples is *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis.

Mycobacteria The key event during *M. tuberculosis* infection is the ability of this pathogen to survive within phagosomes in macrophages. This capability is linked to the aptitude of the live pathogen to block phagosome maturation. Pathogenic mycobacteria survive within macrophages, whereas non-pathogenic mycobacteria are killed by macrophages. Somehow pathogenic mycobacteria block essential mechanisms of killing activated by non-pathogenic mycobacteria. By studying how non-pathogenic mycobacteria are killed, it would be possible to identify the mechanisms of killing that are activated within macrophages.

The project Our research focuses on the mechanisms whereby *M. tuberculosis* arrests phagosome maturation and avoids killing by macrophages. We study the intracellular transport of non-pathogenic and pathogenic mycobacteria in macrophages. We have identified novel proteins involved in vesicular trafficking, particularly phago-lysosome fusion, during mycobacterium infection. These proteins are promising candidates for being involved in the lysosomal-mediated killing process, as well as in the molecular events linking innate and adaptive immune responses. Our studies lead to a better understanding on how macrophages kill mycobacteria and other infectious pathogens. The long-term goal of this project is to identify new targets for development of prophylactic or therapeutic interventions against this important human pathogen.



Intracellular trafficking of non-pathogenic and pathogenic mycobacteria within macrophages. Upon internalisation by macrophages, pathogenic mycobacteria (red) reside in phagosomes in which the fusion with the late endosomal-lysosomal compartment is blocked. As a consequence they survive. In contrast, non-pathogenic mycobacteria (green), such as M. smegmatis, *reside in phagosomes that fuse with the late endosomal-lysosomal compartment and, consequently, bacteria are killed.*



Bone marrow macrophages infected with bacille Calmette Guèrin (BCG). Green: BCG-GFP; Red: Rhodamine-phalloidin; blue: DAPI. *Photo: HZI*

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THE RESEARCH PROGRAMME “INFECTION AND IMMUNITY” IN POF II

Research activities within the Helmholtz Association are organised in six research fields, each consisting of several research programmes. In a five-years frequency the centres of the Helmholtz Association apply for funding in the different HGF-research programmes in a competitive process. In the first period of the so called “Programme-oriented funding” (Programmorientierte Förderung, PoF) the HZI was involved in three programmes:

1. “Sustainable Use of Landscapes”,
2. “Comparable Genome Research”
3. “Infection and Immunity”.

In the second funding phase (PoF II), starting in 2009, our institute will now focus entirely on the programme “Infection and Immunity”, which was substantially restructured and optimised. The aim of the programme is to contribute to solving the grand challenges in infection research by building a strong basis in basic research and applying this knowledge to develop new strategies for prevention and therapy for translation into public health benefits. To address these challenges the restructured programme “Infection and Immunity” will include activities in five areas of infectious disease research which are pooled in the following topics:

- Microbial Pathogenesis
- Host Resistance and Susceptibility
- Inflammation and Immunity
- Strategies for Prevention and Therapy
- Translational Infection Research

Although the division into these topics provides an important conceptual framework for the strategic development of the programme, there is a close interaction and considerable overlap between these topics that is essential for the overall success of the programme to reach its goals.

The topic Microbial Pathogenesis addresses the question of how pathogenic microorganisms cause disease. Adherence to host cells, invasion into the cell, intracellular survival, dissemination and immune evasion are strategies used by microorganisms to establish an infection in a host.

Studies in the topic Host Resistance and Susceptibility focus on the infected host. Here it will be examined which factors determine host resistance or susceptibility to infection by initially studying genetic and cellular factors in mouse model systems and later in human cohorts. The influence of environmental factors such as microbial communities and nutrition will also be investigated.

The topic Inflammation and Immunity addresses the processes involved in eliciting innate and adaptive immune responses against infectious agents. In addition, the pathological consequences resulting from dysregulation of the immune response are investigated, such as toxic shock, allergic reactions and autoimmunity.

The major aim of the topic Strategies for Prevention and Therapy is to identify new targets or compounds that are potential candidates for anti-infectives or vaccines. This will be achieved by developing robust diagnostic and immune monitoring methods, establishing new vaccination strategies and the isolation, characterisation and validation of new anti-infective compounds.

The topic Translational Infection Research will integrate the activities at the translation centre established near the campus of the Medical School in Hannover (MHH). In this centre “Twinning projects” between basic research scientists of the HZI and clinical scientists and clinicians of the MHH will be performed.

To transfer the challenges in infection research into public health benefits the programme involves a number of intensively interacting research disciplines. Basic research in the area of microbiology, cell and molecular biology and immunology will unravel the complexity of infectious diseases and establish a knowledge-based foundation for developing new anti-infectives and vaccines. Taking into account the extremely high complexity of biological processes taking place when a pathogen interacts with the host, systems biology approaches are gaining more and more importance in the programme. The well established research with mouse model systems has been intensified and will be flanked by new approaches in epidemiology.

To support the transfer of results from basic research into clinical application, the programme contains a number of activities in chemical biology, natural product chemistry, medicinal chemistry and vaccinology. A strong structural biology supports the discovery pipeline, as well as basic research. Furthermore, the component of translational research has been strengthened in the programme with the new institute TWINCORE, where basic researchers from the HZI work closely with clinicians from the Medical School Hannover.

01 Microbial Pathogenesis

TOPIC SPEAKER | **Prof. Dr. G. Singh Chhatwal** | **Department of Microbial Pathogenicity** | **gsc@helmholtz-hzi.de**

Challenges In spite of the availability of a large number of different antibiotic and antiviral agents, the disease burden caused by infections is increasing continuously. Chronic persistent infections are becoming more and more apparent and constitute a major challenge for the medical profession. The increasing emergence of antimicrobial resistant bacterial isolates is a major concern. Due to the decreasing therapeutic options, the development of new treatment strategies is urgently required. To meet these challenges a detailed understanding of the mechanisms of pathogenicity is of utmost importance. The topic of Microbial Pathogenesis is designed to meet the challenges and includes three main research areas.

1.1 Elucidation of mechanisms of pathogenicity

Goals:

1.1.1 Study of the host-pathogen interactions at the molecular and cellular level

1.1.2 Analysis of biofilm formation and its regulation

Summary: In the course of their co-evolution with their hosts bacterial pathogens have developed sophisticated strategies to exploit the host cell and immune system for their own benefit. Intracellular survival, dissemination in the host and pathogen persistence require a complex series of interactions within the host cell. We shall study the host cell-pathogen interactions at the molecular and cellular level, using selected microorganisms such as *Lysteria monocytogenes*, *Salmonella typhimurium* and enterohaemorrhagic *E. coli.* In addition, the mechanisms of biofilm formation by bacterial communities including *Pseudomonas aeruginosa* and *Streptococcus mutans* will be elucidated.

1.2 Functional and structural characterisation of virulence factors

Goals:

1.2.1 Identification of novel virulence factors from pathogenic bacteria

1.2.2 Analysis of bacterial virulence using proteomics

1.2.3 Structural analysis of proteins involved in host-pathogen interactions

Summary: Pathogenic microorganisms produce a variety of virulence factors with diverse functions in processes such as adherence, invasion, intracellular survival, evasion of the host immune response and bacterial communication. Their functional characterisation will not only contribute towards our understanding of pathogenicity, but also help in identifying promising candidates for the development of vaccines, diagnostics and novel therapeutics. We shall identify novel virulence factors from streptococci, pneumococci and *Listeria*, as well as different viruses and analyse their structure:function relationship.

1**.3 Investigation of pathogen diversity and epidemiology**

Goals:

1*.3.1 Molecular epidemiology of pathogenic bacteria*

Summary: One major problem in controlling infectious diseases is that most pathogens show a high degree of strain diversity. This, at least in part, explains the different forms of disease caused by the same species. A further complication is that circulating strains often exhibit strong regional differences in terms of antigenic profile and virulence traits. Molecular epidemiological studies are urgently needed to understand these phenomena and to design region-specific control strategies. We will collect several hundred isolates from humans as well as animals from different geographical areas and with different clinical manifestations. These isolates will be characterised with low-density DNA arrays that carry genes encoding classical and putative virulence factors. Selected strains will be subjected to whole genome sequence analysis.

Expected results / milestones:

- Elucidation of mole cular mechanisms of adherence, invasion, persistence, evasion of immune defence and biofilm formation
- Identification and characterisation of new molecular targets allowing the development of pathogen-specific intervention strategies
- Structural analysis of microbial virulence factors to understand their role in pathogenesis
- Understanding host specificity of pathogens in order to design region-specific combat strategies

02 Host Resistance and Susceptibility

TOPIC SPEAKER | **Prof. Dr. Klaus Schughart | Department of Experimental Mouse Genetics | kls@helmholtz-hzi.de**

The course and severity of an infection is determined by a multitude of extrinsic and intrinsic factors that include the virulence and dose of the pathogen, as well as the health, age, gender, genetic make-up, and nutritional status of the host. Although pathogen virulence has been extensively studied, very little is known about the detailed immunological and molecular mechanisms by which host factors modulate the course of an infection. Even less is known about the influence of host genes on the infection outcome.

The major goal is, therefore, to gain a better understanding of host factors that contribute to the generation of an effective immune response against pathogens. Specifically, studies will be directed towards the identification of genetic factors and characterisation of microbial communities influencing the host response to infectious pathogens.

GENETIC FACTORS IN ANIMAL MODELS The relevance of host genetic factors on infection processes has been well documented for viral, bacterial, and parasitic pathogens in humans, farm animals and mammalian model systems. Host factors such as innate and adaptive immune responses, immune deficiencies or auto-immunity have a direct effect on the fitness of the host defence. Thus, the understanding of how host genetic factors influence the molecular and cellular components of the host immune system will be essential for the development of new strategies to prevent and treat infectious diseases.

To identify and understand the mechanisms of action of genetic susceptibility factors in humans, experimental studies in animal model systems are essential. The mouse is one of the most important model systems because its immune system has been extensively studied, its genetics is well known and many mutant and variant strains are available.

The host genetic resistance or susceptibility to infection is usually a complex trait. Complex genetic traits involve multiple genes. They are the basis of most genetic diseases and predispositions in humans. Complex traits are difficult to study in humans since phenotypic variability is not only caused by the genetic background but also by environmental factors.

Identification of genetic factors that determine host susceptibility Genetic factors influencing the host response to an infection will be studied in animal model systems. Populations of mouse strains that differ in their genetic make-up and mouse mutants with defects in specific genes will be infected with pathogens and their primary and secondary responses analysed. We focus our studies on the following pathogens:

Group A streptococci are prevalent human pathogens capable of causing a variety of diseases. We have shown that strains of mice from various genetic backgrounds differ markedly in their susceptibility to *Streptococcus pyogenes*. Using resistant and susceptible mouse strains, we will identify the genes and genetic networks controlling susceptibility to *S. pyogenes*, characterise the immunological functions and underlying molecular mechanisms and design new strategies to enhance resistance in susceptible hosts.

Staphylococcus aureus is one of the most important causes of life-threatening bacterial infections in Western countries. We have recently identified strain-dependent differences in the response to *S. aureus* infections in common laboratory mouse strains. We will

use these differences to determine the genetic, cellular and molecular mechanisms that contribute to the host defence.

Influenza A virus represents one of the most important threats to human health and economics. Thus far, very little is known about host resistance or susceptibility to influenza infections. At the HZI, we have established an infection model for influenza A H1N1 virus in the mouse and identified significant strain differences for survival and weight loss. We are performing comparative studies on the pathogenicity, gene expression patterns in the lung, viral load, and immune response in infected mice from different strains. This information will be used to map quantitative trait loci (QTL) that contribute to infection susceptibility.

The mammalian prion protein, PrP^{C}, is able to switch its conformation from a monomeric soluble form into an aggregated transmissible prion state, PrP^{Sc}. Once a prion is introduced into a susceptible host, it triggers a PrP conversion cascade, which leads to prion disease. A combination of techniques will be used to unravel the structural origin of prion transmission barriers and prion adaptations to new hosts. The 3D structures of mammalian prion associated PrP will be revealed and should provide detailed insight into biophysical mechanisms of the PrP^{C} to PrP^{Sc} conformation conversion.

