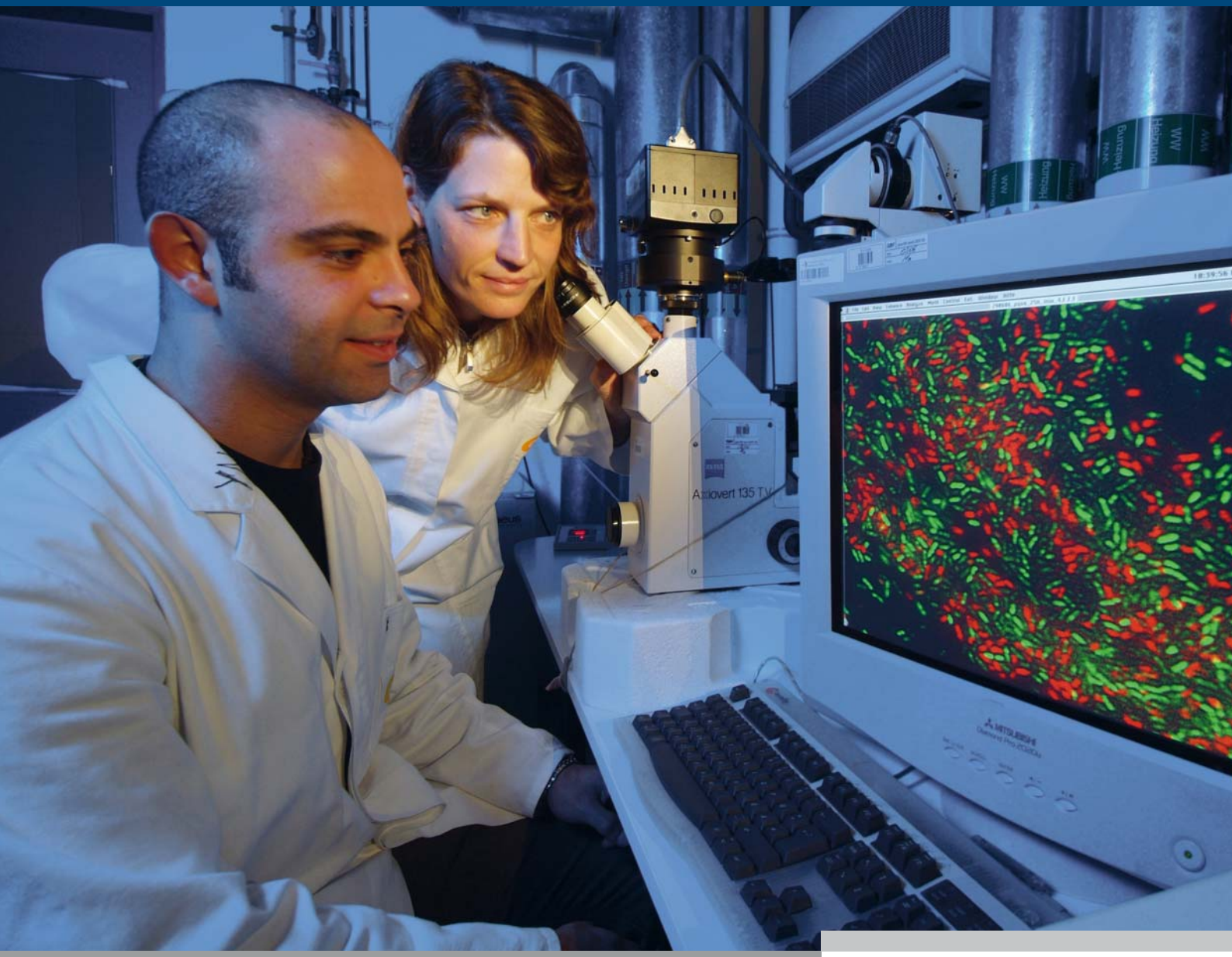
RESEARCH REPORT 2006 / 2007



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SCIENTIFIC REPORTS

FACTS AND FIGURES

The Helmholtz Centre for Infection Research

The Helmholtz Centre for Infection Research (HZI) is the new name of the former GBF, the German Research Centre for Biotechnology. Within the Helmholtz Association it was the first centre to include Helmholtz in its name. The Helmholtz Association of German Research Centres is Germany's largest non-university scientific organization.

The focus of our work is the study and investigation of pathogens which are medically relevant or can be used as models to study infection mechanisms. Ninety percent of the HZI funding is provided by the German Federal Government and the other ten percent by the State of Lower Saxony (Niedersachsen). The HZI has about 600 employees and an annual budget of about 50 million €.

Infections are responsible for a third of all deaths worldwide. Global mobility, international tourism and migration accelerate the spread of infectious pathogens. Because of growing antibiotic resistance, the susceptible immune systems during old age and the reappearance of nearly forgotten diseases, the development of new therapies and medicines to combat infectious diseases is urgently needed. Furthermore, recent research results indicate that infections also are responsible for triggering certain diseases, which were previously thought not to be connected to pathogens, such as cancer, diabetes or allergies.

Here are some of the scientific questions that we address in our work: What turns bacteria into a pathogen? Why are some people highly sensitive and others resistant to infections? How can we intervene in the infection process? Understanding these mechanisms will contribute to combating infectious diseases with new drugs and vaccines.

The human immune system can respond with amazing speed and precision to new pathogens and yet collapses occasionally under the attack by bacteria or viruses. How our natural pathogen defense system works and how these strategies can be used, or even improved, is the focus of HZI-research on the immune system. For this, we also study the immune response in mice, which are very similar to humans.

The HZI works closely with universities and other research institutes both at the national and international level. It is a member of the National Genome Research Network (NGFN). As part of an EU-funded programme for young researchers, the HZI, together with the Hannover Medical School, trains young scientists to become experts in infection research.

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Foreword

Prof. Dr. Rudi Balling | Scientific Director

People who visited the GBF five years ago and who comes back to the campus today may well have difficulties finding their way around. Besides there being a new entrance with a representative reception building, visitors will discover that we have a new laboratory complex in the former biotech innovation center and, a few steps beyond, are building a new animal house for some 30,000 mice.

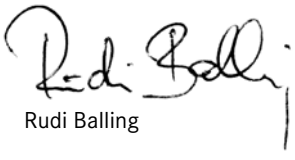
What is behind all these readily obvious changes? Well, for one thing, the GBF is now the Helmholtz Centre for Infection Research – both in name and content. Researchers here are investigating how infections unfold, how pathogens are constructed and how their hosts respond to an infection. All of these processes are analyzed at the molecular and cellular levels as well as in the context of the entire organism. Our goal is to develop new strategies for the prevention and therapies of infections to combat them when they occur.

At the moment much of our work is concentrated on the investigative chain “molecule-cell-organism”. Our new mouse house will be up and running in 2008. This new facility will open up entirely new opportunities for preclinical research. It will improve our ability to recognize the processes involved in an organism when an infection occurs. It will also help us understand why one individual has a highly sensitive reaction to a particular pathogen and the next person does not. This important basic research will help us establish a basis on which to build personalized infection medicine and it will help accelerate further developments toward eventual clinical testing.

At the other end of the research chain is the investigation of molecules; that is to say, their chemistry. The Helmholtz Centre for Infection Research possesses an especially valuable treasure: natural substances. They, in turn, can provide a nearly inexhaustible source of new substances that may be useful later on as drugs or vaccines against infections. These compounds, of course, need to be systematically studied to determine their chemical and biological characteristics. Chemists and biologists together have to study and explain the mechanisms that make these compounds effective and why they are interesting for infection research. We are currently in the process of setting up a “chemical pipeline” of analyses that can be used for everything from structural and cell biology to microbiology and preclinical testing.

All of the development opportunities at our center – whether big or small – face one tremendous challenge: the globalization of science. Our answer to this challenge can only be the internationalization of our campus. To this end, we are already successfully cooperating with world renowned research facilities, such as the Pasteur Institute and Rockefeller University, for whom we are an attractive partner. In addition, the Bill and Melinda Gates Foundation has entrusted us with an exciting research project to develop a vaccine against hepatitis C. Meanwhile, we are holding a guest professorship in China and together with the Helmholtz Association we have founded the first Indo-German Science Centre.

Developments like these must continue if we intend to maintain our high standards of research in the future. So, I‘m looking forward to greeting a growing number of new scientists from abroad at our Stöckheim campus and welcoming home our students and post docs from their research stints in the USA, Canada, Britain, France, India or China.



Rudi Balling

Photos: left: In the mouse facility. Animal care takers controlling the cages with the mice. | centre: Prof. Dr. Rudi Balling and Dr. Georg Frischmann, the directors hoisting the new flag of the Helmholtz Centre for Infection Research on 18 July 2006 | right: Prof. Dr. Rudi Balling together with representatives from the city and state during the symbolic groundbreaking ceremony for the new mouse facility. | *Photos: HZI, Bierstedt (le) | HZI, Gramann (ce) | Radde (ri)*

Studying Infection and Immunity in Animal Models at the Helmholtz Centre for Infection Research

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

Since March 2006, a new construction site at the Helmholtz Centre for Infection Research (HZI) will hardly go un-noticed by visitors and employees alike in the Southern part of our campus. Here, an extension to our animal facilities is being built, which will considerably strengthen our research programme on infection and immunity.

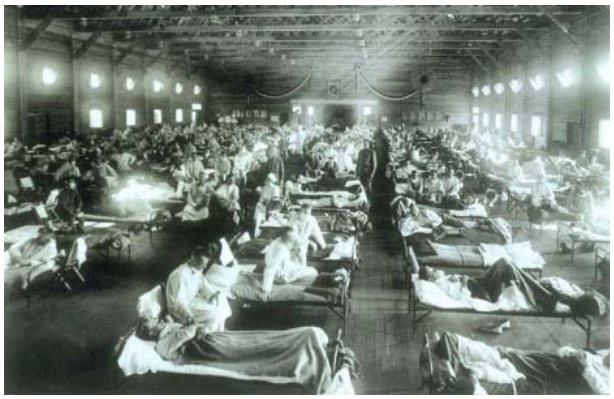
"€ 20 Million for 30,000 mice" was the headline in the local newspapers when the site was officially opened with a groundbreaking ceremony. Representatives from the scientific community, the federal and state ministries of research, and the city's mayor addressed the gathering.

But what exactly will this new building be used for? What scientific questions are to be addressed? Why are so many mice needed and which type of mice? How many people will be working here? These are only some of the questions that visitors and many employees of the HZI may be asking. In the following, I will try to provide some of the answers.

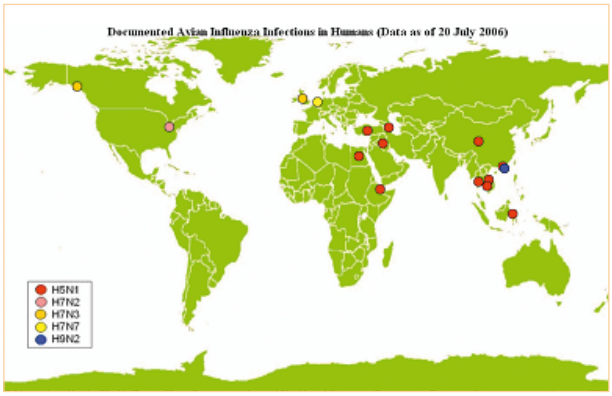
The new mouse facility on the HZI campus: upper photo: During the excavation | lower photo: Construction of the basement.
Photos: HZI, Müller

Background One of the most important determinants of human disease is genetic predisposition. The establishment and progression of many diseases, like neurological and cardiovascular disorders, abnormal immune responses, and in particular, infections, are to a large part determined by variations of multiple genetic *loci* in affected individuals. Therefore, we will need to achieve a much better understanding beyond our currently very limited knowledge of genetic diversity in populations and of the complex, multi-level relationships between genetic variation, the environment and disease phenotypes.

These studies require sophisticated animal models because, for obvious reasons, they cannot be performed on humans. Especially, we will need models over which we have tight genetic, environmental and experimental control and which have a genetic complexity matching that of human populations. Amongst the various animal models available, the mouse represents the best experimental system because its biology and genetics are very well characterized and powerful resources for systematic approaches are available. In particular, a large number of mouse strains has been established that are inbred and therefore genetically identical within a given strain, but differ between strains. Also, several thousand lines of mouse mutants currently exist in the scientific community which carry a deletion or modification in a given gene *locus*. This enormously rich resource allows us to mimic human genetic diversity and to study the *in vivo* function of a given gene in different experimental settings.



The influenza pandemy 1918/19 caused the death of approximately 50 Million people. The photo shows an emergency camp in Funston, Kansas, USA. *Courtesy of the National Museum of Health and Medicine, Armed Forces Institute of Pathology, Washington, D.C. (NCP 1603).*



Cases of human infections with bird influenza A subtypes H5, H7 & H9 *Source: WHO*

Mouse model systems have been used very successfully in the past to determine genetic regions and genes involved in infection susceptibility in humans. The analysis of mouse models for infectious disease in humans will thus lead to important insights into the mechanisms of host-pathogen interactions and the immune response in humans. And the results of this research will allow us to design new therapies and methods for the diagnostics and treatment of infectious diseases in humans.

Infection research in the mouse At the HZI, many basic mechanisms are being studied in cell free systems and in cell cultures. They form the basis for more comprehensive studies at the level of the whole organism. Only here, it is possible to investigate the complex multi-level interactions of a pathogen with its host, which involve numerous organ systems and cell types and complex communications between them. For example, during an infection, the host defense employs cells in the periphery (skin, lung, GI-tract, *etc.*) that sense the presence of pathogens and then send signals to other immune-competent organs. These signaling molecules will trigger immune cells in the blood stream to invade the tissue and destroy the pathogen and infected cells. Other immune cells take up the pathogens and their proteins to transport them to the lymph node or spleen and present them to immune effectors cells, which then produce antibodies or generate highly selective killer cells.

Mice are ideal experimental systems to investigate these processes. The immune system of the mouse has been studied for decades and many important findings that are relevant to humans have been discovered in the mouse laying the foundations for new immune therapies in humans. The genome of several inbred mouse strains has been completely sequenced and many mouse mutants which carry defects in single genes have been generated and are being shared by the scientific community world-wide. Most importantly, the comparison to the human genome reveals that 99% of the genes in the mouse have a homologue in humans, meaning that for almost every human gene a corresponding mouse gene exists.

We can thus expect that studies in mice will allow us to understand the principle mechanisms of pathogen-host interactions, point to the gene circuits involved and allow us to test new intervention strategies. In some cases, there will not be a one-to-one correspondence of mouse and human genes, but the underlying regulatory circuits will be the same, even if the individual players are somewhat different.

Many of the research projects at the HZI involving mouse models are concerned with studies on the genetic susceptibility of infections and the basic understanding of immunity. These projects aim to develop better therapies for the diagnosis or treatment of infectious diseases in humans. For obvious ethical reasons, these studies cannot be performed in humans. In addition, humans are genetically very diverse and numerous environmental factors influence the outcome of a disease or predisposition to it, making it very difficult to unravel the underlying biological and molecular principles.

The mouse integrated resource for infections and immunity research (Mirii) Almost all scientific research projects at the HZI require mouse model systems. These include activities supported by in-house funding of the Helmholtz Programme-oriented Research (PoF) as well as projects funded by the German Ministry of Research and Education (BMBF), the German Research Foundation (DFG), the German National Genome Research Network (NGFN), the EU framework programmes FP6 and FP7, and the Bill-and-Melinda Gates Foundation (BMGF).

Therefore, at the HZI, we have established an integrated scientific concept for using mouse model systems to study infectious diseases and immune responses: the mouse integrated resource for infections and immunity research (Mirii). The Mirii concept comprises high quality facilities, well trained animal caretakers, scientific know-how and a large resource of mouse lines and mutants. Mirii will be continuously developed further to fulfill the current and future needs of the different research projects at the HZI.

We are constantly improving the existing infection models with respect to application methods or parameters to measure the course and outcome of an infection. In addition, we are constantly adding new pathogens and application methods to our repertoire of expertise. As a result, the HZI is one of the very few distinguished research centres in the world with a broad expertise and the necessary infrastructure allowing high-quality research in the field of infectious disease.

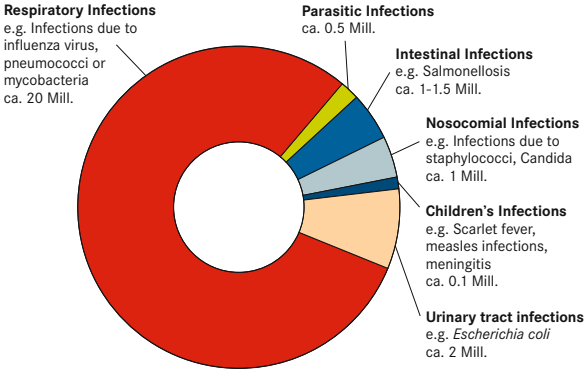
In the following, I will briefly describe some of the scientific questions that we are currently addressing at our research centre. More detailed information is given in the individual project description throughout this report.

Genetic susceptibility to infections The research groups "Infection Genetics (ING)", "Infection Immunity (INI)" and "Experimental Mouse Genetics (EMG)" are interested in understanding genetic factors contributing to the susceptibility of the host to bacterial and viral infections. These activities are funded by the PoF-Programme of the Helmholtz-Association, grants from the German Ministry of Research and Education (BMBF), the National Genome Research Network (NGFN), the European 6th framework programme, and several other external research programmes from public and private sources.

In most cases, the genetic factors that contribute to disease susceptibility in humans are not Mendelian traits (single gene *loci*) but are complex (quantitative, multi-genic) traits. Complex traits are also the basis of many genetic diseases, or genetic predispositions in humans, e.g. obesity, or cardio-vascular, infectious and auto-immune diseases. These traits are extremely difficult to study in humans since phenotypic variability is not only influenced by genetic background but also by environmental factors. The contribution of the latter is only poorly defined in humans and thus generates considerable "background noise", making it often impossible to detect the relevant genotype-phenotype correlations. For the mouse, many diverse genetic populations are available in a reproducible fashion and, therefore, the genetic as well as the experimental parameters can be well controlled and hypotheses on the contribution of single or multiple gene combinations can be experimentally verified.

The currently ongoing research activities in determining genetic susceptibility at the HZI involve infection models with bacterial and viral pathogens.

Streptococcus pyogenes and *Staphylococcus aureus* are Gram-positive microorganisms that are capable of causing a wide spectrum of infections, ranging from mild to very severe diseases. If such an infection is not controlled efficiently in an infected host, it will very rapidly result in a fatal septic shock. Researchers at the HZI have discovered that genetically different populations of mice responded very differently to an infection with these pathogens. And further genetic studies revealed that three regions in the genome are



Infectious diseases in Germany, cases per year

Source: PathoGenoMik Report 2003

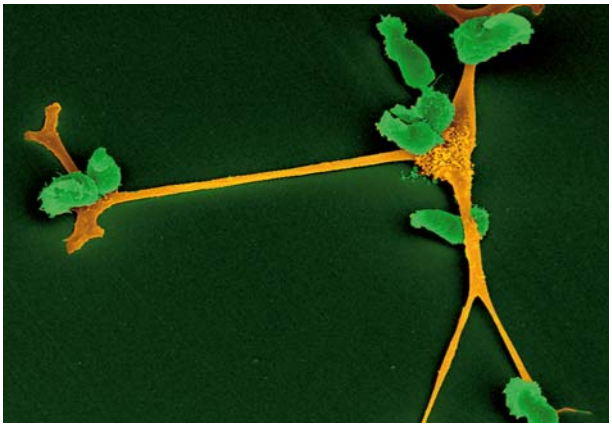
contributing to the susceptibility of the host. These results will lay the basis for subsequent studies to determine the molecular mechanisms leading to a septic shock in humans.

Listeria monocytogenes can cause severe encephalitis in humans. At the HZI, studies in mice have shown that there is a pronounced sex difference in the susceptibility to these pathogens. Females, which are in general more resistant to infections, are highly susceptible in this model to an infection with *L. monocytogenes.* HZI researchers demonstrated that this is mainly due to the production of a particular immune hormone (IL10) which appears to repress the immune response in females.

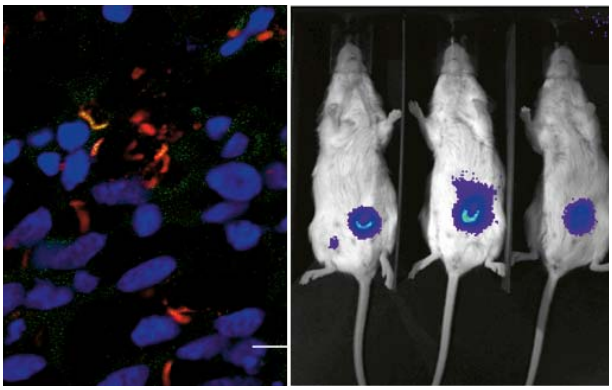
Infections with influenza A viruses represent a devastating disease, causing each year severe illness in about 500 million people worldwide and one million in Germany. About 400,000 people worldwide and 8,000 people in Germany die each year as a result of an infection with influenza A virus. Although the pathogen is studied in great detail by many laboratories around the world, very few have paid attention to the genetics of the host. At the HZI, we have begun to study the response in genetically diverse mouse populations to an influenza A infection. The aim of these studies is to identify the genetic factors that influence the course and severity of an influenza infection in humans.

Establishing immunity The immune system is a highly dynamic cellular network that has evolved to protect the host from a wide variety of foreign, potentially harmful, microorganisms. Its response involves many different cellular components, short- and long-range signaling molecules, effector molecules and cells. To dissect the various components and their molecular basis is one of the most active areas of research performed in the groups “Mucosal Immunity (MI)”, “Immune Regulation (IREG)”, “Experimental Immunity (EI)”, “Molecular Inmunity (MOLI)”, “Immune Dynamics (ID)”, and “Vaccinology (VAC)” at the HZI.

To avoid self-destructive immune responses, one of the major tasks of the immune system is to discriminate between self- and non-self. Peripheral tolerance to self antigens, in addition to clonal deletion and induction of anergy, involves active suppression mediated by regulatory T cells. Therefore, researchers at the HZI study intensively the role of regulatory T cells in the course of infections, but also in the context of auto-immune diseases. They could show in mouse models of experimental type II diabetes that the activation of regulatory T cells can inhibit the self-destruction of pancreatic island cells.



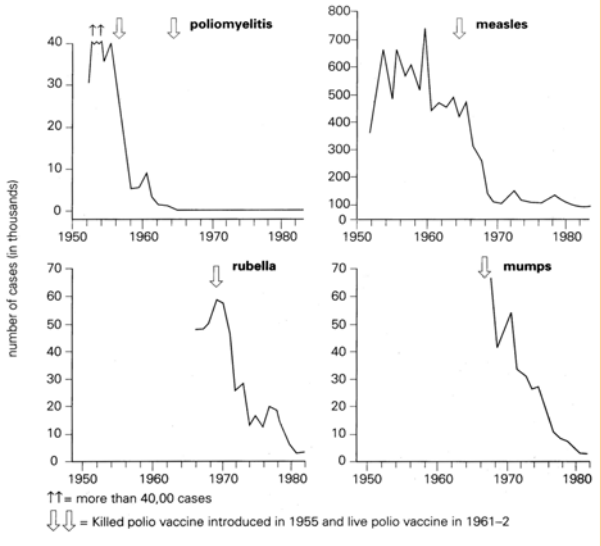
Initiating the immune defense: T cells are being prepared by a dendritic cell for their fight against pathogenic invadors.
Photo: HZI, Rohde



Live imaging of mice. Tumours that were colonized with Salmonella typhimurium *carrying inducible fire fly luciferase were analysed by immunohistology (left). Bacteria are in red, cell nuclei are stained blue. Monitoring tumour colonizing bacteria by* in vivo *imaging (right). Tumour-bearing mice were injected with Luciferase expressing bacteria. These bacteria can then be visualized in a non-invasive fashion within the tumour in life mice.* *Photos: HZI, Westphal, Löβner*

To be able to respond appropriately to the intrusion of a pathogen, the immune system consists of a hierarchical structure of defense mechanisms. The first line of defense is aimed at limiting the systemic spread of the pathogen. It also activates the second defense line which results in the establishment of the long-lasting adaptive immune response. Cytokines – small molecule secreted by the first line immune cells – are crucial during these reactions. Several research groups at the HZI are studying the molecular and cellular effects of cytokines in the mouse in the context of inflammatory bowl disease and infections with *L. monocytogenes and Yersinia enterocolytica.*

The ultimate result of a successfully defeated pathogen is the establishment of immunity in the host. An infection itself will cause immunity, but more importantly, immunity can also be induced through the application of a vaccine. In fact, vaccination has been the most successful medical treatment in the history of mankind to protect people from acquiring an infectious disease. Smallpox has been eradicated and a worldwide programme for measles vaccination has saved the lives of millions of children. Vaccines contain a molecular structure that is specific to the pathogen and include a so-called adjuvant which is needed for the stimulation of an immune response. Researchers at the HZI are studying the induction of immunity in mouse models using different methods of application and testing new strategies as well as new chemical entities for adjuvant activity. For the latter, a new adjuvant, MALP-2, has been identified from the rich source of natural products discovered at the HZI.



The decrease of viral infections after the introduction of vaccination programmes in the USA. Reprinted from "Medical Microbiology", 2nd edition (C. Mims et al, eds.), Mosby publishers, 1998, p.444, with the permission from Elsevier.

Despite the high similarities in the immune responses and gene regulatory networks of mice and humans, there are, of course, differences (*e.g.*, MHC restriction). In addition, some host-restricted pathogens will only infect and replicate in humans. This is the case for the hepatitis C virus (HCV), which represents an enormous health problem worldwide. Infected individuals will often be lifelong carriers of the virus with the high associated risk of chronic liver disease and hepatocellular carcinoma. Vaccines are not currently available, the lack of appropriate animal models being a major bottleneck for their development. To address this need, the Bill & Melinda Gates Foundation is financing a research project at the HZI and its partner institutes in the context of the Grand Challenges in Global Health initiative. The activities are focusing on the development of mice which are able to sustain the generation of the human immune system as well as the efficient engraftment of human hepatocytes. It is expected that these mice will greatly facilitate the development of vaccine candidates against diseases caused by HCV and other hepatotropic pathogens.

The animal facilities For the described research activities, it is necessary to maintain many different mouse strains and mutants in a clean and well-controlled environment. At the HZI, this is ensured by specifically dedicated buildings and, more importantly, a team of well trained and highly motivated animal caretakers.

The personnel The animal facilities and associated sevices are managed by the "Central Animal Facility" (TEE) which belongs to the department of "Experimental Mouse Genetics" (EMG). The head of the group, Dr. David Monner, and his team of currently 22 animal caretakers and cleaning aids, is responsible for the general management and maintenance of the unit. The excellent in-house training of the staff and their dedication to serving our scientists, as well as maintaining the general infrastructure and hygiene status, is one of the most important factors in guaranteeing the best environment for high quality research at the HZI.

Insights into the current mouse facility: upper photo: Animal care takers preparing clean cages | lower photo: Controlling the cages and their inhabitants. *Photos: HZI, Bierstedt*

The team is responsible for the routine animal care, like changing mice into new cages, a task which is performed at least once a week. They set up matings for the maintenance and expansion of mouse strains, wean the offspring, and enter all data into a central database. In addition, the TEE team assists researchers with expertise on animal care and performs routine sampling of tissues and injections.

The people from the TEE-team also help with the import and export of mice, and Dr. Monner assists the researchers at the HZI as the officer for animal ethics (Tierschutzbeauftragter) in filing applications and following up of animal experimentation permits in accordance with the German law.