Regulatory gene network We will study the host response in different Genetic Reference Populations (GRPs) of mice. Families of GRPs will be infected with *S. aureus* and influenza. Whole genome expression patterns will be determined during the course of infection and expression levels correlated with genotypes. In this way, “expression Quantitative Trait Loci” (eQTLs) will be determined that are responsible for regulating other genes in “trans”. We will deduce gene regulatory circuits. These networks will be simulated in computer models, subsequently validated in the mouse and further refined.

GENETIC FACTORS IN HUMANS In humans, several single gene loci have been identified that contribute to host infection susceptibility. Almost all of these have previously been identified as susceptibility loci in mice. The high density genetic map of human haplotypes and large scale SNP-analyses now make it possible to directly map complex genetic loci that are associated with human diseases. Only a few whole genome association studies have been performed in humans in which genetic influences to infection susceptibility have been mapped. The most recent one described three gene loci associated with the onset and course of HIV infections.

We will analyse, in human cohorts, different immunological parameters and individual histories of infections. Phenotype-genotype association studies will be performed to identify genomic regions and gene candidates which may determine the predisposition to infectious diseases. These data will allow the formulation of hypotheses on the molecular mechanisms, genes and gene networks involved in human host defence.

MICROBIAL COMMUNITIES IN THE HOST Besides genetic factors, the physiological status of an individual will greatly influence his defence capabilities of successfully fighting a pathogen. A more detailed knowledge of factors that determine the strength of the host defence against infectious pathogens will be essential to meet future health care needs, especially in a population in which the percentage of elderly people is constantly growing.

Microbial communities often form as biofilms on medical implants and are the cause of inflammatory responses, eventually necessitating removal and replacement of the implant. As yet, very little is known about the composition of such communities on implants. We will thus analyze in detail, the composition and molecular interactions of microbial communities in such biofilms.

Determination of genetic and environmental influences on microbial communities Complex bacterial communities exist in the gut and the lung. The aim of our studies is to understand how genetic factors and nutrition influence the composition of such communities, and how these changes affect the host response to infections. We will analyse the microbial diversity in the mouse gut in different genetic and environmental settings. In addition, we will perform studies to clarify how the composition of the nasal microflora influences the asymptomatic carriage of pathogens such as *S. aureus.*

03 Inflammation and Immunity

TOPIC SPEAKER | **Prof. Dr. Hansjörg Hauser | Division of Molecular Biotechnology | hha@helmholtz-hzi.de**

Innate defence activation and inflammation belong to the first reactions of the immune system upon infection. These events, in turn, are essential in initiating specific and often long-lasting adaptive immunity. The reactions are initiated by the affected cells themselves and are then orchestrated by a variety of leukocytes and lymphocytes. The mediators responsible consist of low molecular weight components such as prostaglandins or small proteins like cytokines as well as molecules that are responsible for cell:cell interactions. Finally, for the clearance of a pathogen, cells of the immune system like T cells and their mediators or antibody producing B cells are responsible. Consequently, the topic “Inflammation and Immunity” deals with the processes which lead to the immediate defence reactions and long-lasting protection. It also deals with negative outcomes of such reactions as toxic shock and autoimmunity, and an as yet not understood influence in oncogenesis and tumour surveillance.

One of the activities within this topic concerns the structure-activity relationships of pathogen receptors and the role of lipid rafts as an entry or exit point for pathogens. The focus hereby is on the analysis of TLRs and intracellular receptors. Biological follow-up events under study comprise intracellular signalling induced by pathogens, PAMPs or inflammatory signals.

A major subject in this topic concerns the understanding of induction and signalling by type I interferons. Online reporters that allow the monitoring of IFN-ß induction, the induction of IFN-stimulated genes and a reporter that monitors the positive feed-back loop of IFN secretion introduced into cells and mice revealed to be key tools to discover new features of the system. IFNs are not only induced by viruses but also to a lower extent by bacteria and PAMPs like TLR2 and 4. It was also found that IFN-ß is produced at very low levels in non-infected mice. Mice lacking this constitutive IFN production are inefficient in T cell stimulation and show higher angiogenesis rates in tumours. The new methods also allowed the studying of location and dynamics of events during virus- and biofilm-induced IFN production. The construction of a time-resolved model of the IFN-network is a major goal of current studies. This is done with the help of systems biology methods and includes the implementation of relevant mathematic and bioinformatic methods for modelling the regulatory networks. First activities aim at studying interferon regulation in T cells.

A key-hypothesis we follow is that inflammatory signalling mediators and mechanisms may be used as a target for the prevention and therapy of inflammatory and infectious diseases. Therefore, relevant signal transduction pathways for infection and inflammation such as TNF-alpha, IL-1- and Toll-like receptors are investigated in cell culture and animal model systems. We have assessed the role of the central inflammatory signalling mediator TAK1 in inflammatory mouse model for rheumatoid arthritis and could show the beneficial role of a systemic TAK1 targeting strategy by the RNAi technology to alleviate rheumatoid arthritis symptoms. Also, a successful intrabody-mediated strategy to interfere with Toll-like receptor-mediated signalling pathways was demonstrated for the treatment of inflammatory diseases in a mouse model.

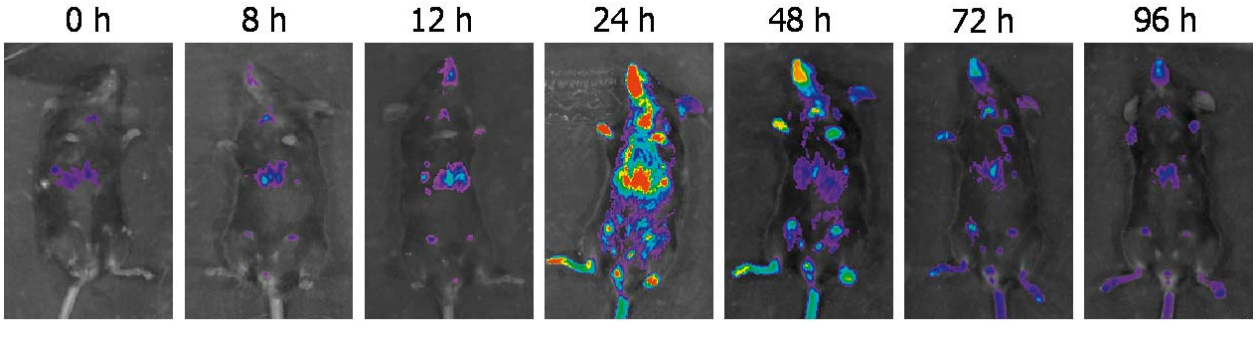
Three projects deal with the interrelationship between infection, oncogenesis and tumour surveillance.

1) Bacteria that are injected into tumour-bearing mice accumulate specifically in the tumour and lead to a partial tumour shrinkage. This reaction could be enhanced by depletion of granulocytes that are attracted by the bacterial invasion. When a complete tumour remission was induced the establishment of an anti-tumour immune response could be found.

2) New studies concern the senescence surveillance in a chronic hepatitis model through inflammatory processes. Early results with this mouse model indicate that the cellular senescence programme together with the innate immune system limit tumour growth. The mechanisms of how cellular senescence is bypassed and the significance of “senescence surveillance” for suppression of hepatocarcinogenesis are investigated.

3) The transcription factor IRF-1 that is normally induced by infections and follow-up inflammatory cytokines by itself induces an unusually rapid immune response and elimination of tumour cells that express IRF-1. The response leads to a T cell mediated protection of these mice from tumorigenesis by the same tumours.

The role of T cells and mesenchymal cells in infection and autoimmunity is part of our studies towards the understanding of “immunoregulatory networks”. One activity aims at dissecting the basic mechanisms underlying the induction and regulation of mucosal T cell responses in the context of autoimmune diseases. Studies to investigate maintenance or breakdown of peripheral T cell tolerance during bacterial or viral infection have been initiated. The function of T regulatory cells, decision makers for tolerance, anergy, autoimmunity or defence is part of these studies.



Infection with VSV-mutant AV2 (1 x 10^6 pfu) Photo: HZI

04 Strategies for Prevention and Therapy

TOPIC SPEAKER | **Prof. Dr. Dr. Carlos A. Guzmán | Department of Vaccinology and Applied Microbiology | cag@helmholtz-hzi.de**

No economy, even in industrialised countries, can cope with the exploding costs associated with the diagnosis, prevention and treatment of infections. The clinical management of infected patients has also become more difficult due to the emergence of multidrug resistant pathogens. Therefore, the main goals of this topic are to: (i) establish new vaccination strategies, (ii) develop robust diagnostic and monitoring methods, and (iii) discover new anti-infectives.

VACCINATION STRATEGIES Vaccination constitutes the most cost-efficient prophylactic intervention against infection. The possibility of using vaccines as a therapeutic tool against both infectious and non infectious diseases is also gaining interest. Therefore, tools and strategies for immune intervention will be developed and subsequently exploited for the generation of candidates against specific diseases. Since most infectious agents need to transit or are restricted to the mucosa, the elicitation of a local response at the portal of entry is highly desirable. Therefore, a discovery programme for the identification of mucosal adjuvants was established. For chronic infections, targeted modulation of the immune system by the transfer of antigen presenting cells, effector cells and regulatory cells can provide new approaches for the immunological control of these diseases. This need will be addressed through the establishment of cellular immune therapies. Finally, to transfer new discoveries from basic research to the clinic, novel strategies are needed for a rapid and cost-efficient screening, selection and prioritisation of candidates. The results obtained in mouse-based systems cannot always be extrapolated to humans and primate-based models are often too expensive and fraught with ethical constraints. Consequently, cost-efficient preclinical validation systems based on humanised mice with high predictive value for humans will be developed.

DIAGNOSTICS Diagnostics are crucial for determining the presence of pathogens and the cause of infections at individual and population levels. They are a cornerstone for correctly assessing the nature and aetiology of a disease, designating an appropriate course of treatment and monitoring the effects of interventions. Recent advances in molecular detection allow detailed studies in terms of taxonomic resolution and *in situ* gene expression. However, new approaches are needed that combine the analysis of viruses, bacteria and lower eukaryotes. This will allow the elucidation of complex poly-microbial occurrences in the course of individual infections and during outbreaks. The same is true for immune monitoring methods, which allow dissection of host responses to natural infections and vaccination. Thus, this topic will develop fast and reliable approaches to: (i) detect and quantify major pathogens in clinical and environmental samples without cultivation, (ii) assess activity and virulence of specific pathogens *in situ* and (iii) perform an efficient immune monitoring of infected or vaccinated individuals.

ANTI-INFECTIVES The development of a new generation of anti-infectives is urgently required. A close interaction of chemistry and biology is a prerequisite for such an endeavour. Therefore, the research activities dedicated to the identification, analysis, chemical synthesis and biological evaluation of bioactive small molecules have been integrated into a “Chemical Pipeline” which co-ordinates the anti-infective research from the discovery of new bioactive compounds all the way through their production, chemical optimisation and functional studies, to their delivery as biochemical tools or anti-infective drugs. In the area of discovery, new chemical entities will be added to the existing collection of chemical compounds at the HZI by further prospecting of novel microbial sources, as well as by the genetic manipulation of existing producer strains. Chemical synthesis will be exploited to

create series of derivatives originating from selected natural product core structures. Bioactive molecules will be modified for use as biochemical tools or lead structures for drug development. The active pharmacophore substructures of the compounds will be identified. Chemical syntheses and specific modifications of the parent structures will then confirm particular structural details and identify the structural elements involved in their activity. This will enable the selection of suitable sites to either modify their properties, attach labels and tags, or to simplify their structures for easier synthetic production of active derivatives. Further studies will enable elucidation of the mode of action of the discovered compounds. The most promising bioactive compounds will be further developed as new anti-infectives for clinical application.

Expected results

- Development of high resolution molecular genotyping, quantification and virulence assessment methods
- Establishment of a pre-clinical platform based on humanised mice for vaccine and anti-infective testing
- Establishment of proof of principle for at least one vaccine candidate
- Discovery of novel antimicrobial chemical entities and transfer of at least one into clinical development

05 Translational Infection Research

TOPIC SPEAKER | **Prof. Dr. Ulrich Kalinke | Twincore | Kalinke.Ulrich@mh-hannover.de**

Translational research goes beyond the level of basic discovery and transforms the knowledge gained into real public health benefits. Unfortunately, the process of “translation” is not yet well developed. The research field “Health” of the Helmholtz Association is committed to supporting the process of translating discoveries from basic research into clinical application. Often, we observe a lack of “interdisciplinary research culture” between basic researchers and clinicians. The infrastructure to carry out efficient translational research is fragmented and different career structures and reward systems hamper collaboration between basic and clinical researchers. As a consequence, basic sciences and clinical research are often not well integrated within health research programmes. Many basic researchers do not appreciate the realities of clinical research. For instance, a basic scientist who has discovered a promising new drug target might not understand the process of passing a successful discovery through drug development into clinical trials. On the other hand, clinicians often find it difficult to set aside sufficient time for research. As a result, the “translational gap” hinders the smooth movement of basic findings to public health applications. In addition to these general roadblocks there are a number of problems specific to the field of infectious disease translational research. These include a lack of a well-structured career path for clinical scientists and the decline of industrial engagement in this field. In order to overcome these problems and to develop an efficient infectious disease translational research programme, the HZI has entered into a long-term strategic alliance with the Hannover Medical School (MHH). Together, both institutions founded TWINCORE, Centre for Experimental and Clinical Infection Research GmbH, located in Hanover.

Over the next years TWINCORE in co-operation with MHH and HZI will develop a translation research programme in infection research. In 2009 the topic starts initially with two projects, both dealing with HCV infections. The Department of Gastroenterology & Endocrinology at the MHH is one of the leading centres for the clinical treatment of HCV infections and is co-ordinating many of the late-phase clinical trials for new therapeutic interventions. Drug resistance against HCV antivirals is a major problem in the treatment of HCV infections and existing drugs often display severe side effects. Therefore, new preventive and therapeutic inventions are urgently needed. The first project aims to identify key molecular interactions between viral proteins and host factors during a HCV infection and implement screening systems for identification of inhibitory compounds. The second project is focused on a better knowledge of type I interferon function in the context of infection and therapy of HCV, including the identification of new type I interferon associated mechanisms in virus control within the central nervous system and understanding the role of type I interferon as a natural adjuvant to enhance long-lived antibody responses and to trigger T cell immunity.

TWINCORE, Centre for Experimental and Clinical Infection Research GmbH

TWINCORE
Zentrum für Experimentelle und Klinische Infektionsforschung

MANAGING DIRECTOR | **Prof. Dr. Ulrich Kalinke**

HEAD OF EXPERIMENTAL INFECTION RESEARCH | Prof. Dr. Ulrich Kalinke
HEAD OF EXPERIMENTAL VIROLOGY | Prof. Dr. Thomas Pietschmann
HEAD OF INFECTION IMMUNOLOGY | Prof. Dr. Tim Sparwasser
HEAD OF PATHOPHYSIOLOGY OF BACTERIAL BIOFILMS | Prof. Dr. Susanne Häußler
HEAD OF CELL AND GENE THERAPY | Prof. Dr. Michael Ott
HEAD OF INFECTION AND CANCER | Prof. Dr. Tim Greten

TWINCORE, the translation centre of HZI and MHH

TWINCORE, Centre for Experimental and Clinical Infection Research GmbH (TWINCORE) is a joint venture between the Helmholtz Centre for Infection Research (HZI), Braunschweig, and the Hannover Medical School (MHH). The building of the former Max-Planck-Institute was acquired by HZI in 2005. This was followed by renovation of the premises and the recruitment and appointment of staff. On August 1, 2008 Prof. Dr. Ulrich Kalinke was appointed Managing Director and the official inauguration of TWINCORE was celebrated on August 29, 2008. TWINCORE has been recorded in the commercial register since October 17, 2008. The goal pursued with the founding of TWINCORE is to promote and further develop the outstanding expertise of HZI and MHH in the field of infection research in a joint centre with a focus upon translational research. The purpose of translational research is a dual one: on the one hand facilitating the path of the latest findings from basic research to the patients and, conversely, enabling unanswered questions from clinical practice to make their way back to researchers. A key part of the work at TWINCORE is also the scientific investigation of regulatory concerns regarding approval and implementation of clinical trials. Complex issues frequently arise in advance of clinical trials, for example regarding the relevance of preclinical experiments for the safety and efficiency of new medicines for use in humans. TWINCORE helps to enable the development of new treatment options for the prophylaxis and therapy of infectious diseases, ensuring that a solid scientific basis for the minimisation of risk exists prior to the testing of new approaches on humans.

RESEARCH PROFILE OF TWINCORE

Research at TWINCORE has 4 focuses, the analysis of pathogen-host interactions, new mechanisms of pathogen inhibition, new vaccination strategies and new preclinical models. Major points within the foci are detailed briefly below.

1. Analysis of pathogen-host interactions During long periods of co-evolution, pathogens and hosts developed complex strategies to enable survival of both the host population and the pathogen population. At the cellular level intrinsic immune mechanisms play a role. At TWINCORE investigations are conducted to establish the influence such factors have on host- and tissue-specificity of pathogens. For some years now it has been acknowledged that, in addition to the recognition of “foreign”, the communication of “danger signals” via pattern recognition receptors (PRR) plays a central role in the induction of protective immunity. The analysis of how innate immunity is triggered via stimulation of PRR and the consequences on pathogen-specific immunity are subject of intensive investigations. In this process both acute and chronic courses of infection are examined. Pathogens have developed various strategies to evade host immunity. Pathogen-encoded factors that modulate immune responses are sought. Furthermore, the influence of regulatory cells on the course of infection is also analysed. An important subject is the examination of pathogen-mediated tumour formation as for example observered in the context of chronic hepatitis C virus (HCV) infection that frequently may cause the development of liver carcinoma.

2. New mechanisms of pathogen inhibition Following the triumphant march of antibiotics in the treatment of bacterial infections, over the past decades key breakthroughs have been made in the development of antiviral substances. New approaches to the inhibition of pathogen reproduction are sought at TWINCORE. In collaboration with the HZI and the University of Hannover, biological compound libraries are being examined for antiviral and antibacterial substances.

This involves the utilisation of new cell culture methods, for example permitting a targeted search for inhibitors of HCV replication. A further core focus is the search for inhibitors of bacterial biofilm formation, which occurs in chronic infections. Similarly, new gene-therapy approaches are also being sought for the treatment of infectious diseases. Moreover, it is also being investigated whether pathogen-encoded immune modulators constitute potential target structures for new therapeutic approaches.

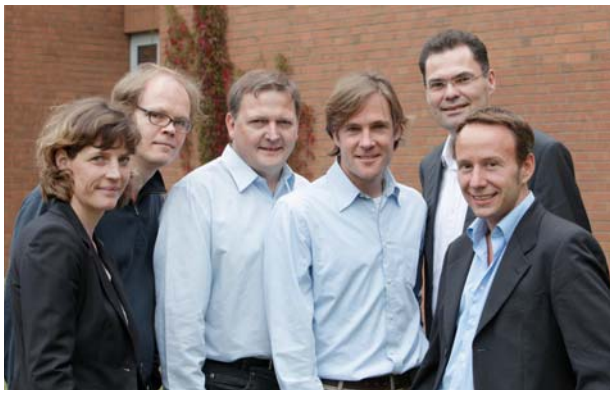
3. New vaccination strategies

To the general public, vaccination is one of the most successful medical advances. Nevertheless, there are still numerous infectious diseases for which no vaccination is available. Consequently, at TWINCORE new vaccination strategies are being developed, with virus-like particles investigated for utilisation as vaccine vectors, together with the specific in vivo charging of specialised cross-presenting dendritic cells with antigens. One interesting option is the reinforcement of immune responses via the influencing of regulatory T cells. There are currently comparatively few approved adjuvants for the reinforcement of immune responses following vaccination. Therefore, together with partners at HZI new adjuvants are being investigated. Similarly, it is also investigated whether cytokines are suitable natural adjuvants for certain vaccination protocols. A further key focus is the analysis of mechanisms that support the induction of long-lasting IgG responses.

4. New preclinical models

New therapeutic or prophylactic approaches developed in basic research need to be subjected to extensive preclinical testing prior to first being tested on humans. At TWINCORE new models are being developed that should enable improved prediction with regard to reactions in humans. An important point in this respect is the humanisation of mice. To this end on the one hand, mice are treated with human cells to enable components of the human immune system to develop in the animals. On the other hand, mouse models are also being developed in which human precursor cells contribute to the formation of liver tissue. A further aspect is the genetic humanisation of mice for example via bacterial artificial chromosome (BAC)-mediated transgenesis. By this method human receptors and allelic forms of them found in the human population may be expressed in order to investigate their function in an animal model. A further aspect is the investigation of effects that are mediated by constant portions of human antibodies. In parallel to experimental research regulatory research is performed to learn more about how licensing procedures that are in force within the European Community impact on the development of new medicinal products.

The main entrance of TWINCORE, Centre for Experimental and Clinical Infection Research GmbH. Alongside modern laboratories and superb equipment, TWINCORE also offers optimal rooms in which to hold scientific meetings and seminars. Photo: HZI, Gramann



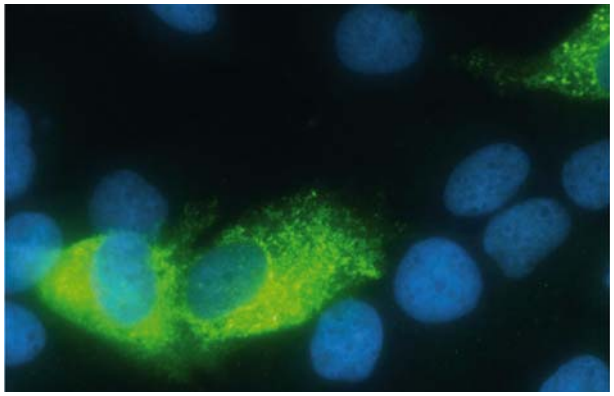
The TWINCORE research group leaders (from left to right): Profs. Susanne Häußler, Tim Greten, Michael Ott, Thomas Pietschmann, Managing Director Ulrich Kalinke, and Tim Sparwasser. *Photo: HZI, Gramann*

RESEARCH GROUPS AT TWINCORE

In 2008 there were established three TWINCORE research groups that were led by Prof. Kalinke, Prof. Pietschmann and Prof. Sparwasser. In January 2009 Prof. Häußler was appointed as a new group leader at TWINCORE, reinforcing TWINCORE's translational research activities in the field of chronic bacterial infections. In addition, TWINCORE offers an optimal research environment to translational research groups from MHH. The translational research groups of Prof. Ott and Prof. Greten of the Clinic for Gastroenterology, Hepatology and Endocrinology, directed by Prof. Manns, are currently seconded to TWINCORE. Brief reports of these six research groups are listed below. TWINNING-projects are a key instrument in integrating individual researchers from MHH clinics or HZI departments into the work of TWINCORE research groups. TWINCORE currently has a number of these TWINNING-projects and there are plans to establish even more in the future.

Research group Prof. Kalinke | kalinke.ulrich@mh-hannover.de Following viral infection, within hours type I interferon responses are usually induced, which secure the initial survival of the host. It is only days later that adaptive immunity is activated to an extent that it is able to eradicate pathogens. In earlier projects we have shown that following an infection with the vesicular stomatitis virus (VSV) a small number of highly specialised cells, also addressed as plasmacytoid dendritic cells (pDC), are activated via PRR triggering to produce large quantities of protective type I interferon. Interestingly, practically all closer examined viruses developed countermeasures that inhibit the induction of such type I interferon responses. A key focus of our work is how various different viruses induce type I interferon responses and which type I interferon-inhibiting factors they encode. Local conditions of type I interferon responses decisively influence the course of disease. In that line we are investigating how type I interferon inhibits the spread of pathogens within the central nervous system. In more recent investigations we discovered that type I interferon can also have direct effects on immune cell functions. Currently we are studying how type I interferon induced upon vaccination with virus-like particles influences the formation of long lasting IgG antibody responses. Antibodies are comparatively large molecules, the variable parts of which bind antigens specifically, whereas the constant parts may be bound via so-called Fc receptors expressed by certain immune cells and other body cells and confer antibody function. However, thus far there is only partial comprehension regarding which Fc receptors bind antibodies of the different subclasses and what influence this binding has on immune functions. This issue is currently being investigated also for monoclonal antibodies because such reagents play an increasingly important role as innovative therapeutic agents in the treatment of tumours, autoimmune diseases and infections. The predictability of functions of new medications within the human body is a key theme. In addition to the application of classical cell culture and tissue techniques we are also attempting to mature human immune cells in immune deficient mice in order to be able to better investigate complex interactions of human immune cells. Finally, also regulatory research is performed in collaboration with the Paul-Ehrlich-Institut, Germany.