In addition, special services are offered on request, like re-derivation using embryos generated either conventionally or by *in vitro* fertilisation (IVF) and strain archiving by sperm and embryo cryopreservation.

The mouse resources The different laboratory strains of mice that are currently maintained in research institutes and breeding facilities of the HZI exhibit a genetic diversity that is comparable to the contemporary human population. They were derived from a mixture of the *Mus musculus musculus,* an Asian *Mus musculus* subspecies, and *Mus musculus domesticus*, the European/North American/African *Mus musculus* subspecies. Members of different subspecies can interbreed and it is therefore possible to generate new strains with genetic mixtures of European and Asian subspecies. In addition, some inbred laboratory strains have been derived from catches in the wild which represent other mouse species, *e.g. Mus spretus, Mus hortulans, Mus pahari.* Breeding a *Mus musculus* strain with *Mus spretus* generates offspring will cover a genetic diversity range that would correspond to the genetic diversity between a *Homo sapiens* and *Homo neanderthalensis.*

In experimental research, inbred mice are mostly preferred. They are derived from continuous brother-sister mating over at least 20 generations, resulting in families of mice in which all individuals are genetically identical. At present, about 100 different inbred mouse strains have been generated in different laboratories around the world representing a very rich resource for genetic and functional studies.

To make this resource available for the different ongoing research projects in our centre, several mouse inbred strains, about 20 at present, are maintained as two to three generation families at the animal facility of the HZI. A minimum of about five cages is required to keep a live colony of a particular strain.

For infection experiments, the colonies have to be expanded. For one infection study, groups of about 6 to 10 mice of the same age, sex and strain are required for each dose of infectious pathogen to obtain statistically significant results. In general, between three to five doses of a given pathogen are delivered. Most of the mice needed for experimentation are bred in our facilities.

Modern mouse technology allows to specifically inactivate a given gene in the genome and study its functional role during an infection or the immune response. At the HZI, we are currently keeping about 60-80 different mouse mutant

strains carrying gene mutations, or variations thereof. But, as in humans, a given gene mutation may be influenced by other gene defects or variations of the general genetic background. Since these genetic interactions are also very important denominators of infection susceptibility and immunity, we are also generating new families by breeding these mutations into different mouse inbred strains.

Within our present animal facilities, we can perform infection experiments at the bisosafety level BSL2 (S2 according to the German gene technology law). The BSL3 laboratory and animal rooms in our new animal facility (see below) will provide the high security level infrastructure to also investigate important viral and bacterial pathogens, such as Hepatitis C virus, high pathogenic avian influenza or mycobacteria.

The environment To perform reproducible studies with mice, not only the genetic background but also the environment has to be well controlled. For mouse colonies, the most important environmental factor is their hygiene status. The Specific Pathogen Free (SPF)-status is an internationally accepted standard for the health status of laboratory animals. This status is defined and continuously reviewed by the Federation of European Laboratory Animal Science Associations (FELASA). At the HZI, we keep all our mice at this SPF-hygiene status.

To maintain the SPF-status, all the mice in our facility are kept in a special micro-environment, so-called Individually Ventilated Cages (IVC). IVC cages carry an individual air intake and outlet nozzle. The incoming and outflowing air is passed through particle-tight filters, guaranteeing that sterile air enters the cages and pathogens are filtered out from the exhaust air. In this way, each cage represents an isolated unit in itself, avoiding cross contamination and protecting the environment and people from the pathogenes.

A routine health monitoring programme has been put in place as a quality control system to follow the health status of all the mice in the different HZI facilities. In addition, mice from other laboratories with a lower hygiene status, *e.g.* those infected with a murine viral or bacterial pathogen, will not be allowed to enter our facilities. If such strains are absolutely necessary for our research projects, we will receive and keep them in a small, specialized quarantine unit which is far away form the other facilities and managed completely independently. From this unit, it is then possible to sanitize the imported mouse strains via embryo transfer into the central facilities. Embryo transfer allows us to free the offspring from all infectious agents.

The existing buildings The present animal facilities at the HZI consist of three units: a main building (Building T), the infection unit (D-Annex), and in a separate building, the quarantine unit (Building K). Each of the units has its own infrastructure of cleaning kitchens, barriers and animal caretaker teams. In addition to SPF barrier housing and infection experiments, space is also provided for the generation of mutant mice.

Building T consists of three laboratory and 11 animal rooms on two floors. It has a total capacity of about 3,300 cages or 7,000-10,000 mice and is mainly used for maintenance of inbred strains and mutant lines.

Infection experiments up to Biosafety level 2 (or S2) are performed in the D-Annex. This unit went into service in October 2003, and has a capacity of about 1,700 cages or 4,000-5,000 mice – all in one room.

The quarantine unit has a very small capacity of a few hundred cages and is only used for the sanitation of mouse strains.

The new mouse facility under construction The HZI originally began as the German Research Centre for Biotechnology (GBF) in 1965. When the focus of the centre changed in the year 2000 to infection research, the infrastructure also had to be adjusted to fulfill the needs of a modern health research centre. One of the most important aspects in health research is to validate biological mechanisms and new treatment strategies *in vivo*, in the whole organism. Therefore, the existing mouse facilities had to be extended to serve the needs of the new research programmes.

This urgent requirement was also recognized by an international board of expert scientists in mid-2003, during the evaluation of the HZI's scientific programme. The board recommended the building of a new mouse facility.

The planning of a new facility started shortly thereafter. A group of experts from the HZI consisting of the head of the TEE, the head of the Technical Services, two scientists, and members of the HZI management designed the first concepts which were discussed and approved by the German Ministry of Education and Research (BMBF) in January 2004. During the year 2005, the planning group and a team of architects further defined the details of the construction plans which were then approved by the BMBF and the Ministry of Economy and Culture (MWK) of the State of Lower Saxony in January 2006; construction of the building began in March of 2006. The current timetable foresees the new facility going into operation in July 2008.

Upper photo: 30 October 2006: The new mouse facility under construction. *Photo: HZI, Jonas* | *lower photo: 27 March 2007: The concrete building of the new mouse facility is finished.* *Photo: HZI, Bierstedt*

The new building will provide the urgently needed expansion of space for experimentation with mice in order to perform *in vivo* validation experiments in the context of the current PoF-programme and the many externally funded projects at the HZI. The present facilities do not provide enough space to maintain and breed the many strains and mutant lines of mice needed for the identification of new genes involved in susceptibility to infection, the analysis of the immune system, the validation of new vaccines, or alternative treatment and prevention strategies. In addition, the new facility will have a special unit with laboratories and animal facilities at biosafety level 3 (BSL 3), which is presently not available on campus.

The design of the new facility follows the general principle of the other animal facilities at the HZI: SPF hygiene status and barrier-maintained mouse colonies. On a total area of about 2,000m², the new facility will provide space for about 10,000 cages of mice, offices for staff and technicians, and a meeting room on the ground floor. The basement and half of the 4th floor are completely occupied with technical rooms to maintain the proper ventilation, temperature and humidity. Several autoclaves and sterilization locks ensure that all equipment and material enters germ-free into the facility. Personnel entering the facilities behind the barriers will change clothes in specific locks and pass through air showers to make sure that no infectious material is introduced.

To ensure proper maintenance of the mouse colonies, general management of the facility, and support for scientists, about 30 people, animal caretakers, cleaning personnel, technical personnel, and scientists, will be working in the new facility.

Concluding remarks In summary, the sophisticated technical and architectural infrastructure, dedicated animal caretakers, highly experienced scientific research groups and special mouse strain resources, all focusing on infection research, make the HZI a very unique and well suited place for experimental and preclinical infection research.

Acknowledgements I would like to express my many thanks to all the people (the project team, architects, animal caretakers and many more) who have been involved in establishing and running the current animal facilities and designing as well as realizing the new facility. Our special thanks go to Dr. David Monner, who will retire in 2007, for all his dedication and hard work as the Head of the Central Animal Facilities.

Klaus Schughart born in 1956, studied biology at the University of Köln and received his PhD at the Institute of Genetics in 1986. 1987-1989 Postdoctoral Fellow at Yale University, New Haven, USA. 1990-1994 Research group leader at the MPI of Immunobiology, Freiburg. 1994 Habilitation in Genetics, University of Freiburg. 1995-1996 Research group leader at the GSF, Munich. 1997-2001 Head of the Department of Molecular and Cellular Biology, Transgene S.A., Strasbourg, France. 2002-2006 Head of Research and Development and the Scientific and Technical Services at the HZI. Since 2006 Head of the Department of Experimental Mousegenetics at the HZI and joint professorship at the University of Veterinary Medicine in Hannover.

Highlights 2005-2007

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Medal for Research into Soil Bacteria Gerhard Höfle and Hans Reichenbach were honoured for their scientific achievements and presented with the Hans Herloff Inhoffen Medal in March 2006. Höfle and Reichenbach have both worked for many years at the Helmholtz Centre for Infection Research (then the GBF) on myxobacteria, which live in the soil, in order to develop uses for the benefit of mankind. In their research into this microorganism, Höfle, Reichenbach and their team discovered a cancer drug – epothilon – which is currently undergoing clinical testing. The prize, awarded each year by the Association of the Friends of HZI, is to be presented at the annual Inhoffen Lectures, a public event, jointly sponsored by the Helmholtz Centre for Infection Research and Braunschweig Technical University.

During the ceremony for the Hans Herloff Inhoffen Medal: Prof. Balling is giving his congratulations to Prof. Höfle. From left to right: Prof. Joachim Klein, Dr. Mark Ergezinger (winner of the Fritz-Wagner Prize), Prof. Gerd Höfle, Prof. Hans Reichenbach, Prof. Rudi Balling, Silke Wenzel and Annika Steffen (both winner of the doctorate prize) *Photo: HZI,Sierigk*

Experts Help to Explain Influenza The Helmholtz Centre for Infection Research invited experts from around the world to attend a symposium on the influenza virus. The special guest at this "Afternoon on Flu" on 2 March 2006 was Prof. Adolfo Garcia-Sastre from the Mount Sinai School of Medicine in New York. The virologist is credited with reconstructing the pandemic 1918 "Spanish Flu" virus from frozen historical tissue samples, bringing them back to life in the laboratory. The symposium, organized by Dr. Siegfried Weiss and Dr. Sabine Kirchhoff, found a broad echo in the media with reports appearing in *Die Welt, Berliner Morgenpost, Technology Review, stern.de* and *Yahoo,* among others.

Prof. Dr. Adolfo Garcia-Sastre from the Mount Sinai School of Medicine, New York, during his visit at the HZI FORUM
Photo: HZI, Hübner

Foundation-Laying for the New Animal House Together with special guests, the directors of the Helmholtz Centre for Infection Research, Prof. Rudi Balling and Dr. Georg Frischmann, held a symbolic foundation-laying on 14 March 2006 for the new animal facility. The new building will house the mice used by Helmholtz scientists for their research into the mechanisms of infectious disease. The information obtained from these studies will form the basis for new diagnostic and therapy procedures in the fight against pathogenic bacteria and viruses.

During the Foundation-laying ceremony for the new mouse facility *Photo: HZI*

International Cell Biology Conference in Braunschweig Some 500 scientists from home and abroad met for the annual meeting of the German Society for Cell Biology (DGZ) in Braunschweig in March 2006. The host of the conference was Braunschweig Technical University. Local coordinator of the meeting was Prof. Jürgen Wehland, Head of the Department of Cell Biology at the Helmholtz Centre for Infection Research. The majority of the approximately 60 invited speakers came from abroad. The annual meeting's opening lecture was given by the American researcher, Prof. Jennifer Lippincott-Schwartz from the National Institute of Health in Bethesda, Maryland, USA. Lippincott-Schwartz is one of the leading researchers in the area of protein visualization in individual living cells. The German Society for Cell Biology, founded 30 years ago, is an organization with some 1,300 biochemists, geneticists, molecular biologists, medical doctors, zoologists and botanists in the German-speaking world.

The Carl Zeiss Lecture was given by Prof. Jennifer Lippincott-Schwartz (National Institutes of Health, Bethesda, USA), one of the leading scientists in the field of intracellular protein dynamics. *Photo: HZI, Haas*

Award-Winning Vision of "Cell Laboratories" The future of biomedical research could look something like this: a single cell precisely targeted with wafer-thin ‚nano needles' under continuous surveillance by analysis chips and high-performance microscopes. For this visionary model of the "lab-in-a-cell", the immunologist, Prof. Jan Buer, was awarded first prize and €10,000 in the contest "Imagining the Future" at a special ceremony in Starnberg, Bavaria. The worldwide competition was sponsored by Roche Applied Sciences, a unit of the Roche Pharmaceutical Company. Prizes were awarded to the best scientifically-based forecasts for the future of biomedical research and its applications for the development of new drugs and diagnostics. Prof. Buer is a researcher at the Helmholtz Centre for Infection Research and teaches at the Hannover Medical School (MHH).

Prof. Dr. Jan Buer (ri) receiving the cheque for the 1st prize of the "Imagining the Future" competition *Photo: Roche*

"Girl's Day" – This Time for Boys, Too The Helmholtz Centre for Infection Research opened the doors of its laboratories for curious youngsters once again for Germany's nationally observed "Girl's Day". Female students between the ages of 14 and 16 had the opportunity to learn more about careers available at a large research centre. A new aspect was introduced this year: for equality's sake, a parallel project day called "New Paths for Boys" was held and naturally, the Helmholtz Centre was open to them as well. The two events were integrated into a single, joint programme.