Research group Prof. Pietschmann | tpi07@helmholtz-hzi.de Infection with HCV, which belongs to the family of flaviviruses, is one of the major causes of chronic liver disease. According to WHO estimates, up to 170 million people have had HCV contact worldwide. Of these, around 100 to 130 million people are considered to be chronically infected. We develop new cell culture techniques for the investigation of HCV replication. The goal of this research is to investigate the molecular mechanisms of HCV replication in liver cells. In particular, early stages of HCV infection are studied, which are critically involved in virus entry into liver cells. We are also analysing processes that lead to the packing of the viral genetic material in progeny viruses and their release from the host cell. In this manner we aim to draw up the fundamental basis of the infection strategy of this human-pathogenic virus, to subsequently generate new approaches and perspectives for the development of therapies. In TWINNING-projects with partners at the MHH and the HZI we employ the HCV cell culture system to identify new agents that inhibit HCV replication. Furthermore, within the Helmholtz Alliance on Immunotherapy of Cancer we are involved in the development of a new immuno therapy approach for the treatment of chronic HCV infection and HCV-associated hepatocellular carcinoma. (Further details look at page 89)



HCV infected human hepatoma cells visualized by indirect immunofluorescence of a viral protein (green). The granules represent viral replication complexes engaged in synthesis of HCV genome progeny. (Fig. 2 from Prof. Pietschmann's contribution, see page 89) Photo: HZI

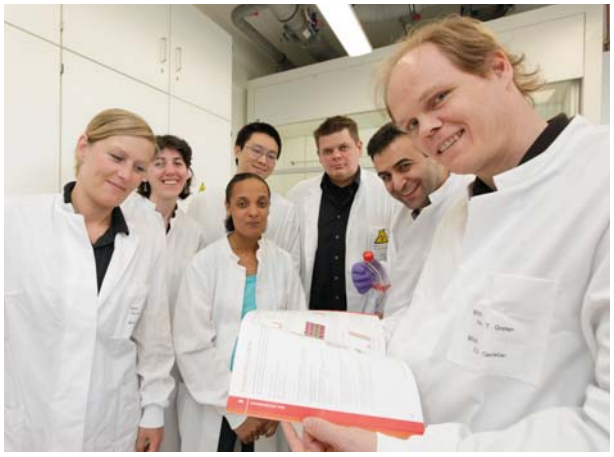
Prof. Sparwasser and his team. Photo: HZI, Gramann

Research group Prof. Sparwasser | sparwasser.office@mh-hannover-de Our focus lies upon the investigation of the significance of PRR, for example from the family of *Toll-like* receptors (TLRs) and the C-type lectins for the activation of the most important positive regulators of the immune system and initiators of adaptive immunity, i.e. the dendritic cell system (DCs). A further focus is upon regulatory T cells (Tregs), which may be regarded as the principal counterparts to DCs: Tregs use mechanisms as yet not completely understood to inhibit an overshooting immune response and limit the proliferation of T effector cells. Optimal vaccination strategies against pathogens may comprise the activation of specific DC subpopulations whilst avoiding Treg expansion or induction. Vaccination studies aimed towards Tregs and DCs in the murine model system have several limitations, in that in vivo analysis of Tregs and DC subpopulations are extremely difficult. For example, subpopulations of DCs exist in extremely low numbers in various lymphatic organs that have highly specialised tasks in several cases, including the induction of tolerance. As usually these *"regulatory immune cells"* react highly sensitively to *ex vivo* isolation and have thus far proved only inadequately manipulable *in vivo*, the knowledge of function and significance of Tregs and DC subpopulations for adaptive immunity remains incomplete. A further complicating factor is the expression of different pattern recognition molecules or different expression profiles of PRRs to DC subpopulations between humans and mice. A key objective is therefore the development of molecular tools that allow the genetic manipulation of DCs and Tregs. We wish to use these models to investigate the function of Tregs and subpopulations of DCs in infection, allergy and tolerance. In *"humanised"* models the role of PRRs such as human TLR9 and DC-SIGN is analysed and vaccination strategies aimed at these molecules tested *in vivo.* Within the Sparwasser group Dr. Jan Dudda is to establish a junior research team on the analysis of "interaction of dermal DCs and Tregs".

Research group Prof. Häußler | sus@helmholtz-hzi.de
Pseudomonas aeruginosa is the most dominant bacterial pathogen causing chronic lung infection in cystic fibrosis (CF) patients. Although most patients are colonised with only one *P. aeruginosa* clone, various morphotypes can often be isolated. Our research focuses on the elucidation of the molecular mechanism underlying this diversity. In CF patients who are chronically colonized with *P. aeruginosa* so-called small colony variants (SCVs), which form biofilms particularly efficiently, are frequently recovered from the respiratory tract. This phenotype is characterised by the expression of a certain gene cluster that is regulated by a bacterial signal molecule, cyclic di-GMP. Our screening for mutations conferring the SCV phenotype has revealed even single nucleotid exchanges. In the future we aim at studying clinically relevant mutations that occur in *P. aeruginosa* under *in vitro* biofilm growth conditions and *in vivo* in the course of a chronic infection. The knowledge of the genotypes that are selected at different stages of infection can be helpful for the development of new, promising therapy strategies. *P. aeruginosa* produces three interbacterial signal molecules, one of which is referred to as the Pseudomonas quinolone signal (PQS). PQS is involved in cell density-dependent virulence factor regulation and the establishment of biofilms. Furthermore, we found that PQS plays a key role in the generation of morphological diversity under biofilm conditions. PQS is an anti-oxidant and, at the same time, produces oxygen radicals. The pro-oxidant activity damages DNA, resulting in double strand breaks that are only insufficiantly repaired. As a consequence genetic variants are induced that then may be selected under biofilm conditions. The investigation of the link between interbacterial communication and the generation of specific biofilm phenotypes is an exciting challenge for the future. (Further details look at page 55)

Research group Prof. Ott | ott-mhh@gmx.de
We develop cell and gene therapy procedures for the treatment of hereditary liver disease. Another focus lies on the development of mouse models with chimeric human/murine liver tissue and human immune system for researching vaccination strategies against HIV and HCV. The repopulation of the liver with human liver cells and the transplantation of human blood stem cells into immune-deficient mice continue to represent a major scientific challenge. In order to investigate vaccination strategies in the alb-uPA transgene immune deficient (RAGyc) mouse it is necessary that human cells of a donor are utilised in the repopulation of the mice. Where primary tissue is used as starting material the isolation of both cell populations using fetal liver tissue exclusively is possible. In our transplantation experiments it emerged that the transplantation of fetal hepatoblasts is significantly less efficient than that of adult primary hepatocytes. Alternatively, the research group enabled the transplantation of fetal human liver tissue beneath the capsule of the recipient liver to be tested for the first time. The first combined transplantations of human blood stem cells and fetal liver tissue to a newly-developed mouse strain are set to be performed shortly. Due to the lack of availability of primary human cell material for the "humanisation" of mice, as well as for cellular therapies in humans, the research group is conducting intensive research into alternative cell sources. In association with other MHH groups and international partners, research is underway into hepatic differentiation protocols for embryonic stem cells and iPS cells. In another project the risk of insertional mutagenesis in lentiviral gene transfer is analyzed. Serial transplantation of *ex vivo* genetically transduced hepatocytes enables the incidence of liver tumours in dependence on the number of lentiviral insertions to be investigated. Furthermore, the research group is supervising a clinical study on cell transplantation in patients with urea cycle disorder. This project, marks the world's first controlled study on cell therapy of hereditary metabolic diseases of the liver.

Prof. Ott and his team. *Photo: HZI, Gramann*



Prof. Greten and his team. *Photo: HZI, Gramann*

Research group Prof. Greten | greten.tim@mh-hannover.de The goal of the research group is the development of immunological therapies for the treatment of tumours in the gastrointestinal tract. In this process we are concentrating on the investigation of patients with infection-associated tumours. Worldwide, almost 1/4 of all tumour illnesses are infection associated. A typical example of this is the hepatocellular carcinoma, which may develop on the basis of chronic viral hepatitis. In this respect we are investigating how the immune system reacts to the creation of tumours in patients. In order to be able to study the individual cellular and molecular procedures in more detail we are also developing mouse models that simulate the tumour illnesses of patients as closely as possible. On the basis of the findings that we acquire from these studies we are developing new therapy protocols which we then test in the scope of controlled clinical studies of patients with carcinoma of the gastrointestinal tract.

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Publications 2007-2009

Div. Cell and Immune Biology | Prof. Dr. Jürgen Wehland

Dept. Chemical Biology | Dr. Ronald Frank

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Dept. Cell Biology | Prof. Dr. Jürgen Wehland

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RG Microbial Communication |

Prof. Dr. Irene Wagner-Döbler

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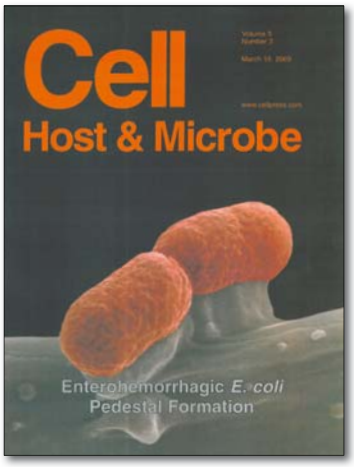

Cover picture of the journal Cell Host & Microbe, Vol. 5(3), 2009, on the occasion of the publication of the article by Weiss, S.M., Ladwein, M., Schmidt, D., Ehinger, J., Lommel, S., Städing, K., Beutling, U., Disanza, A., Frank, R., Jänsch, L., Scita, G., Gunzer, F., Rottner, K. & Stradal, T.. IRSp53 links the enterohemorrhagic E. coli *effectors Tir and EspFu for actin pedestal formation.* ***Cell Host & Microbe****, 2009;* ***5(3)****: 244-258. The permission of Elsevier Ltd. is gratefully acknowledged.*

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JRG Chronic Pseudomonas Diseases |

Prof. Dr. Susanne Häußler

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JRG Chronic Infections and Cancer | Dr. Lars Zender

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Former RG Mucosal Immunity (until 2008) |

Prof. Dr. Jan Buer

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Prof. Dr. Matthias Gunzer

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(until 2008) | Prof. Dr. Jutta Eichler

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Div. Molecular Biotechnology | Prof. Dr. Hansjörg Hauser

Dept. Genome Analysis | Dr. Helmut Blöcker

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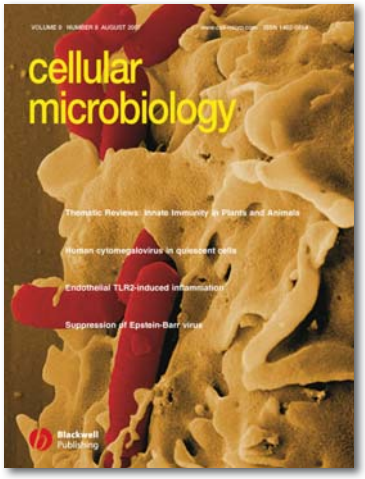

Cover picture of the journal Cellular Microbiology, Vol. 9, 2007, on the occasion of the publication of the article by Gekara, N.O.; Westphal, K.; Ma, B.; Rohde, M.; Groebe, L., and Weiss, S.. The multiple mechanisms of Ca^{2+} signalling by listeriolysin O, the cholesterol-dependent cytolysin of Lysteria monocytogenes. ***Cellular Microbiology. 2007;* 9:** *2008-2021. The permission of Blackwell Publishing is gratefully acknowledged.*

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(until 2009) | Prof. Dr. Jürgen Bode

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Div. Structural Biology | Prof. Dr. Dirk Heinz

Dept. Molecular Structural Biology | Prof. Dr. Dirk Heinz

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RG Biophysical Analysis | Dr. Victor Wray

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Dr. Wolf-Dieter Schubert

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JRG Structure-based Infection Biology | Dr. Torsten Lührs

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RG Recombinant Protein Expression |

Dr. Joop van den Heuvel

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Dept. Microbial Pathogenicity | Prof. Dr. G. Singh Chhatwal

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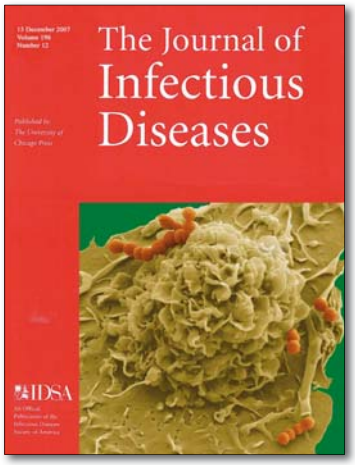

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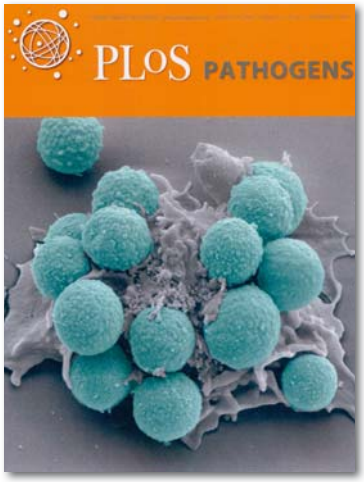

Cover picture of the journal PLoS Pathogens, Vol. 3(2), 2007, on the occasion of the publication of the article by Behnsen, J.; Narang, P.; Hasenberg, M.; Gunzer, F.; Bilitewski, U.; Klippel, N.; Rohde, M.; Brock, M.; Brakhage, A.A., and Gunzer, M.. Environmental dimensionality controls the interaction of phagocytes with the pathogenic fungi Aspergillus fumigatus and Candida albicans. ***PLoS Pathogens.*** *2007;* ***3(2):*** *ev03.i02.doi:10.1371/image.ppat.v03.i02 (February 23, 2007). The photograph was provided by Narang, P.; Rohde, M., and Gunzer, M.; Helmholtz Centre for Infection Research.*

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Cover picture of the journal Bioorganic & Medicinal Chemistry, Vol. 15(23), 2007, on the occasion of the publication of the article by Menche, D.; Arikan, F.; Li, J.; Rudolph, S., and Sasse, F.. Efficient one-pot synthesis of biologically active polysubstituted aromatic amines. ***Bioorganic & Medicinal Chemistry****. 2007;* ***15(23)****: 7311-7317. The permission of Elsevier Ltd. Is gratefully acknowledged.*

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- Pietschmann,T. (2009) Virology: Final entry key for hepatitis C. *Nature* **457,** 797-798.

Lectures 2008-2009

Lectures held at the HZI by external scientists

- Alexopoulos, Leonidas, Dr. *Broad exploration of signaling pathways in liver cells*; Division of Biological Engineering, Massachusetts Institute of Technology (MIT), Cambridge, MA. Cambridge, USA; 2008 December 2.
- Ammon, Andrea , Dr. *The Role of ECDC in the Prevention and Control of Infection Diseases in the EU*; European Center for Disease Prevention and Control. Stockholm, Sweden; 2009 February 13.
- Andersson, Siv, Prof. Dr. *Extreme Symbiotic Bacterial Genomes*; Dept. Molecular Evolution, University Uppsala. Uppsala, Sweden; 2009 January 23.
- Ball, Andrew, Prof. Dr. *Development of commercial bioremediation strategies for the treatment of oil tank bottoms*; Flinders University, School of Biological Sciences. Adelaide, Australia; 2009 April 28.
- Bartosch, Birke, Dr. *Hepatitis C virus host cell interactions*; Laboratoire de Physiopathologie Moléculaire et Nouveaux Traitments des Hépatites, Virales. Dardilly, France; 2009 May 13.
- Baumann, Frank, Dr. *In vivo Dissection of functional Domains of PrP: New Lessons thaught by Transgenic Mice*; Hertie-Institut für klinische Hirnforschung, Universität Tübingen. Tübingen, Germany; 2008 December 5.
- Baumgraß, Ria, Dr. *Impaired TcR signaling promotes Treg cell differentiation - from molecular mechnisms towards clinical application*; Deutsches Rheuma-Forschungszentrum. Berlin, Germany; 2009 May 8.
- Bautsch, Wilfried, Prof. Dr. *Methicillin-resistant Staphylococcus aureus (MRSA): a hygienic problem of the entire health system*; Institut für Mikrobiologie, Immunologie und Krankenhaushygiene, Städtisches Klinikum. Braunschweig, Germany; 2009 April 24.
- Becker, Christian, Dr. *Regulatory T cells: mechanism of function and selective activation*; Universitätshautklinik. Mainz, Germany; 2008 February 15.
- Benecke, Mark. *Kriminalbiologie*; International Forensic Research & Consulting., Köln 2009 March 5.
- Benham, Craig J., Prof. Dr. *Superhelically induced duplex destabilization (SIDD) in DNA, and its roles in regulation*; Dpts. of Mathemtics and of Biomedical Engineering, UC Davis, USA 2009 March 26.
- Bernhard, Frank, Prof. Dr. *Protocol development for the preparative scale synthesis of difficult proteins in high quality by cell-free expression strategies;* Universität Frankfurt, Germany, 2009 June 15
- Blankenstrein, Thomas, Prof. Dr. *The immune response to sporadic cancer*; Max-Delbrück-Centrum, Dept. of Molecular Immunology and Gene Therapy. Berlin, Germany; 2008 February1.
- Bocharov, Gennady, Prof. Dr., *Mathematical Modelling of Experimental Virus Infections;* University of Moscow, Russia, 2009 May 7.
- Böhm, Markus. *Illumina Genome Analyzer Powered by Solexa Sequencing. Basic principle of technology, system components, workflow, applications, outlook*; Fa. Illumina. 2008 February 13.
- Bollati, Mariela, Dr. *Screening of compounds affecting type I IFN activities*; Cellular Biology Unit, Inst. Pasteur de Montevideo. Montevideo, Uruguay; 2008 November 3.
- Bryceson, Yenan, Dr. *Triggering and mechnanisms of NK cell-mediated cytotoxicity;* Center for Infectious Medicine (CIM), Karolinnska Institute. Stockholm, Sweden; 2009 April 24.
- Bröker, Barbara M., Prof. Dr. *The antibody response against Staphylococcus aureus in health and disease*; Abt. für Immunologie, Universität Greifswald. Greifswald, Germany; 2009 May 15.
- Camp, David, Dr. *A natural toolbox for the HTS paradigm*; Queensland Compound Library, Eskitis Institute, Griffith University. Nathan, Queensland Australia; 2008 May 28.
- Cantz, Tobias, Dr. *Stem Cell Biology: New Applications for Studying Hepatic Diseases*; MPI. Münster, Germany; 2008 August 22.
- Caruso, Arnaldo,Prof. Dr. & Fiorentini, Simona, Prof. Dr. *Development of a therapeutic anti-AIDS vaccine / Functions of the HIV-1 matrix protein p17*; University of Brescia, Italy, 2009 June 18
- Dalpke, Alexander, Prof. Dr. *Regulation of local immunity by airway epithelial cells*; Dept. of Hygiene and Medical Microbiology, Universität Heidelberg. Heidelberg, Germany; 2008 April 25.
- Dersch, Petra, Dr. *Regulating yersinia virulence: Environmental sensing on the post-transcriptional level*; Technische Universität . Braunschweig, Germany; 2008 Sep 12.
- DeSalle, Robert, Dr. *How evolutionary Trees are made, what they mean and how they have advanced modern evolutionary Thought*; The American Museum for Natural History. New York, USA; 2008 December 12.
- Dübel, Stefan, Prof. Dr. *Engineering the front and far ends of human antibodies to generate novel protein therapeutics*; TU Braunschweig, Inst. für Biochemie und Biotechnologie. Braunschweig, Germany; 2008 March 28.
- Déglon, Nicole, Dr. *Gene Transfer Approach for the Modeling and Treatment of Neurodegenerative Diseases*; Atomic Energy Commission, Inst. of Biomedical Imaging Research Center. Orsay, France; 2008 October 10.
- Fry, Jeremy, Dr. *Rapid CD4+ and CD8+ T cell epitope discovery and validation*; Prommune. 2008 May 20.
- Gessner, André, Prof. Dr. *Inflammation and anti-bacterial defense in Lyme disease*; University Clinic of Erlangen, Institute of Microbiology. Erlangen, Germany; 2008 May 28.
- Groll, Michael, Prof. Dr. *Natural and synthetic inhibitors of the 20S proteasome: Crystallographic knowledge in drug design strategies*; TU München. München, Germany; 2008 April 7.
- Hall, Brian. *Imaging Flow Cytometry*; Amnis Corp. 2008 September 22.
- Hanski, Emanuel, Prof. Dr. *Regulation of streptococcal virulence by the autoinducer peptide*; Dept. of Clinical Microbiology, The Hebrew University Hadassah Medical School. Jerusalem, Israel; 2008 May 16.
- Hebenstreit, Daniel, Dr. *Histone modifications in T helper cells*; MRC Cambridge. Cambridge, UK; 2009 March 24.
- Höfer, Thomas, Dr. *Nonlinear Dynamics of Gene Regulatory Circuits in T Helper Cell Differentiation;* DKFZ. Heidelberg, Germany; 2008 November 7.
- Hoffmann, Ralf, Prof. Dr. *Design of novel peptidomimetics with a broad activity spectrum for in vivo treatment of Gram-negative pathogens*; Institute of Bioanalytical Chemistry, University of Leipzig, Germany, 2009 June 19.