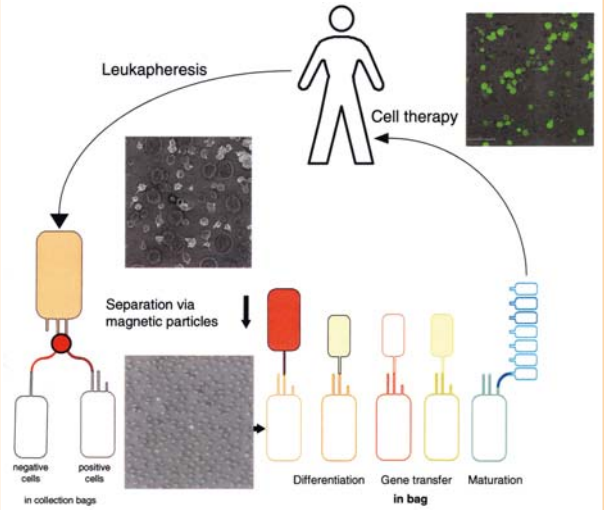
Pupils from schools getting deeper insights into the work in a laboratory at the HZI during the Girl's Day/New Paths for Boys *Photo: HZI*

Funding and Honours for Braunschweig's Student Laboratory The Biotechnology Student Laboratory, BioS, on the campus of the Helmholtz Centre for Infection Research, is among the best in Germany – especially in the area of teacher training. This was the conclusion of an independent evaluation committee, which assessed student labs on behalf of the Leibniz Institute for Educational Training in the Natural Sciences (IPN) in Kiel. As a result, BioS was awarded a grant of €25,000 in May 2006, which in turn guaranteed that a large-scale teacher training project envisaged for the following year could go ahead as planned. Since 2002, BioS has been open to secondary school students in the upper grades, who are interested in complex experiments. Students are able to learn about genetic and immunological work in BioS courses which have more advanced equipment than is available in ordinary schools.

Prof. Dr. Ralf Mendel (ce) from the TU Braunschweig in discussion with Arntraud Meyer (le) and Dr. Iris Eisenbeiser (ri) from the Biotechnology Student Laboratory, BioS
Photo: HZI, Ammerpohl

An Alliance for Antibody Production New, improved production methods for developing antibodies – this is the goal of a cooperative effort between the Helmholtz Centre for Infection Research and Roche, the international pharmaceutical company. In cooperation with Roche, Helmholtz scientists are studying those animal production cells responsible for generating antibodies. In a large number of test runs, the researchers hope to find out in which culture media and under what conditions these cells grow best. Additionally, it aims to improve the economies of scale and the total yield from production. Antibodies are widely used in medicine. These protein molecules, which serve as a defensive "weapon" in the human body against the invasion of disease-causing pathogens, exhibit very specific binding behavior. This is why they can be used to target other molecules and to neutralize them.

Helpful Cells for Modern Medicine "Therapies using living cells" was the topic of a medical conference for doctors and researchers from Germany and abroad that was held in Braunschweig in June 2006. The event, called "Innovative Cellular Therapies on the Way to the Clinic", was sponsored by the Helmholtz Centre for Infection Research and the Professional Association of German Transfusion Doctors (BDT), represented by physicians from Braunschweig Hospital. The conference was held to mark the conclusion of a research project, coordinated by Helmholtz scientist Dr. Hansjörg Hauser and funded by Germany's Federal Economics and Technology Ministry, which studied the extraction of immune cells for tumour therapies.



A closed bag system was developed for GMP-compliant production of dendritic cells for immunotherapy. From leukapheresis samples of patients progenitor cells can be isolated, differentiated and genetically modified. Portions are frozen in a formulation suitable for therapeutic application.

European Experts Honour HZI-Researcher Dr. Theresia Stradal was honoured with a European-wide award for her work on signal molecules in mammals. Dr. Stradal, the leader of a scientific working group at the Helmholtz Centre for Infection Research, received the "FEBS Letters Young Investigator Award" worth €10,000 at a ceremony in Istanbul on 26 June 2006. The prize is awarded annually by the Federation of European Biochemical Societies (FEBS) for the best scientific paper by a young investigator published in the group's magazine, FEBS Letters. It was awarded at the Federation's annual meeting.

FEBS is the umbrella organization for European bioscience societies with more than 40,000 members in 36 groups and six associated organizations across Europe.

Dr. Theresia Stradal *Photo: HZI, Hans*

A New Name for the GBF: Helmholtz Centre for Infection Research On 18 July the former German Research Centre for Biotechnology (GBF) was given a new name. From that day onwards, the institute is known as the Helmholtz Centre for Infection Research. The switch was made to better illustrate the scientific focus of the centre. A second aspect of the name change was to make the brand „Helmholtz Association“ more recognizable in Germany and the scientific community worldwide. The Helmholtz Association is Germany‘s largest scientific organization and, besides the Helmholtz Centre for Infection Research, includes 14 other research centres with a total of 25,000 employees. In conjunction with the new name, a new logo has been designed. The three arcs represent the three core elements of the Helmholtz strategy: essential contributions to important scientific fields, research with advanced technologies and complex infrastructures, and the transfer of scientific findings to social and economic applications.

Prof. Dr. Rudi Balling and Dr. Georg Frischmann, the directors, hoisting the new flag of the Helmholtz Centre for Infection Research on 18 July 2006 *Photo: HZI, Gramann*

The Government of Lower Saxony Meets in “Twincore” On 5 September 2006 the cabinet of the Lower Saxony State Government convened one of its sessions in “Twincore”, the new centre for experimental and clinical infection research in Hannover. The institute is jointly operated by the Helmholtz Centre for Infection Research and the Hannover Medical School (MHH). Members of the press were also invited to attend the official welcome for the state‘s ministers by MHH president, Prof. Dieter Bitter-Suermann, and the Helmholtz Centre‘s scientific director, Prof. Rudi Balling. “Twincore” houses teams of basic researchers who work together with clinical scientists on joint projects. The institute is a pioneer in cooperation between university and non-university research.

Prof. Dr. Rudi Balling, HZI, Prof. Dr. Dieter Bitter-Suermann, MHH, and the Government of Lower Saxony (Niedersachsen) in front of the “Twincore”-Building *Photo: HZI, Gramann*

Tschira Prize for HZI-Researcher Florian Bredenbruch, a young *Pseudomonas* researcher at the Helmholtz Centre for Infection Research, was the 2006 winner of the Klaus-Tschira-Prize for making science understandable. The €5,000 award is given to young investigators who succeed in making the topics of their doctoral theses understandable for the general public. Bredenbruch achieved this in an article for the magazine “Bild der Wissenschaft”. His thesis dealt with *Pseudomonas aeruginosa*, a bacterium that can be very dangerous for those suffering from lung diseases. Bredenbruch studied a signal molecule – pseudomonas quinolon signal, or PQS. Apparently, PQS is responsible for killing part of a bacteria community living in a biofilm.

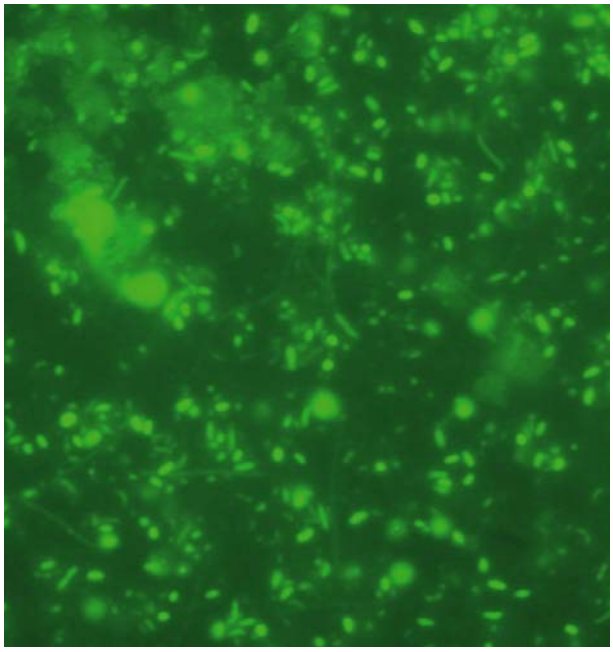
Under stress, part of this microbial community sacrifices itself to make resources available to the survivors.

Florian Bredenbruch, winner of the 2006 Klaus-Tschira Prize, during his work in the laboratory *Photo: HZI, Bierstedt*

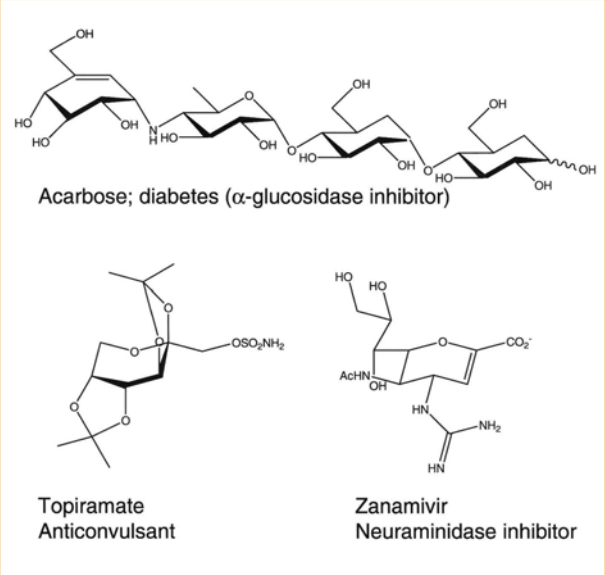
New Plastics for Improved Cell Breeding The development of novel plastic surfaces in order to optimize the growth and control of human and animal cells was the aim of nine partners who met in November 2006 to cooperate on the "Innosurf" research project. Coordinator of the project is Dr. Kurt Dittmar, a molecular biologist at the Helmholtz Centre for Infection Research. The Federal Economics and Technology Ministry selected the project for its "Innonet" grant programme and will help to fund this cooperative effort to the tune of one million euros. Besides the Helmholtz centre, the "Innosurf" alliance includes Braunschweig Municipal Hospital, Braunschweig Technical University, the Fraunhofer Institute for Coating and Surface Technologies in Braunschweig and the University of Tübingen. Additionally, four private companies support the project with research and funding.

Dr. Kurt Dittmar (ri) in discussion with Mr. Bin Ma (ce) and Dr. Werner Lindenmaier (le) *Photo: HZI, Hübner*

Safer Drinking Water across Europe The EU project "Healthy Water" focuses on understanding the kinds of pathogenic microorganisms living in our drinking water and what diseases they can trigger in humans if their numbers are high enough. Since the end of 2006 this project has been coordinated by the Helmholtz Centre for Infection Research. The project is financed over a three-year period by €2.4 million. Braunschweig scientists, headed by Dr. Manfred Höfle, are supported by eight EU partners from industry and research. The consortium, based on successful work in the preceding EU-project "Aqua-Chip", looks forward to further develop and validate molecular detection technologies for the assessment of all pathogenic viruses, bacteria and protozoa which may prove to be a risk for drinking water, by using advanced DNA chip-technology and real-time PCR. These detection technologies will be applied to specific drinking water supply systems that are considered to bear an increased infection risk. In parallel, epidemiological studies for the human populations at risk will be conducted. The European Union expects that the "Healthy Water" project will supply crucial information on how to streamline its drinking water guidelines and how to improve European drinking water supplies.



Drinking water microflora obtained by a novel concentration device tested in the Healthy Water project enabling a concentration by a factor of 20,000 within a few minutes (epifluorescence microscopy after staining with SYBRgreen). *Photo: HZI*



Uridyltriphosphate is the basic building block of many substances

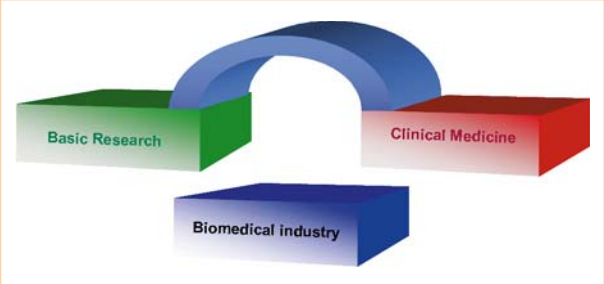
Building Blocks in Sugar for Medical Needs The cost-effective production and industrial suitability of uridyltriphospate, or UTP, is the goal of the new research project "QuantPro" that is being coordinated by the Helmholtz Centre for Infection Research. UTP is the basic building block of many so-called oligosaccharides, which consist of several sugar molecules. A number of successful drugs for the treatment of common ailments, such as diabetes or thromboses, have been developed on the basis of these polysaccharides. With the help of partners from the universities of Stuttgart and Regensburg, Helmholtz researcher Dr. Vitor Martins Dos Santos and his colleagues are looking into the cost effective production of UTP in large quantities with the aid *E.coli* bacteria reprogrammed through Systems and Synthetic Biology approaches. The UTP-producing bacteria are to be fed with a simple nutrient, like glucose, a normal household sugar. The group is confident that this could be a useful method for biotechnological production, since UTP is essentially nothing more than an activated, energy-rich form of glucose.

What Mouse Genes can Tell us about Disease Susceptibility Experts working on the cooperative "GeNeSys" project since early 2007 are looking for answers to the important medical question as to how complex genetics are involved in disease. "GeNeSys" is a virtual institute financed by the Helmholtz Association. It encompasses several laboratories at different locations in Germany having its own management structure. "GeNeSys" (German Network for Systems Genetics) has been set up to analyse closely related mouse families and to study the relationships between their genotype and their actual characteristics that appear in them, the so-called phenotype. The bioinformatic evaluation of the data should enable the researchers to draw conclusions about the genetic causes of diseases with complex hereditary patterns. Particular emphasis has been placed on the susceptibility to infectious diseases. The network project is being coordinated by Prof. Klaus Schughart, department head at the Helmholtz Centre for Infection Research. Numerous other research institutes and universities are participating in the project.

Talking about Mice Since summer 2005 the Department of Public Relations has been coordinating the BMBF-supported discussion project "Animal Experimentation in Research". The issue of using animals for research is a sensitive and emotional one that makes an open and honest discussion on the reasons for conducting such experiments very difficult. The aim of the project has been to develop communication forms that allow to discuss this subject in larger groups. In two school events involving more than 200 students in total, pupils learned to conduct a rational discussion on the subject of animal experimentation. Other aspects of the discussion project included a video about the work at the Helmholtz Centre's animal facility and a forum with researchers, ethics experts and representatives from the authorities that issue permits and conduct controls. The forum is compiling methods for a long-term public dialogue on the subject, once the BMBF project is concluded.