- Honigman, Alik, Prof. Dr. *CREB, Hypoxia and Tumorogenesis; Out of Breath is not out of Function*; Faculty of Medicine, The Hebrew University. Jerusalem, Israel; 2008 October 7.
- Ivics, Zoltan, Dr. *Relics from the past: molecular biology and applications of resurrected vertebrate transposons*; Max Delbrück Center for Molecular Medicine. Berlin, Germany; 2009 February 17.
- Joost, Insa, Dr. *Adhesion, Inflammation and Survival: The multifaceted interactiones of S. aureus Eap protein in endovascular*; Inst. für Medizinische Mikrobiologie, Universitätsklinikum des Saarlandes. Homburg, Germany; 2008 April 7.
- Kaaks, Rudolf, Prof. Dr. *Development and uses of large prospective cohorts with bio-repositories; the example of the 'EPIC' study*; German Cancer Research Center, Heidelberg, Germany. 2008 March 17.
- Kaderali, Lars, Dr. *Reconstructing Regulatory and Signalling Networks from High-Throughput Data*; Deutsches Krebsforschungszentrum . Heidelberg, Germany; 2008 November 21.
- Kalinke, Ulrich, Prof. Dr. *Type I interferons in viral infection, tumor disease and autoimmunity*; Twincore. Hannover, Germany; 2008 September 12.
- Klavonn, Frank. *How to analyze data obtained from high throughout technologies?*; Fachhochschule BraunschweigWolfenbüttel. Wolfenbüttel, Germany; 2009 April 3.
- Koegl, Manfred, Dr. *Applying automated Yeast two-hybrid screening: About nuclear receptors and host-pathogen interactions*; Translational Research, and Genomics and Proteomics Core Facilitie, German Cancer Research Center. Heidelberg, Germany; 2008 July 18.
- Kolmar, Harald, Prof. Dr. *Serendipity, design and evolution: Towards novel miniproteins with antiinfective and therapeutic potential*; TU Darmstadt. Darmstadt, Germany; 2009 April 6.
- Köster, Reinhard, Dr. *Bridging cell biology and development genetics: Bio-imaging of neuronal migration i n the developing zebrafisch cerebellum*; Helmholtz Zentrum München. München, Germany; 2008 February 21.
- Kretschmer, Karsten, Prof. Dr. *Molecular and cellular aspects of regulatory T cell generation*; Dana-Faber Cancer Institute und Center for Regenerative Therapies (CRT). Boston und Dresden; 2008 February 8.
- Kues, Wilfried, Dr. *Reprogramming events in early mammalian embryos are reflected by circular plasmid encoded marker genes*; Friedrich-Loeffler-Institut für Nutztiergenetik. Mariensee, Germany; 2008 October 21.
- König, Renate, Dr. *HIV'S Journey to the Nucleus - Discovering Host Factors by High-throughput Systems-based Analysis*; Burnham Institute for Medical Research. San Diego, USA; 2008 October 31.
- Laible, Goetz, Dr. *Biopharming in dairy cattle: Production of valuable proteins in milk*; AgResearch, Ruakura Research Centre. Hamilton, New Zealand; 2008 September 2.
- Leier, André, Dr. *Methods and challenges in modeling cellular signaling pathways - Examples from Hes1, mTOR and IFN pathways*; Dept. of Biosystems Science & Engineering, ETH Zürich. Zürich, Switzerland; 2009 May 18.
- Leong, John, Dr. *Actin up; pedestal formation by pathogenic E. coli*; University of Massachusetts. Worcester, USA; 2008 November 12.
- Li, Yin, Prof. Dr. *Understanding the probiotic effect of Lactobacillus through genomics*; Institute of Microbiology, Chinese Academy of Sciences (CAS), China 2008 February 14.
- Ljunggren, Hans-Gustaf and Malmberg Kalle, Drs. *New insights into the activation and inhibition of human NK cells*; Karolinska Institute, Centre of Infectious Medicine. Stockholm, Sweden; 2009 May 11.
- Lohmann, Volker, Dr. *Viral and host factors governing hepatitis C virus replication*; Heidelberg, Germany; 2008 May 11.
- Machner, Matthias, Dr. *How the intravacuolar pathogen Legionella pneumophila exploits host cell Rab GTPase function*; Tufts University School of Medicine, Dept. of Molecular Biology and Microbiology. Boston, USA; 2008 February 5.
- Majumdar, S. , Dr. *Development of an easily adaptable death-less techniques for generating transgenic mice*; Deivision of Embryo Biotechnology, National Institute of Immunology. New Delhi, India; 2008 November 3.
- Marquez Lago, Tatiana, Dr. *Stochastic modeling of an tunable synthetic mammalian oscillator*; Dept. of Biosystems Science & Engineering, ETH Zürich. Zürich, Switzerland; 2009 May 18.
- Matthews, Brian, Prof. Dr. *Structure isn't everything but sure helps*; University of Oregon, Eugene, USA, 2009 June 22.
- Müller, Arno, Dr. *Coordination of Cell Movement by Fibroblast Growth Factor Signaling in the Drosophila Embryo*; University of Dundee. Dundee, United Kingdom; 2008 November 27.
- Müller, Christian D., Dr. *Discovery by High Content Screening of new molecular tools to study the "hot" link between inflammation and cancer*; Institut Gilbert Laustriat, Université Louis Pasteur-Strasbourg. Straßburg, France; 2008 December 11.
- Noll, Thomas , Prof. Dr. *Functional genomics as a tool for cell culture process developmen*t; University of Bielefeld, Cell Culture Technology. Bielefeld, Germany; 2008 March 7.
- Pasupuleti, Mukesh, Dr. *Structure and functional studies of antimicrobial peptides*; Center for Innate Immunity, Lund University. Lund, Sweden; 2009 May 13.
- Peschel, Andreas, Prof. Dr. *Staphylococcus aureus colonization, infection and immune evasion*; University of Tübingen, Tübingen, Germany, 2009 May 27.
- Pessler, Frank, Dr. *Transcript Profiling of Crystal-Induced Inflammation and Innate Immunity in a Minimal Tissue*; Twincore, Zentrum für Experimentelle und Klinische Infektionsforschung GmbH. Hannover, Germany; 2008 November 10.
- Philipp, Bodo, Dr. *Cell-cell interaction of Pseudomonas aeruginosa*; University of Konstanz, Konstanz, Germany, 2009 May 7.
- Piehler, Jacob, Prof. Dr. Dyna*mics of the type I interferon receptor complex: Biophysical analysis and functional implications*; Johann Wolfgang Goethe University . Frankfurt, Germany; 2008 May 16.
- Preissner, Klaus, Prof. Dr. *Inflammation, degeneration and defence in the cardio-pulmonary system: new players, new games*; University of Gießen. Gießen, Germany; 2009 March 12.
- Quentmeier, Hilmar, Dr. *JAK2V617F activating mutation and silencing of JAK inhibitor SOCS2 pathogenic mechanisms for myeloproliferative disorders*; DSMZ. Braunschweig, Germany; 2008 May 30.
- Rausch, Sebastian, Dr. *Tregs control pathology and effector responses induced by an intestinal nematode infection*; Institut für Biologie, Lehrstuhl für Molekulare Parasitologie, Humboldt-Universität zu Berlin. Berlin, Germany; 2009 April 17.
- Röhrs, Sonja, Dr. *Epigenetic alterations in lymphomas*; DSMZ. Braunschweig, Germany; 2008 June 10.
- Rudolph, Lenhard, Prof. Dr. *Teleomeres, stem cells and aging;* Institute of Molecular Medicine and Max-Planck Research Group; University of Ulm, Ulm, Germany, 2009 June 12
- Saftig, Paul , Prof. Dr. *An acidic life: Lysosomes in health and (infection)-disease*; University of Kiel. Kiel, Germany; 2008 May 16.
- Schambach, Axel, Dr. *From retroviral biology to retroviral therapy*; MHH Hannover. Hannover, Germany; 2008 February 12.
- Schwab, Martin, Prof. Dr. *Repair of the injured spinal cord or brain: A biomedical approach*; Brain Research Institute, University of Zürich and ETH Zürich, Zürich, Switzerland, 2008 June 25.

- Schwille, Petra, Prof. Dr. *Creating to understand: The Virtues of bottom-up Biology*; Institut für Biophysik, TU Dresden. Dresden, Germany; 2009 February 6.
- Sensen, Christoph, Prof. Dr. *From 2D to 4D bioinformatics*; Dept. Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada, 2008 January 24 .
- Söll, Dieter, Prof. Dr. *The genetic code revisited: Four decades after Francis Crick*; Yale University, USA. 2008 November 10.
- Steinert, Michael, Prof. Dr. *Die Legionärskrankheit: Ein Bakterium geht fremd*; TU Braunschweig. Braunschweig, Germany; 2008 December 10.
- Trauner, Dirk, Prof. Dr. *Pericyclic Reactions in Natural Product Synthesis*; Ludwig-Maximilians-Universität, Dept. Chemie und Biochemie. München, Germany; 2008 November 28.
- Uphoff, Cord . *The neglected menace of mycoplasma contamination of cell cultures*; DSMZ. Braunschweig, Germany; 2008 May 20.
- Vallan, Claudio, Dr. *Flow Cytometry Analysis*; Celeza GmbH, Germany, 2008 March 6.
- Van der Gast, Christopher, Dr. *Spatial and temporal scaling of bacterial taxa*; NERC Centre for Ecology and Hydrology. Oxford/UK; 2008 February 20.
- Welte, Tobias, Prof. Dr. *Networks of Excellence - Results and Advantages for Translational Research*; Medizinische Hochschule Hannover. Hannover, Germany; 2008 July 4.
- Wendt, Ulrich, Dr. *Biostructural Research in drug Discovery*; Sanofi-Aventis GmbH, Frankfurt, Germany, 2008 January 11.
- Wessler, Silja, *Dr. Heliobacter plyori: Disruption of the epithelial barier function*; Paul-Ehrlich-Institut, Berlin, Germany, 2009 May 29.
- Wilting, Jörg, Prof. Dr. *Development and malformation of lymphatic vessels*; Universitätsmedizin Göttingen. Göttingen, Germany; 2008 April 8.
- Winkler, Thomas, Prof. Dr. *Protective antibody responses by virus specific memory B cells*; Universität Erlangen, Lehrstuhl für Genetik, Inst. für Mikrobiologie, Biochemie und Genetik. Erlangen, Germany; 2008 January 18.
- Wittinghofer, Alfred, Prof. Dr. *Nucleotide-dependent molecular switches and how they function*; Max Planck Institute of Molecular Physiology. Dortmund, Germany; 2008 December 3.
- Wittmann, Valentin, Prof. Dr. *Combinatorial approaches to probe carbohydrate-protein and carbohydrate-RNA interactions*; Department of Chemistry, University of Konstanz. Konstanz, Germany; 2008 February 22.
- Yusibov, V. M., Dr. *Plant based production of vaccine antigens*; Fraunhofer Center for Molecular Biotechnology. Newark / Delaware, USA; 2008 December 4.
- Zeilinger, Carsten, Dr. Deve*lopment of functional assays with ion channels als targets – from bench to appliance*; Institute of Organic Chemistry, Leibniz University Hannover. Hannover, Germany; 2009 February 20.

Photos from left to right: Eva Zimmermann giving instructions on IT-programmes to pupils during the Future Day at the HZI | Discussion during the visit of the Indian Minister of Health with his delegation and HZI scientists and politicians at the HZI FORUM | During a workshop on Technology Transfer and Networking in Biotechnology organized by HZI, InWEnt and BPPT at the BPPt facilities in Jakarta, Indonesia *Photos: HZI, Krämer (le) | HZI, Krämer (ce) | HZI, Jonas (ri)*

FACTS AND FIGURES

Facts and Figures

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In 1965 the HZI was founded as “Centre for Molecular Biological Research” (GMBF) with financial support by the Volkswagen Foundation. In 1976 the Federal Government through the Ministry for Research and Technology (BMFT) together with the State of Niedersachsen took over the Centre, now called “German Research Centre for Biotechnology” (GBF). Since then the BMFT/BMBF as well as the State of Niedersachsen have jointly financed the GBF /HZI. In 2006 it was the first research centre of the Helmholtz Association to change its name into a Helmholtz Centre institution: the Helmholtz Centre for Infection Research – HZI.

Research Financing In 2008 the total costs of the HZI amounted to 54.4 Mio. € with more than three quarters, 41.6 Mio. €, devoted to the programme “Infection and Immunity”.

External Funding More than 75% of the external funding came from national research programmes. About 11% and 6% were from EU programmes and industry, respectively.

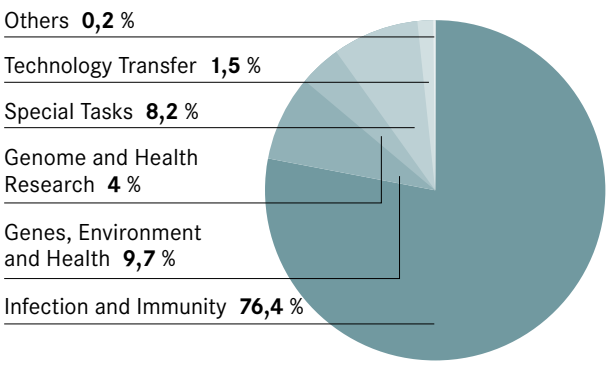
Costs per programme (in T€)

Research Area	Programme	Full Cost
Health	Infection and Immunity	41 586
	Genome and Health Research	2 207
Environment and Health	Genes, Environment and Health	5 253
Technology Transfer		802
Special Tasks		4 442
Others		140
Total Sum		**54 430**

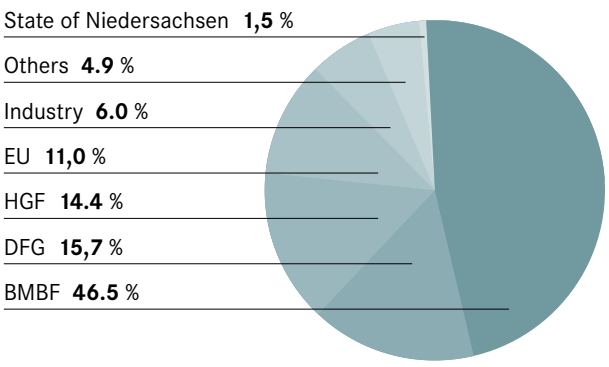
External financing (in T€)

Source	Full Cost
BMBF	7 747.91
DFG	2 620.75
EU	1 834.02
Industry	995.55
HGF	2 403.21
State of Niedersachsen	249.64
Others	813.31
Total Sum	**16 664.38**

Full Costs 2008



External funding 2008 – by source



Property Rights / Licences In 2008, eleven patents were applied for, ten of them outside of Germany. All these patents were originated in the research area "Health".

Patents and property rights, licences, year 2008

	Total number	Germany	
Priority based applications (2008)	11	1	10
Priority based applications, total number	96	43	53
Granted patents (2008)	130	9	121
Total number of held property rights	523	47	476
Licence agreement, total number	368	26	342
Licence proceeds* (in T€)	500	140	360

** Including revenues from other "know-how" transfer agreements*

Publications, Professorships, DFG-Programmes, and Guest Scientists The HZI has further increased the impact of its scientific output in recent years. Several articles have been published in highly renowned journals of the Nature-group.

Many HZI scientists are participating in important national and international research programmes.

Participation of HZI scientists in national and international research programmes

DFG (German Research Foundation)	
SFB 431	Membrane Proteins
SFB 566	Cytokin-Receptors
SFB 578	From Genome to the Product
SFB 587	Lung Immunity
SFB 599	Permanent Implantates
SFB 621	Pathobiology of the Intestinal Mucosa
SFB 738	Optimization of Conventional and Innovative Transplants
SSP 1150	Signal Pathways to the Cytoskeleton and Bacterial Pathogenecity
SSP 1160	Colonisation and Infection through Human-Pathogen Fungi
SSP 1230	Bacterial DNA Vectors
Excellence Cluster 42	REBIRTH
FOR 119	Hepatocellular Carcinoma
FOR 471	Cell Differentiation
FOR 629	Antibodies and Proteinanalysis

BMBF	
Basic Innovation Genome Research	Candida Therapy
Basic Innovation Genome Research	Tuberculosis Therapy
ERA-Net	SPATELIS
FORSYS	Metabilic Balance in *E. coli*
FORSYS	T cell Talk
Genomic	Metagenome of Biofilms
Genomic	*Sorangium cellulosum*
MEDSYS	Jekyll & Hyde
MEDSYS	BioInSys
NGFN II	Infection and Inflammation
NGFN II	Ecological Genomics
NGFN II	Mammalian Models
NGFN II	Protein
NGFN II	DNA
NGFN II	Cell
NGFN II	Antibody Factory
NGFN Plus	German Mouse Clinic
NGFN Plus	Adipositas Network
Susceptbility & Resistance	SkinStaph
Susceptbility & Resistance	PROGRESS
Susceptbility & Resistance	Resistence Susceptibility
Zoonosis / Systemsbiology	SysMo
Zoonosis / Systemsbiology	ZooMAP
Zoonosis / Systemsbiology	PBA-Zoo
Zoonosis / Systemsbiology	Influenza

Quantitative Parameters	Category	2006	2007	2008
Publications	ISI-listed Papers published by the Centre	247	240	205
	Peer-reviewed non-ISI publications in journals	7	10	5
	Books/other non-ISI-listed publications	18/3	9	4
	Total number	**275**	**259**	**214**
Habilitations		0	3	2
Dissertations		15	25	40
PhD Students		97	181	219
Full Professorship Offerings (W2/W3)	Calls for professorship	4	5	4
Special DFG-Programmes	DFG-SFBs, Transregios, Excellence clusters	6	8	8
	DFG-Research Focus	5	4	2
	Graduiertenkollegs	3	3	3
	Total number	**14**	**15**	**13**
Guest Scientists		132	121	98

EU Frame Programmes	
CA	CASIMIR
CP	FAST-XDR-DETECT
CP	FLUINHIBIT
CP	BACSIN
CSA	TARPOL
Infrastructure	EATRIS
Infrastructure	Infrafrontier
Infrastructure	ProteomeBinders
Marie Curie IOF	APPI
Marie Curie EST	MIDITRAIN
Marie Curie RTN	IMDEMI
NoE	Marine Genomics
NoE	EUROPATHOGENOMICS
NoE	MUGEN
NoE	CliniGene
NEST	PROBACTYS
NEST	EMERGENCE
STREP	Healthy-Water
STREP	ASSIST
STREP	EPI-VECTOR
STREP	FPLFEX
STREP	New TB Drugs
STREP	Fastest TB
STREP	PANFLUVAC

Graduate Schools	
International PhD Programme	“Infection Biology”
Marie Curie Graduate School	“MIDITRAIN”
International Graduate School	“Molecular Complexes”
DFG-Graduate School GRK 653	“Pseudomonas”
DFG-Graduate School GRK1273	“Chronical Infections”

Technology Transfer The HZI has a great potential for the development of innovative products, processes and services, especially in cooperation with industrial partners. Therefore, an important goal is to foster the transfer of research results into industrial applications through technology transfer. Thus, the establishment of spin-off and start-up biotech companies, licence agreements as well as service contracts with industrial partners are important elements for the transfer of R&D results. In order to further support technology transfer activities, the HZI is a member of BioRegioN and the “Transferkolleg Biotechnologie e.V.”.

The HZI-Biotech Campus In 2006 the HZI started with the construction of the new mouse house, which will be inaugurated in August 2009.

Intellectual Property Since 2002 the *Ascenion Ltd. Co.* offers services principally for the four Helmholtz Research Centres in the area of health care: GSF, HZI, MDC, and DKFZ. The headquarters are in Munich, but an office with two employees works on the HZI-Campus.

Ascenion Ltd. Co. manages the following areas for the HZI:

- Acquisition and management of intellectual property
- Evaluation of the commercial potential of an invention before patent filing
- Development and employment of strategies for the exploitation of the HZI patent folio

Biotech Fair on the HZI Campus On 18 September 2008 OMNILAB organized, with the support of HZI and DSMZ, its 5th biotech-fair and symposium in the FORUM. About 60 enterprises, the OMNILAB divisions, OAS, OCS, OMNICHEM and Jürgens Export, and the big research institutions of the region presented themselves to about 550 visitors, a new record. From Hannover and Magdeburg came about 150 visitors. More than 220 persons participated in 16 lectures, which gave insights into new developments in the biotech area. The next fair is foreseen to take place at the HZI facilities on 16 September 2010.

During the exhibition of the Biotech Fair organized by OMNILAB at the HZI FORUM in September 2008 Photo: OMNILAB

Biotech Network ASEAN-MERCOSUL – Germany The internet platform of the ASEAN-South American-German Biotechnetwork (www.asag-biotech.net) was reorganized from the beginning of 2008. Now it is possible to search for most of German institutes that are working in the biotech area, *sensu latu*. Furthermore, several institutes from Latin American countries as well as from ASEAN countries are listed. Also biotech-indutries and events can be searched for. The platform allows direct acces to the most important institutions in Germany and other countries, to organisations that offer fellowships, calls, and possibilities of networking.

The publication data basis of the Helmholtz Institutions, several patent data basis, some university libraries can be accessed easily. In 2008 HZI together with InWEnt and other partners organized several workshops on "Technology Transfer" and "Networking" in Bangkok, Hanoi, Manila, Jakarta, Kuala Lumpur and São Paulo. Except the workshop in São Paulo, they were supported by the Ministry of Economics, Labour and Traffic of Niedersachsen.

The participants of the actual InWEnt-HZI-course for Industrial Biotechnology (2008/9) in front of the Reichstag. The main purpose of the visit was to get insights into the work of the Bundestag (parliament) through a discussion with Dr. Carola Reimann, MdB from Braunschweig and who is a biotechnologist. *Photo: HZI, Jonas*

Open Day at HZI On Saturday, 9 May 2009, HZI opened its doors for everybody to have a look at the activities of the infection research centre. More than 1,400 visitors from the region came to get to know more details about infections, vaccines, biofilms, etc.

Prof. Kalesse shows what you can do with liquid air
Photo: HZI, Gramann

Crèche and Kindergarten for Children of HZI Employees The Helmholtz Centre for Infection Research (HZI) in cooperation with the Sterntaler Kindergarten in Braunschweig-Stöckheim offers childcare for those aged one year and older. At the end of 2008 eleven children were admitted to the kindergarten, four of them in the crèche and the others in the kindergarten.

Initiative „House of the little scientists" Since February 2008 once a month 2-3 PhD students are conducting small experiments for the children in the Kindergarten Sterntaler. Ten PhD students are involved in that project. The children should easily come into contact with the natural sciences through those experiments.

HGF-Mentoring Programme 2008 On 29 April 2008 the final event of the 3rd HGF-mentoring programme took place in Köln. The 4th programme (2008/9) started a month later. From the HZI one mentree as well as three female mentor and one male mentor are participating. Until now seven mentrees and nine mentors from the HZI have participated in all programmes.

Audit "berufundfamilie" The first annual report with regard to the audit "berufundfamilie" was presented on 25 November 2008. From 1 January 2009 an office has been installed at the HZI. The possibility of a short-term care for children of HZI employees is included.

Future Day for girls and boys In 2008 as well as 2009 seventy-eight pupils between 12 and 16 from various types of schools visited the HZI at the Future Day. In 2008 there came 49 girls and 29 boys, whereas in 2009 43 girls and 35 boys came to the HZI. They visited nine different departments and laboratories in the Centre.

Girls and boys are very curious to learn more about what HZI is doing in its research. *Photo: HZI, Krämer*

Personnel At the end of 2008 the HZI staff comprised 639 persons with full time and part time occupation. Additionally, 178 guests worked in various projects, receiving their payment from third parties. Along with 103 HZI-senior scientists, 219 PhD-students, nearly 100 guest scientists and 18 engineers were working at the HZI.

Boards and Assemblies of the HZI The boards and assemblies of the HZI are the Board of Trustees, the Supervisory Board, the Scientific Committee and the Managing Directors.

Board of Trustees The Board of Trustees is formed by the two trustees of the HZI, the Federal Republic of Germany and the State of Niedersachsen, represented by their respective departments, the Federal Ministry of Education and Research (BMBF) and the Finance Ministry of Niedersachsen.

Supervisory Board The Supervisory Board (SB) oversees the legality, expedience and economy of the management. It decides on general research goals, the principal research policy and financial affairs of the centre. It consists of a maximum of 15 members.

Scientific Advisory Committee The Scientific Advisory Committee (SC) consists of members of the Supervisory Board and external scientific experts. It advises the Supervisory Board with regard to the R&D programme as well as the general research strategy of the HZI.

Managing Directors The Managing Directors of the HZI: Research & Development: Prof. Dr. Rudi Balling Administration: Dr. Georg Frischmann

Prof. Dr. Rudi Balling (ri), Dr. Georg Frischmann (le)
Photo: HZI, Gramann

Scientists Assembly The scientists assembly of the HZI advises the Management in scientific matters. It consists of the heads of the departments and reseqarch groups and elected scientists, 33 in total. The Managing Directors, the heads of the sections and the junior research groups as well as a representative of the PhD-students are guests of the assembly. Chairman is Dr. Wolf-Rainer Abraham (since May 2003). Vice-chairman is Dr. Siegfried Weiß.

Direktorium The “Direktorium” advises the Managing Directors of the HZI in all important questions of the Centre. Members are the Managing Directors, the heads of the divisions and the independent departments, a representative of the junior research groups and the chairman of the Scientific Assembly.

Staff Council The Staff Council has certain consultation and co-determination rights in personnel and social questions. It consists of 11 members, elected by the HZI staff. Chairman is John Aubert.

Equal Opportunities Officer is Evelyn Rohn-Stenzel.