Training Young Investigators for Science Training top-notch young scientists from Germany and abroad for cutting-edge biomedical research is the goal of the "Helmholtz Study Group for Infection Biology", a doctoral programme launched in Braunschweig in January 2007. This demanding training programme for 20 young academics has been jointly organized by the Helmholtz Centre for Infection Research, the Hannover Medical School (MHH) and the University of Veterinary Medicine Hannover (TiHo). The Helmholtz Association supports the programme with €1.8 million from its Impulse and Networking Fund. Besides laboratory training and work on doctoral theses, participants in the graduate programme will learn special topics in a variety of symposia, lectures, weekend seminars and summer schools. Furthermore, they acquire key skills in economics, patent law and management.

New Infrastructure for European Research
EATRIS – European Advanced Translational Research Infrastructure for Medicine – is one of six projects in the biosciences within the framework of the European Strategy Forum for Research Infrastructure (ESFRI) that will apply for the next EU frame work call. The coordinator of the project is Prof. Rudi Balling, scientific director of the Helmholtz Centre for Infection Research. Over the next two years, the participating partner institutes aim to set up a pan-European management structure. EATRIS is designed to accelerate the transfer process by moving discoveries faster from basic medical research to applications for the patient.



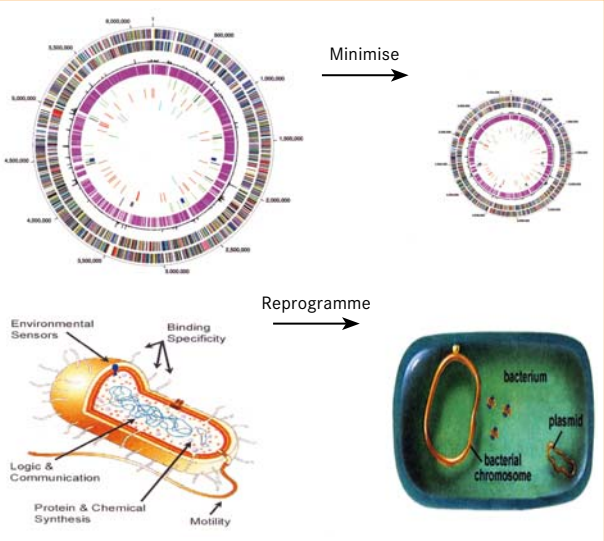
The EATRIS concept

Biotech Training for Researchers from ASEAN Countries
This Biotech training was coordinated by the Helmholtz Centre for Infection Research and InWEnt with some support from Germany's employment office, UNESCO and BioRegioN. The programme was designed to promote scientific and industrial cooperation between Germany and Southeast Asia. It included numerous lectures, lab visits, scientific demonstrations, excursions and a course on technology transfer at the Helmholtz centre. Participants also enjoyed a four-month individual internship in a biotech company or institute, as well as a German language course, an introduction to German culture and a course in management. An Internet-based virtual network also was established as a direct result of several of the courses.

The participants during the Farewell ceremony at the Mission of the State of Lower Saxony (Niedersachsen) in Berlin
Photo: HZI, Jonas

Programming bacterial catalysts *à la carte* "Simplify and Reprogramme". This is the motto of the new research project „PROBACTYS", which is being coordinated by Dr. Vitor Martins Dos Santos at the Helmholtz Centre for Infection Research. The goal of the project is to streamline bacterial cells by stripping them of large chunks of superfluous genetic material so to create a little "factory chassis" that is subsequently plugged in with a range of highly-coordinated, versatile genetic circuits. Proof positive of the approach in this initial project should lead on to the development of ranges of catalysts for use in the tailored development of new items of interest in the environmental, medical, and biotechnological fields. This EU-funded project is one of the first European projects in the emerging field of Synthetic Biology, whose pursuit is both the design and fabrication of

biological components and systems that do not already exist in the natural world as well as the re-design and fabrication of existing biological systems. The highly multidisciplinary consortium includes, besides the HZI, the Pasteur Institute in Paris, the Imperial College of London, the CSIC in Madrid, the TU Delft and the Beijing Genome Institute, as well as BioMedal, a Biotech SME.

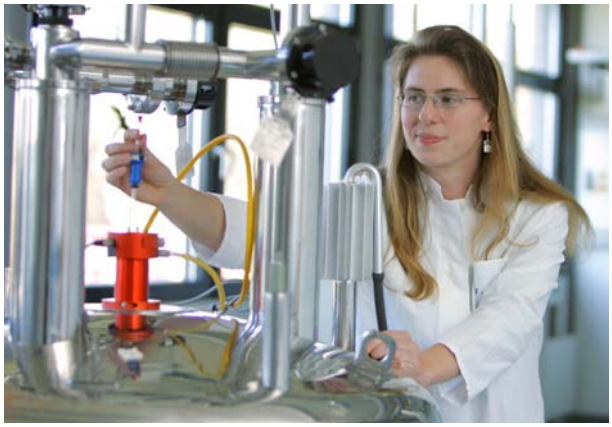


Project concept: minimise and reprogramme *Graphic: HZI,*

Systems Biology of Stress in Pseudomonas Understanding how bacteria respond to stresses is essential to design new microbial-based production processes or to develop new intervention strategies against pathogens. The goal of the PSYSMO project, coordinated by Dr. Vitor Martins dos Santos and Prof. Ken Timmis at the Helmholtz Centre for Infection Research (HZI), is to develop a Systems Biology framework intertwining mathematical modelling and experimentation to study how *Pseudomonas putida*, a versatile soil a bacterium of great biotechnological importance, reacts to stresses in a range of industrially-related processes. Owing to its systemic nature, this integrated framework will laying the basis for the development of novel biotechnological processes and will be also valuable for the understanding of cellular processes involved in the interactions of hosts with infectious opportunistic pathogens such as the dangerous *Pseudomonas aeruginosa*. The ERA-NET funded PSYSMO project is highly multidisciplinary and involves 18 institutions in 5 countries.

Setting up of Helmholtz University Junior Research Groups In May 2005 the first Helmholtz University Junior Research Group was set up at the HZI (then GBF), headed by Dr. Susanne Häußler. This group has been linked to the Hannover Medical School.

On 1 March 2007, a second Helmholtz University Junior Research group was established at the HZI, headed by Dr. Christiane Ritter, who is coming to the HZI from the Salk-Institute, La Jolla, California, USA.



Dr. Christiane Ritter during her work at the NMR
Photo: HZI, Gramann

FOCUS RESEARCH REVIEWS SPECIAL FEATURES

Photos: left: Mathias Müsken analysing images of Pseudomonas aeruginosa *bacteria grown within biofilms with fluorescence microscopy | centre: Antje Ritter preparing absolute tetrahydrofuran | right: Larissa Jundt isolating active compounds through HPLC | Photos: HZI, Bierstedt*

SCIENTIFIC REPORTS FACTS AND FIGURES

"Sociomicrobiology": New Approaches to Understand Chronic Infectious Diseases

AUTHOR | **Priv.-Doz. Dr. Susanne Häußler | Helmholtz University Junior Research Group Chronic Pseudomonas Diseases | sus@helmholtz-hzi.de**

Achievements in medical care in industrialised societies are markedly impaired by chronic opportunistic bacterial infections that remain a major challenge for the medical profession and are of great economic relevance because traditional antibiotic therapy is usually not sufficient to eradicate these infections. One major reason for persistence seems to be the capability of bacteria to grow within biofilms that protect them from adverse environmental factors.

Pseudomonas aeruginosa is not only an important opportunistic pathogen and causative agent of emerging nosocomial* or hospital infections, but can also be considered as a model organism for the study of diverse bacterial adaptation processes. In this context, the elucidation of the molecular mechanisms responsible for the switch from planctonic growth to a biofilm phenotype and the role of interbacterial communication in this process should provide new insights into the capability of *P. aeruginosa* to establish a chronic persistent state of disease.

Moreover, moving one step further, it seems necessary to analyse bacterial behaviour also in the context of multicellular communities and from an ecological and evolutionary perspective. This approach should advance our knowledge on bacterial biofilm development, contribute to a better clinical management of chronically infected patients and lead to the identification of new drug targets for the development of alternative anti-infective treatment strategies.

Chronic infections are biofilm infections Our diagnostic and therapeutic strategies serve us well in the eradication of acute epidemic infectious diseases which can be directly attributed to the use of potent vaccines and antibiotics that were developed to control planktonic bacteria. However, clinicians who deal with device-related and other chronic bacterial infections increasingly face a new category of infectious diseases that differs radically from the acute epidemic bacterial diseases.

These diseases are much less aggressive than acute infections, often persist for months or years, and progress through periods of latency that alternate with periods of acute exacerbation. The pathogens are common environmental organisms and their pathogenic mechanisms are often diffuse and poorly defined. These environmental organisms appear to be sensitive to conventional antibiotics, but these antibiotics fail to resolve the bacterial infections, although they give some relief during acute exacerbations. A main reason for this seems to be that the infecting bacteria grow in the affected tissues in matrix-enclosed communities closely resembling the biofilms that are the predominant form of bacteria in industrial and environmental ecosystems.

The simple fact that the organisms that cause device-related and other chronic infections grow in biofilms seems to be the key to bacterial persistence. Thus, understanding the molecular mechanisms underlying biofilm formation and maintenance will be a major step on the way to developing new therapeutic strategies in order to control chronic biofilm infections.

***Pseudomonas aeruginosa:* a model organism to study bacterial adaptation to chronic persistent infections** In the last decade *P. aeruginosa* has evolved as a model organism to study bacterial adaptation to a chronic persistent state of disease. *P. aeruginosa* is a ubiquitously found gram-negative bacterium that is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. It inhabits terrestrial and aquatic environments and can infect a broad range of host organisms - from plants and insects, to humans. The ecological success of this opportunistic bacterium can be attributed not only to its broad

** nosocomial comes from the Greek word nosokomeion meaning hospital (nosos = disease and komeo = to take care of)*

metabolic versatility enabling survival in varied habitats, but also to its well-regulated release of a large arsenal of virulence factors.

P. aeruginosa is primarily a nosocomial pathogen and a serious problem in patients with burns. Moreover, the pathogen has an exclusive role in the pulmonary infection in cystic fibrosis (CF). More than 90% of CF patients acquire *P. aeruginosa* during early childhood and in these patients chronic infection due to *P. aeruginosa,* repeated exacerbations, and progressive deterioration in lung function remain a major cause of morbidity and mortality, despite current antimicrobial therapy.

We frequently observe a change from a nonmucoid to a mucoid exopolysaccharide alginate-overproducing *P. aeruginosa* phenotype that is associated with an increased inflammatory response and clinical deterioration in CF. At this stage, *P. aeruginosa* adopts a biofilm mode of growth with microcolonies embedded in an exopolysaccharide matrix. These microcolonies act as a protective niche and help the bacteria to evade the host immune response and to withstand antimicrobial therapy. It has been estimated that with the conversion from a non-mucoid to a mucoid phenotype it becomes impossible to eradicate the infection.

Fig. 1. P. aeruginosa *diversity. Various morphological distinct phenotypes can be isolated from the respiratory tract of chronically infected CF patients.* Photo: HZI

***P. aeruginosa* biofilm morphotypes** While in the context of chronic persistent disease much attention has been paid to the mucoid conversion, it has been increasingly recognized that in chronic disease other bacterial morphotypes can be recovered from the respiratory tract of CF patients although most CF patients are colonized with only one or a few *P. aeruginosa* clones (Fig. 1). Slow growing *P. aeruginosa* sub-populations, also termed small colony variants (SCV) because they produce small colonies on nutrient agar, can be frequently recovered from the chronically infected CF lung. Approximately 3% of the *P. aeruginosa* positive CF sputum probes are positive for SCVs. In a prospective study, *P. aeruginosa* SCVs were found in 33 of 86 patients and the detection of SCVs correlated with poor lung function parameters and inhaled antibiotic therapy.

Because studies initially demonstrated a markedly diminished virulence potential, SCVs were categorized as morphological variants which play only a secondary role in infectious diseases. However, slow-growing microorganisms, such as norcardia and mycobacteria, have long been described as causative agents of persistent and recurrent bacterial infections. Moreover, there is increasing evidence that slow-growing subpopulations of a number of bacterial pathogens, which usually tend to cause acute infections, merit consideration in connection with chronic persistent and recurrent infections.

Interestingly, *P. aeruginosa* SCVs, isolated from the lung of chronically infected CF patients, not only exhibit an increased antibiotic resistance profile, but a subgroup of clinical *P. aeruginosa* SCV isolates was shown to be hyperpiliated, to exhibit autoaggregative properties, to adhere to surfaces particularly well and to be involved in the development of biofilms. Moreover, as demonstrated in cocultivation experiments, these highly adherent SCV seemed to have a selective advantage in the late stationary phase of liquid cultures. Thus, limited nutrient conditions and increased oxidative stress might favour growth of biofilm forming hyperpiliated SCVs encapsulated within microcolonies in the CF lung.

In recent years, much has been learned about various factors involved in biofilm formation and it has become particularly clear that adherence is a crucial step. The assembly of *P. aeruginosa* fimbrial adhesins, other than type IV pili, has been considered important for biofilm formation in *P. aeruginosa,* and mutants devoid of a functional chaperone usher pathway (cupA) gene locus were shown to be defective in the formation of biofilms.

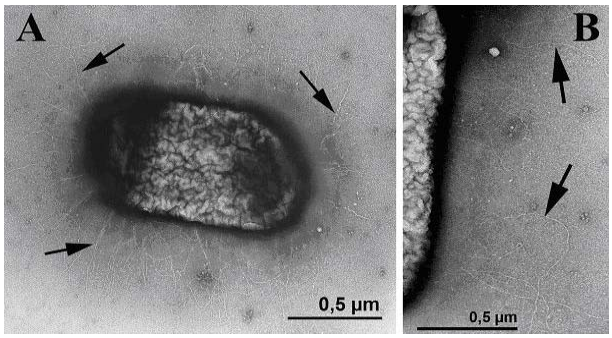


Fig. 2. cupA encoded fimbriae expression in P. aeruginosa.
Photo: HZI, Rohde

Sequencing and functional analysis of selected mutants that exhibited a switch to a biofilm phenotype revealed that the autoaggregative biofilm forming SCV phenotype of *P. aeruginosa* is linked to the expression of the cupA gene cluster. Using transcriptome analysis, electron microscopic imaging and Western blots developed with a poly-clonal serum generated against CupA1, we demonstrated that, like in *E. coli*, this cupA gene cluster is essential for the assembly of fimbria at the bacterial surface of *P. aeruginosa* (Fig. 2). Furthermore, we provided evidence that fimbria expression is regulated via the modulation of the newly identified bacterial signal molecule, cyclic di-GMP, in an antagonizing way (Fig. 3): a rise in the intracellular c-di-GMP level is mediated by activated GGDEF domain containing proteins and is linked to cupA expression, whereas proteins encoding an EAL domain seem to be responsible for the decrease of intracellular c-di-GMP and thus cupA expression in *P. aeruginosa.*

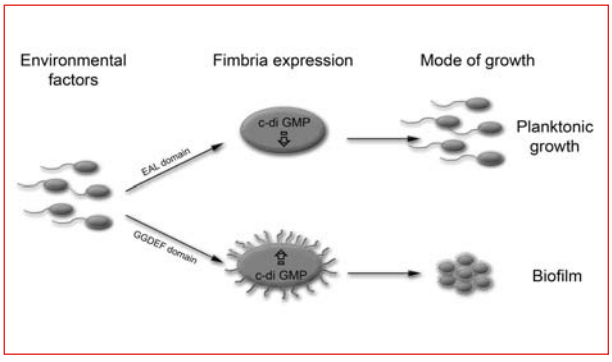


Fig. 3. Environmental factors influence via the modulation of the intracellular c-di-GMP level fimbriae expression in P. aeruginosa.

Future work will concentrate on the identification of cupA expression in clinical isolates and in *P. aeruginosa* within explanted CF lungs. For that purpose a monoclonal antibody has been generated that is able to detect the native CupA1 protein and that can be used for immunohistological studies.

Morphological diversity guarantees survival The appearance of a morphologically diverse *P. aeruginosa* population that develops within biofilms and that exhibits increased resistance to environmental stress has recently been interpreted as support of the “insurance hypothesis”. This hypothesis describes an ecological model predicting that a diverse population is more likely to survive environmental perturbations than a homogenous population. Intriguingly, a morphologically diverse *P. aeruginosa* population is not only a typical microbiological finding of CF specimens, but is also rapidly established in *P. aeruginosa* cultures grown in vitro within biofilms, as opposed to planktonic communities.

However, the molecular mechanisms underlying the origination of diversity remain poorly defined. Strong driving forces for the establishment of population diversity in a heterogeneous habitat seem to be mutation and selection. In order to analyse what kind of mutations are selected for in the biofilm habitat and which genes are affected, we are currently establishing a microarray hybridisation-based method to map mutations within the *P. aeruginosa* genome in a joint project with Affymetrix. Very recently this technique has been successfully applied to detect mutations responsible for metronidazole resistance in the bacterial genome of *Helicobacter pylori.*

Interbacterial signalling involved in biofilm formation Free-floating bacteria usually adapt to distinct environmental conditions with a characteristic change in their gene expression pattern. Different stresses may induce diverse but often overlapping stress responses. However, although many of the survival strategies operate at the individual cell level, others operate at the population level. Living in populations provides a species with additional mechanisms of survival. At a higher level of organisation, bacteria within biofilms benefit from cooperation.

The complexity of multicellular behaviour in bacterial life is also supported by the identification of signalling molecules mediating cooperative traits and a coordinated behaviour.

Bacteria have to integrate both information about their extracellular environment and their intracellular physiological status in order to accomplish appropriate responses. This enables the bacterial population to react to changes in the environment with complex processes, such as biofilm formation and the coordinated release of virulence factors. Extracellular signalling is largely managed by cell-to-cell communication (quorum sensing), which involves the production, release and detection of small signal molecules called autoinducers.

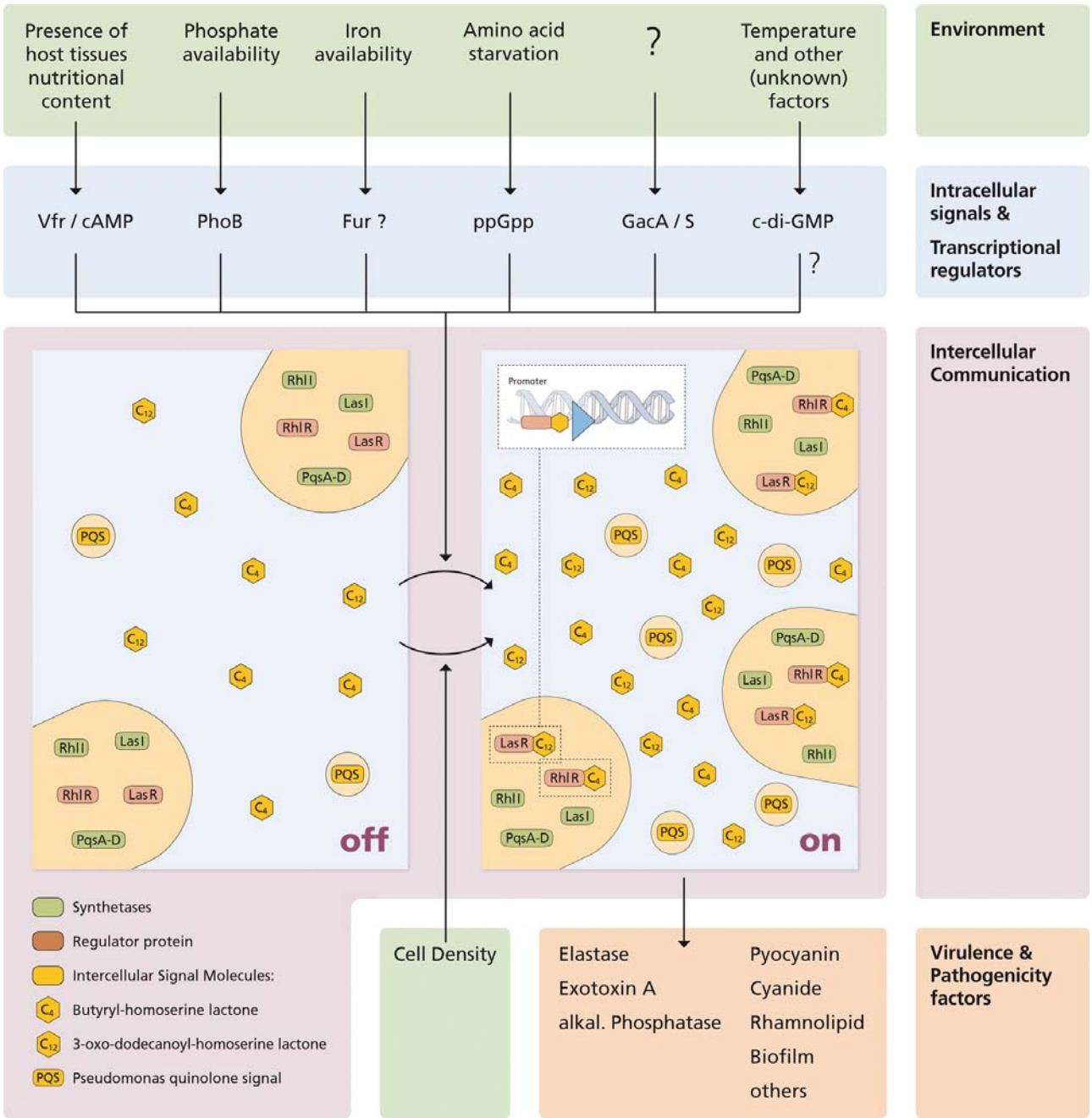


Fig. 4. The role of the environment on the expression of intra- and intercellular signal molecules in P. aeruginosa. *Graphic: HZI, Klimek*

P. aeruginosa possesses two hierarchically organized acetyl homoserine lactone (AHL) mediated quorum sensing systems and produces a third intercellular signal that is involved in virulence factor regulation. This latter signal, 2-heptyl-3-hydroxy-4-quinolone (referred to as the Pseudomonas quinolone signal (PQS)), is a secondary metabolite that is part of the *P. aeruginosa* quorum-sensing hierarchy and is required for the production of rhl-dependent exoproducts at the onset of the stationary phase.

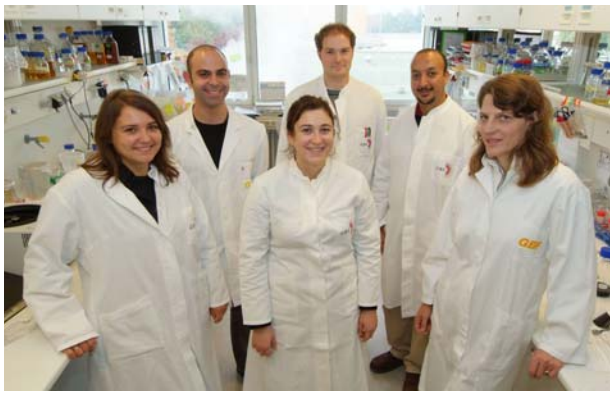
We have dissected the biosynthetic pathway of PQS and demonstrated that PQS plays a significant role in bacterial iron homeostasis. The addition of exogenous PQS to early log phase cultures led to a depletion of iron in the growth medium due to its iron chelating effect. The global transcriptional profile in response to PQS revealed a marked upregulation of genes belonging to the tightly interdependent functional groups of iron acquisition and oxidative stress response. Remarkably, not only most of the differentially regulated genes, but also the induction of a lacZ transcriptional fusion of rhlR could be traced back to a powerful iron chelating effect of PQS. Nevertheless, although iron deficiency *per se* induced rhlR, there seem to be PQS specific effects that are independent of the PQS effect on *P. aeruginosa* iron homeostasis. The detailed elucidation of the links between QS, the PQS regulon and genes involved in iron acquisition in *P. aeruginosa* will be a challenging task for the future.

Linkage of inter- and intra-bacterial signalling pathways
Complex bacterial adaptation processes will require not only information on the extracellular environment including cell density as delivered by cell-cell communication, but also information on the intracellular physiological status. C-di-GMP, cyclic adenosine 3',5' monophosphate (cAMP) and guanosine-3,5-bis(pyrophosphate) (ppGpp) are common second messengers in bacteria, which activate regulatory systems responding to environmental factors. Interestingly, the intracellular signalling pathways are not only directly influenced by environmental cues, but also seem to be directly linked to the quorum sensing systems in *P. aeruginosa* (Fig. 4). This implicates that virulence factor production in *P. aeruginosa* is not only dependent on cell density, but is also strongly influenced by environmental cues due to the tight linkage of intra- and interbacterial signalling pathways.

One for all – all for one In the last decade the orthodox view of bacterial populations as being a homogenous collection of sibling cells has been abandoned. Instead, proof of bacterial interactiveness has accumulated from the observation of complex multicellular development phenomena such as biofilm formation and has forever changed the paradigm towards a more organic view of bacterial life.

The term "sociomicrobiology" has been introduced only recently by P. Greenberg. Quorum sensing and biofilm development are social behaviours of bacteria, and the relative contributions of genetic determinants *versus* environmental conditions on bacterial behaviour has become a main research focus. Cooperation is a difficult behaviour for evolutionary biologists to explain. Why should an individual carry out a costly cooperative behaviour for the benefit of other individuals or the local group? This seems to go completely against the Darwinian idea of ‚survival of the fittest'. However, cooperation can provide a benefit at the population level. In the future, bacteria may gain importance as a model for dissecting social behaviour at the genetic level. As outlined above, the establishment of population diversity in biofilm communities seems to be essential for bacterial persistence. Mutation and selection have been shown to play a role in this process, however, it has also been suggested that the generation of diversity itself may be a subject of regulation in response to environmental conditions. As we observe PQS dependent diversification in the *P. aeruginosa* population under biofilm growth conditions, we intend to comprehensively analyse a putative programmed bacterial response which is dependent on the signal molecule PQS. These studies may lead to a more profound insight into the biological phenomenon of morphological diversity observed within biofilms, as well as its ecological and evolutionary significance.

Perspective We are at the very early stages of understanding the bacterial mechanisms that contribute to chronic persistent infections. In this context, bacterial mechanisms of geno- and phenotypic adaptation, influence of environmental factors on biofilm formation and intra-and interbacterial communication are studied intensively at the molecular level. The integration of the data and the identification of the regulatory networks and dynamic interplay of the genes/proteins and possibly signal molecules involved in bacterial interaction within biofilms will be a challenging task for the future and should be backed by a systems biology approach. However, it seems necessary to move one step further and to analyse bacterial behaviour in the context of multicellular communities. In this respect, the identification of genes involved in the fitness of a bacterial population is desirable in order to also gain insights into biofilm development from an ecological and evolutionary perspective.