Members of the Supervisory Board (SB) and the Scientific Advisory Committee (SC), Status: 15.06.2009

Function	Name, Title	Organisation	Locality
Chairman SB	Lange, MinDir Dr. Peter	BMBF	Berlin
Vice-Chairman SB	Gevers, MinDirig Dr. Heiko	NMWK	Hannover
SB	Warmuth, MR Dr. Ekkehard	BMBF	Berlin
SB	Kuhny, Reg. Direktorin Corinna	Ministry of Finances State of Lower Saxony	Hannover
SB	Strätz, Dr. Michael	HZI	Braunschweig
SB	Weiß, Dr. Siegfried	HZI	Braunschweig
SB + SC	Zettlmeissl, Dr. Gerd	Intercell AG	Wien
SB + SC	Bitter-Suermann, Prof. Dr. Dieter	MHH	Hannover
SB + SC	Müller-Goymann, Univ.-Prof. Dr. Christel	MHH	Hannover
SB + SC Vice-Chairman SC	Schendel, Prof. Dr. Dolores	GSF – Institute of Molecular Immunology	München
SB + SC	Kurth, Dr. Bärbel-Maria	Robert-Koch-Institute	Berlin
SB + SC	Daniel, Prof. Dr. Hannelore	Wissenschaftszentrum Weihenstephan	Freising
SB + SC Chairman SC	Pfeffer, Prof. Dr. med. Klaus	Universitätsklinikum	Düsseldorf
SC	Hacker, Prof. Dr. Jörg	University	Würzburg
SC	Hackenberg, Prof. Dr. Regine	Technical University	Kaiserslautern
SC	Apweiler, Dr. Rolf	EBI	Cambridge
SC	Wilmanns, Dr. Matthias	EMBL	Hamburg
SC	Birchmeier, Prof. Dr. Walter	MDC	Berlin-Buch
SC	Hämmerling, Prof. Dr. Günter	DKFZ	Heidelberg

Chart of Organisation, Status 01 June 2009

Scientific Advisory Commitee (WK)
Prof. Dr. med. K. Pfeffer, Chairman

Supervisory Board (AR)
MinDir Dr. P. Lange, BMBF, Chairman
MinDirig H. Gevers, Niders. MWK, Vive-Chairman

Scientific Director (GFW) Prof. Dr. R. Balling
Administrative Director (GFA) Dr. G. Frischmann

Board of Scientific Directors (DR)

Staff Council (BR)
Chairman J. Aubert

Representative Body for Disabled Employees (VPS) B. Dojka

Assembly of Scientists (WV)
Dr. W.-R. Abraham

Occupational Safety Specialist (FaSi)
G.-W. Piehl

Equal Opportunity Commissioner (GB)
E. Rohn-Stenzel

Division of Cell and Immune Biology (ZIB)
Prof. Dr. J. Wehland

- Dept. Chemical Biology (CBIO) Dr. R. Frank
- Dept. Cell Biology (ZB) Prof. Dr. J. Wehland
- RG Cytoskeleton Dynamics (CYD) Dr. K. Rottner
- RG Microbial Communication (KOM) Prof. Dr. I. Wagner-Döbler
- RG Signalling and Motility (SIM) Dr. T. Stradal
- RG Immunoregulation (IREG) Dr. D. Bruder
- RG Cellular Proteom Research (CPRO) Dr. L. Jänsch
- JRG Chronic Pseudomonas Diseases (CPI) Prof. Dr. S. Häußler
- JRG Chronic Infections and Cancer (CHIK) Dr. L. Zender

Division of Molecular Biotechnology (MBIO)
Dr. H. Hauser

- Dept. of Genome Analysis (GNA) Dr. H. Blöcker
- Dept. of Gene Regulation and Differentation (RDIF) Dr. H. Hauser
- RG Molecular Immunology (MOLI) Dr. S. Weiß
- RG Model Systems of Infection a. Immunity (MSYS) Dr. D. Wirth
- RG Systems and Synthetic Biology (SSBI) Dr. V.A.P. Martins dos Santos

Division of Structural Biology (SB)
Prof. Dr. D. Heinz

- Dept. of Molecular Structural Biology (MOSB) Prof. Dr. D. Heinz
- RG Biophysical Analysis (BA) Dr. V. Wray
- RG Molecular Host-Pathogen Interactions (MHPI) Dr. W.-D. Schubert
- JRG Macromolecular Interactions (MMIA) Dr. C. Ritter
- JRG Structure-based Infection Biology (SBIB) Dr. T. Lührs
- RG Recombinant Protein Expression (RPEX) Dr. J. van den Heuvel

- Dept. of Microbial Pathogenicity (MPAT) Prof. Dr. G. S. Chhatwal
- RG Infection Immunology (INI) PD Dr. E. Medina
- RG Biodegradation (DEG) PD Dr. D. Pieper
- Dept. of Vaccinology and applied Microbiology (VAC) Prof. Dr. Dr. C.A. Guzmán
- RG Chemical Microbiology (CMIK) Dr. W.-R. Abraham
- JRG Phagosome Biology (PHAB) Dr. M. Gutierrez

- Dept. of Experimental Mouse Genetics (EMG) Prof. Dr. K. Schughart (Prokurist)
- RG Experimental Animals Unit (TEE) Dr. H. Riedesel
- RG Environmental Microbiology (UMW) Prof. Dr. K. N. Timmis
- RG Directed Evolution (DirEv) Prof. Dr. J. Collins
- RG Biologic Systems Analysis (BISA) Prof. Dr. R. Balling

- Dept. of Medicinal Chemistry (MCH) Prof. Dr. M. Kalesse
- RG Microbial Drugs (MWIS) Prof. Dr. R. Müller
- Dept. Experimental Immunology (EXIM) Prof. Dr. J. Hühn

- Dept. of Immune Control (IMMK) Prof. Dr. B. Schraven
- RG System-oriented Immunology and Inflammation Research (SIME) Prof. Dr. I. Schmitz
- Dept. Molecular Infection Biology (MIBI) Prof. Dr. P. Dersch

Scientific Groups

Abbreviations:

Dept	*Department*
RG	*Research Group*
JRG	*Junior Research Group*
PWC	*Scientific Controlling*
BCO	*Financial Controlling*
DMC	*External Funding Controlling*
DSB	*Data Security Official*

Scientific and Technical Services (WTD)
Prof. Dr. R. Balling

- Scientific Information (WI) Prof. Dr. R. Jonas
- Library (BIB) A. Plähn
- Computer Centre (RZ) Dr. N. Bedorf
- Safety and Environmental Affairs (SU) Dr. E. Grund

Administration and Infrastructure (VIN)
Dr. G. Frischmann

- Personnel Affairs (PA) J. Schinkel
- Financial Affairs (FA) H. Ohrdorf
- Purchasing Department (EM) B. Balster
- Legal Affairs (JUR) Dr. C. Kügler-Walkemeyer
- Patents (PS) D. Meseke
- Technical Services (TB) O. Rabe

Staff Units

- Public Relations (ÖA) H. Schlender
- Strategic Alliances and Cooperations (SAKO) I. Strebl
- Controlling (CO)
  - Dr. M. Strätz (DMC)
  - H. Ohrdorf (BCO)
  - Dr. R.-J. Müller (PWC)
- Organisation (ORG) N.N.
- Internal Auditing/SAP (IR/SAP) R. Lomberg(DSB)

Infrastructure

Further Impressions of the Open Day 2009

Photos: HZI, Gramann

HZI-InWEnt Workshops in 2008

Photos: HZI, Jonas

People at the HZI

Photo: HZI, Krämer

Photo: HZI, Krämer

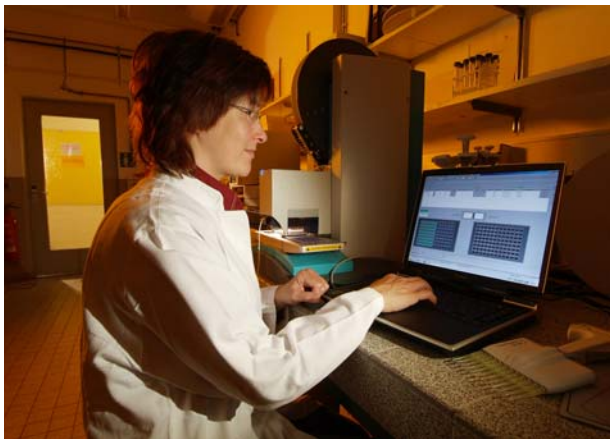


Photo: HZI, Bierstedt

Photo: HZI, Krämer

Photo: HZI, Kluge

Photo: HZI, Schlender

Research Report 2008/2009

Published by
Helmholtz Centre for Infection Research GmbH (HZI)
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Member of
Helmholtz Association of National Research Centres

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Translations: AD REM Sprachdienstleistungen
(Konstanze Plötz & Co-workers)

Photographs
The portraits and further photos were taken by
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Frank Bierstedt – pages: 57, 59, 69, 73, 74, 78, 82, 83, 85-87, 91, 92, 95, 101, 102, 104, 119, 121, 166
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Heinz Gramann – pages: 7, 18, 23, 39, 50, 68, 72, 81, 89, 111, 123, 124
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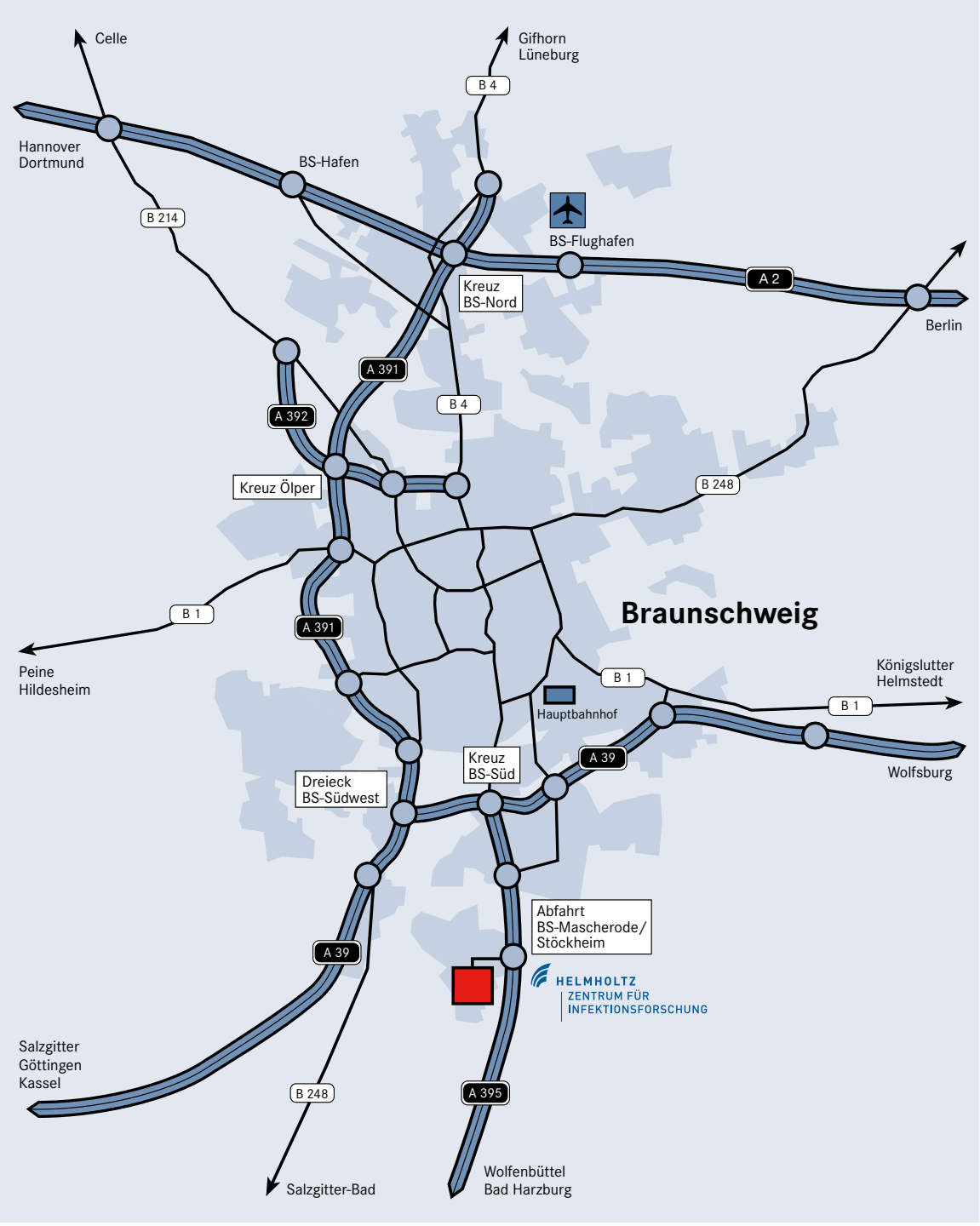
The following portraits were made available by the authors –
pages: 106 (Zender), 109 (Hühn), 110 (Dersch)
For all other photographs, the authors are mentioned below the legends.

Layout and design:
UNRUH Designbüro, Braunschweig
unruh@artmax.de | www.unruhdesign.de

Printed by:
Ruth Printmedien GmbH,
Hinter dem Turme 7, D-38114 Braunschweig
ISSN 1865-9713



Site plan of the Helmholtz Centre for Infection Research

ISSN 1865-9713

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