The „Chronic Pseudomonas Diseases" Team (from left to right): Vanessa Jensen, Yusuf Nalca, Caroline Zaoui, Mathias Müsken, Ahmed Haddad, Susanne Häußler

Foto: HZI, Bierstedt

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The Chemical Pipeline – A Research Programme and Infra-Structure for the Discovery and Evaluation of New Anti-Infectives

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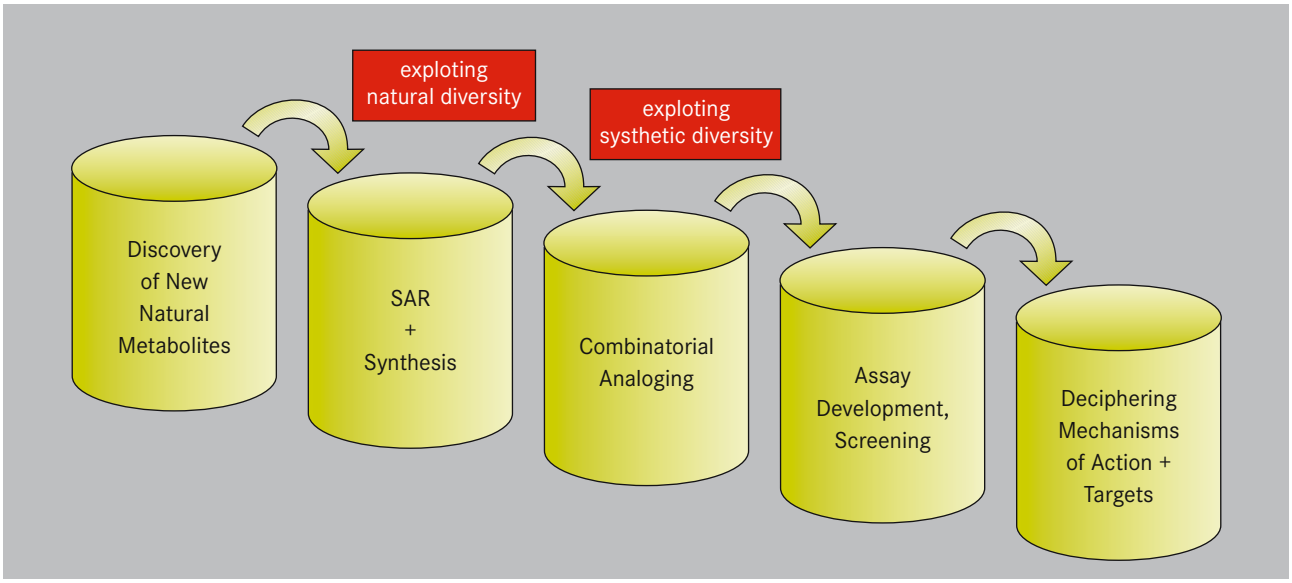
Advances in the biomedical sciences and their applications are achieved by elucidating molecular mechanisms underlying biological processes. The identification of molecular partners interacting in cellular and organismal processes requires the experimental perturbation of such processes, genetically as mutations, and biochemically, by using chemical agonist/antagonist ligands of protein targets. Obviously, the latter provides chemical compounds that are potential drug candidates, and their discovery directly creates a link to pharmaceutical drug development and translation into the clinic. The number of available chemical ligands for biochemical studies, however, is severely limited. Current experimentation at the HZI in the areas of natural products, genomics and combinatorial chemistry/biochemistry serves as a powerful combination that can provide the desired ligands, as well as a competitive advantage for research on infectious diseases. Five research projects dedicated to the identification, analysis, chemical synthesis and biological evaluation of bioactive small molecules have been integrated into the "Chemical Pipeline" in order to speed up the systematic discovery of novel anti-infective substances and new principles for medical intervention.

The mission of the Chemical Pipeline A National Centre for Infection Research is a very effective institution for advancing measures aimed at disease prevention and therapy. Antibiotic therapy is currently the most important means of combating bacterial infections, but is increasingly compromised by the emergence and spread of resistance to antibiotics. The development of new generation antibiotics and anti-infectives is an urgent need, since non-treatable pathogens are emerging rapidly. The search for novel, biologically active substances is therefore of utmost importance, and chemistry is one of the pillars on which the HZI has built its infection R&D programme.

The integration of chemistry into biology and biotechnology research has a long tradition at the centre and represents a unique opportunity among the health research centres of the Helmholtz-Association (HGF). The former "Division of Natural Products", headed by G. Höfle und H. Reichenbach, has built up an extremely valuable expertise in the discovery of biologically active secondary metabolites from bacteria. It has discovered a number of new drug candidates, some of which show promise for clinical application. The department "Chemical Biology" pioneered nucleic acid and peptide-based combinatorial chemical syntheses and biological screening methodologies that were applied to systematic studies in immunology and functional proteomics.

In 2005, the "Chemical Pipeline" was established to ensure continued access to bioactive substances, and is now dedicated to the study and treatment of infectious diseases. The pipeline is directly interfaced with essentially all departments and research groups at the centre. These include the „DFG-Sonderforschungsbereiche" in which the centre participates, the Technical University of Braunschweig, and other institutes of the HGF. It also has other dynamic interfacing possibilities with many groups, institutes and industries, nationally and internationally. This pipeline also participates, as one of the initiating partners, in the "ChemBioNet" (www.chembionet.de), a national network of infrastructure and expertise to support chemical biology research in academia.

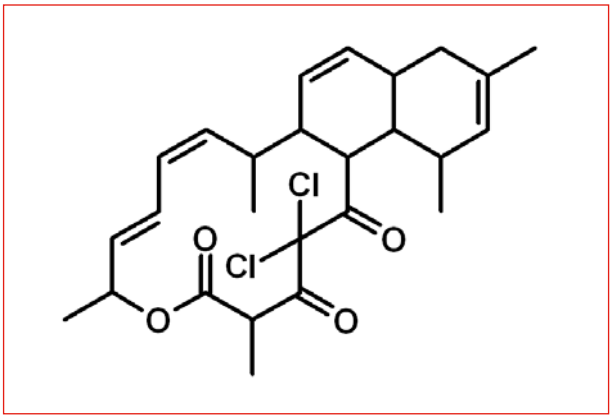
As an integrated programme, the "Chemical Pipeline" aims to discover, create and characterize new bioactive small molecules and to identify their cellular targets and mechanisms of activity. This will be achieved by exploring both natural and synthetic molecular diversity, combining research on natural products, analytical and synthetic chemistry, and combinatorial chemistry/biochemistry, with new developments at the centre in microbial pathogenesis and cell biology.

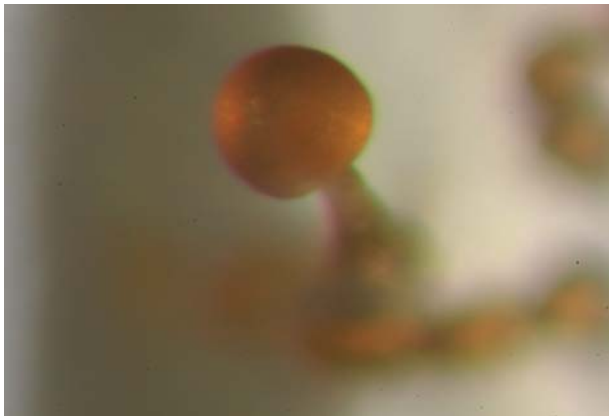


The Chemical Pipeline

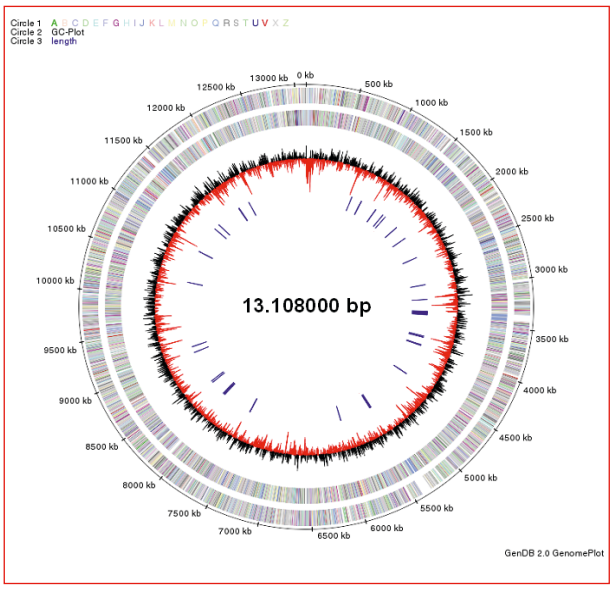
Microbial Diversity and Natural product discovery The research group on "Microbial Drugs" has taken on the task of generating truly novel molecular diversity derived from a broader range of natural product sources. New and modified chemical structures are fed into the pipeline to provide cooperation groups with promising lead compounds that can be optimised for the fight against pathogens, using, for example, combinatorial, synthetic and derivatization chemistry, as well as mode of action studies.

Nowadays, more sophisticated and sustainable approaches are needed to further mine the biochemical potential of this valuable group of gliding bacteria. And the question remains: where are new antibiotics and antifungals going to come from?



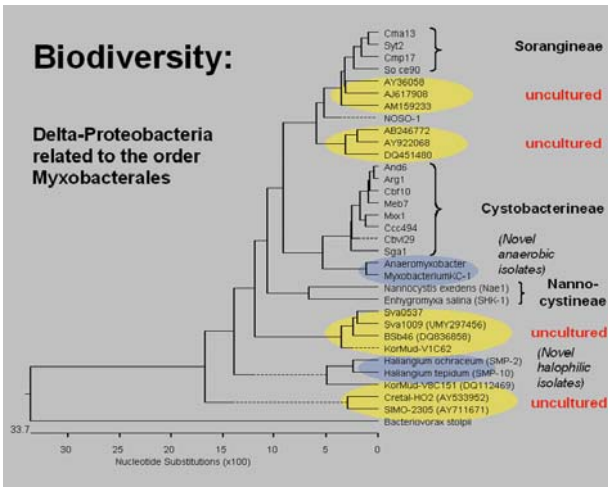


Due to our efforts at the HZI, myxobacteria have been established as a valuable resource for the identification and development of novel and improved clinically relevant secondary metabolites like chlorotonil A (see left) (Reichenbach and Höfle 1999) (Gerth et al. 2003). Myxococcus stipitatus, *a rare member of myxobacteria (see right), was detected as a producer of a novel metabolite in the course of a previous screening programme . It is a generally accepted rule: the more metabolites of a certain producer group are known, the higher efforts must be shouldered in screening and isolation for the detection of novel natural products. This aspect is becoming more important with myxobacterial research, too.* *Photo: HZI, Gerth*



From our knowledge of two completed myxobacterial genome sequences, i.e. of Myxococcus xanthus *and* Sorangium cellulosum, *we gained new insights into the potential of these unique gliding bacteria. The genome of* Sorangium cellulosum *So ce56, the largest bacterial genome known to date was sequenced and annotated in the BMBF funded GenoMik project (R. Müller, K. Gerth, A. Pühler). More polyketide synthase gene-clusters were found than secondary metabolites produced by this strain. Such"silent" polyketide synthase and nonribosomal peptide synthetase gene clusters seem to be common with myxobacteria and offer a promising resource which can be explored in the future* i.e. *by molecular biological tools enabling the targeted expression in the harbouring strain or by heterologous expression of the complete biosynthetic pathway (Bode and Müller 2006).*

The answer is functional genomics and system biology: Our research, funded by the BMBF programme "GenoMik-Plus", has extended the opportunity to continue and broaden our previous studies and to investigate the global regulation of secondary metabolism. The close and highly productive cooperation with the Department of Pharmaceutical Biotechnolgy at the University of the Saarland in Saarbrücken efficiently combines our experience in the cultivation of myxobacteria, yield optimisation, fermentation, natural product isolation, and structure elucidation with the expertise in molecular biology, genetic engineering, analytical biochemistry and genomic know-how of our partners.



Some of these groups of microorganisms seem to depend on marine habitats, others are of terrestrial origin. They are assumed to be closely related to the known suborders of the myxobacteria, e.g. the Sorangineae, Cystobacterineae *and* Nannocystineae, *and they are also related to novel groups of* Myxobacterales *(the facultative anaerobes or anaerobes and the marine isolates, which were detected some years ago). The known groups of myxobacteria are excellent producers of novel metabolites and different strains of the new halophilic* Haliangium *species produce a recently detected antifungal activity, Haliangicin (Fudou et al. 2001). We believe that the isolation of "novel groups of myxobacteria" will open the door to mine further promising resources for novel bioactivities.*

Microbial diversity as a source of innovation:A different, but no less fascinating approach, is the exploration of microbial species that have not been cultivated for unknown bioactive metabolites based on the discovery and isolation of novel species. Ecological studies on the metagenome of diverse habitats on the basis of 16S rDNA sequences revealed many, until now, uncultured groups of δ-proteobacteria which are related to the order of the *Myxobacterales*, and thus indicate another unexplored potential for novel secondary metabolites with biological activity. The difficulty of handling such isolates can be overcome by the increasing know-how in the heterologous expression of complex biosynthetic pathways in several surrogate organisms, such as "thermophilic myxobacteria". Respective studies have already been started.

New targets and our collection of myxobacteria: Short-term supplies of active metabolites can be assured by exploiting hitherto overlooked compounds in our screening extracts.

On the one hand, LC-MS based high-throughput chemical screening methods will be established to search for new chemical entities. On the other hand, alternative screening assays - i.e. the search for inhibitors of "antibiotic efflux pump systems"- will allow the detection of antibiotics with novel mechanisms of action, which are highly relevant for infectious disease therapies.

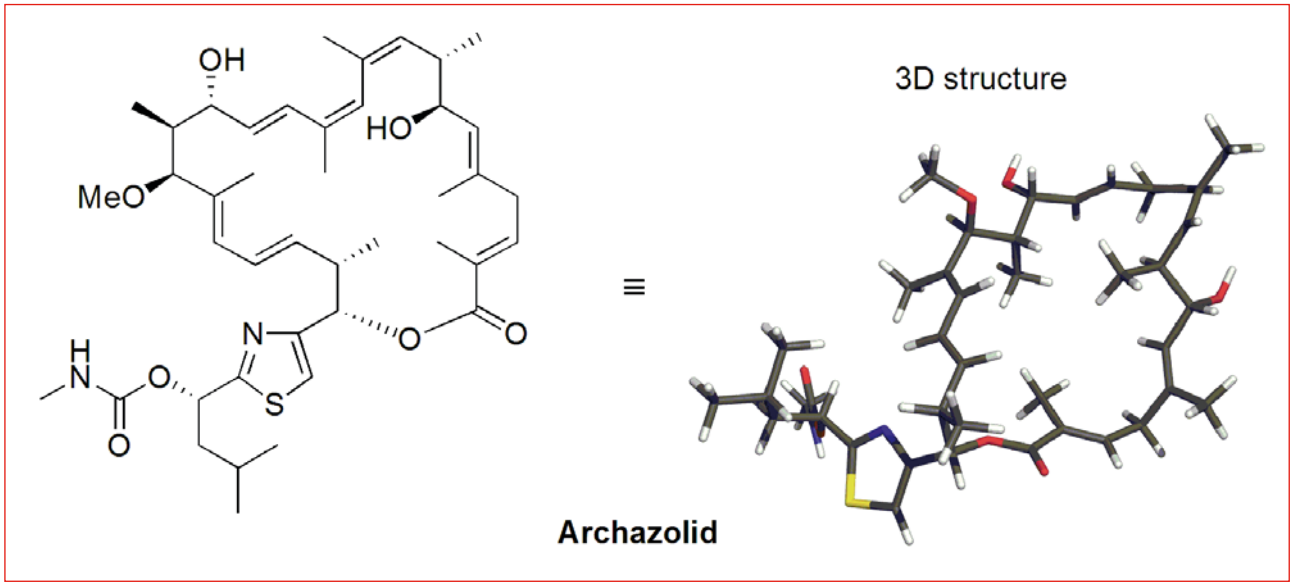
Natural product chemical synthesis Research in the department "Medicinal Chemistry" and the junior research group "Structure and Function of Antibiotics" focuses on the chemical development of natural product derived antibiotics.

We are elucidating the detailed 3D-structure of potent lead structures in order to unravel in detail the mode of action. We are also studying methods for the synthesis of such structures and examining the structural predicaments for activity, in order to develop modified natural product-derived structures with improved chemical and biological properties for further preclinical development. A particular promising source for novel bioactive leads is myxobacteria, which have a long tradition at the Helmholtz Centre for Infection Research.

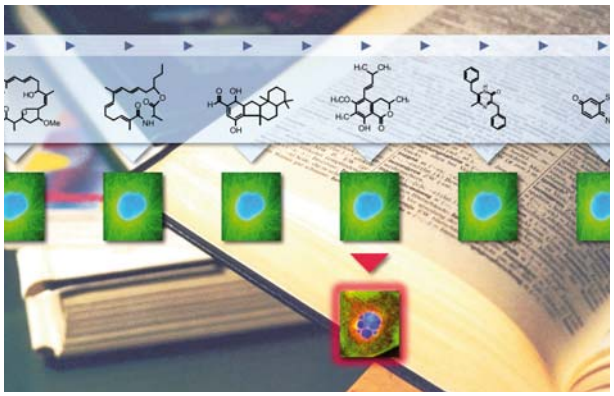
For the development of new promising drugs, it is indispensable that the molecular architecture of such compounds is fully understood. Their pharmacophoric groups need to be identified and their overall framework, which is often necessary for selectivity and specificity, needs to be unravelled. This can be done using modern spectroscopic methods. A more reliable approach, however, is by providing analogues through chemical synthesis. This analoging requires first the synthesis of the very natural product. This paves the way for the implementation of automated synthesis which, in turn, can provide a fine pattern of similar structures around the privileged compound.

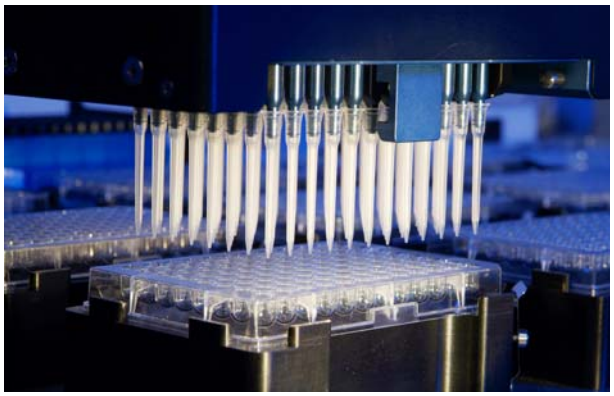
Along these lines, we have recently elucidated the 3D structure of the macrolide antibiotics archazolid, etnangien, chivosazol and the novel anti-tuberculosis lead, thuggacin. Therefore, the synthesis of natural products will be our starting point for medicinal chemistry. We will focus on bacterial RNA inhibitors, but also work on the new TB drug, thuggacin, as well as drugs that interfere with the actin skeleton. The so-established syntheses not only aim to provide derivatives through classical chemical synthesis, but allow the implementation of automated synthesis such as the SPOT method.

A thorough understanding of the spatial arrangement of these new lead structures will have a significant impact on medicinal chemistry. This is the juncture where Medicinal Chemistry and Chemical Biology merge to combine both automation and information for optimizing promising new compounds.



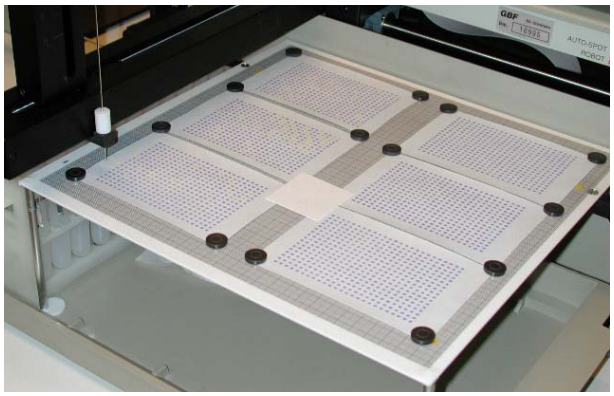
The 3D-solution structure of archazolid, a potent macrolide antibiotic from the myxobacterium Archangium gephyra.

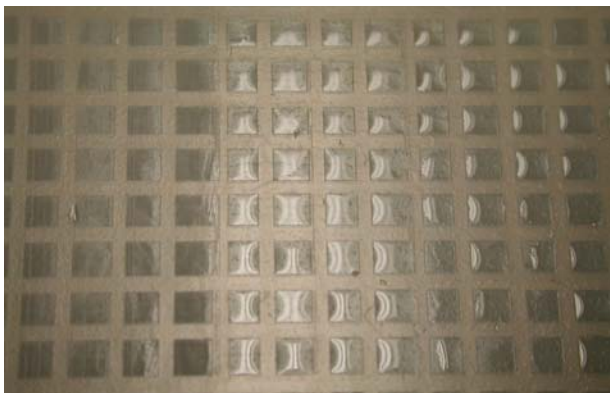




For the search of a bioactive compound a suitable bio-assay is developed which detects the desired activity and large collections of diverse chemical substances as well as series of analogues from common core structures (chemical libraries) are systematically tested in miniaturized high-throughput screening (HTS). *Collage: HZI, Klimek; Photo: HZI, Bierstedt*

Searching through synthetic diversity The discovery of new compounds possessing specifically desired biological activity is more or less an empirical process. The department of "Chemical Biology" approaches this by systematically searching large compound libraries. This involves the collection of diverse, drug-like compounds for screening, and, dedicated assays for the biological process under investigation. Complementing the natural product discovery and synthesis described before, we utilize chemical synthesis to create series of derivatives made around selected core structures – molecular scaffolds. Large series – chemical libraries – of analogues can be rapidly made through the combinatorial assembly of molecules. Our bioassays are primarily designed to address and read-out selected molecular processes, or targets, that have been identified before to be involved in the process under investigation. For the deciphering of the detailed molecular mode of action of the identified active compounds we employ modern affinity proteomics and chemical genetic screens with a complete set of 4,700 single gene deletion mutants of the surrogate organism Saccharomyces cerevisiae. We also develop and maintain the required infrastructure and methodologies to provide access for the whole centre to competent compound collections, high-throughput screening (HTS) logistics and robotics, HTS compatible assay development and the bio-/cheminformatic tools for data evaluation. The project itself is pursuing studies on bacterial adhesion, invasion and biofilm formation, as well as cell proliferation.

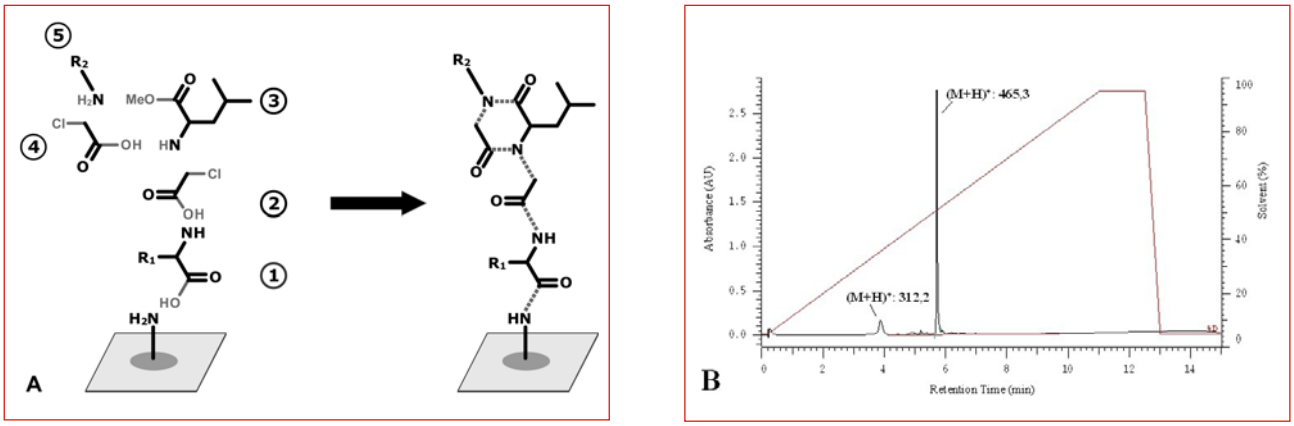




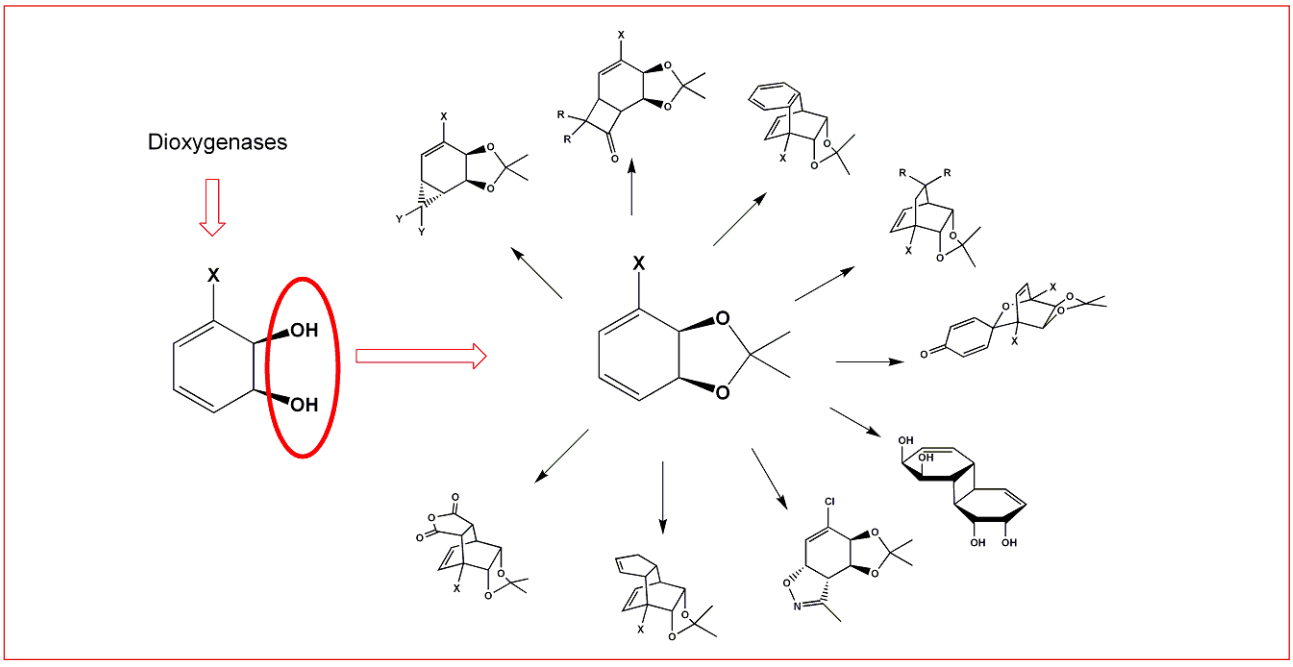
Our method developments for parallel combinatorial chemical synthesis and screening are based on the SPOT synthesis (Frank 1992) performed on cellulose membranes (top); variations of this include the patch array of separated hydrophilic areas on a hydrophobic polypropylene sheet (Beutling et al. 2004) for parallel cell-based screening with solubilized compounds (middle); the BioDisc-synthesizer (Dikmans et al. 2004) to create arrays of compounds on a CompactDisc surface(bottom). *Photos: HZI, Bierstedt*

For the efficient synthesis and screening of compound libraries we particularly exploit the use of planar solid support materials in simultaneous multiple and parallel chemical synthesis. A major workhorse for our library synthesis programme is the SPOT synthesis developed in 1990, which allows us to perform in parallel up to 20,000 chemical reactions by dispensing sub-microliter volumes of reagent solutions to an array of small individual synthesis locations, so called spots, on a continuous cellulose membrane support. These membrane-bound compound arrays can be directly applied in screening experiments, such as affinity capture or enzyme transformation assays. Alternatively, spots can be separated and compounds cleaved from the support for other types of assays which require solution phase compounds, such as cell-based assays. In order to further miniaturize the arrays and to prepare multiple copies with only one synthesis run, we have developed a special novel process for manufacturing synthetic peptide/compound libraries in the form of chemical mini- or microarrays (the SC^2 process®). This is an extension of the SPOT synthesis and keeps the advantageous features of cellulose-bound molecules. Peptide arrays are made available in-house to all other projects through the peptide synthesis platform. Our technology oriented work focuses on full automation, miniaturization, compatibility with increasingly more complex cellular assay systems and high-throughput performance.

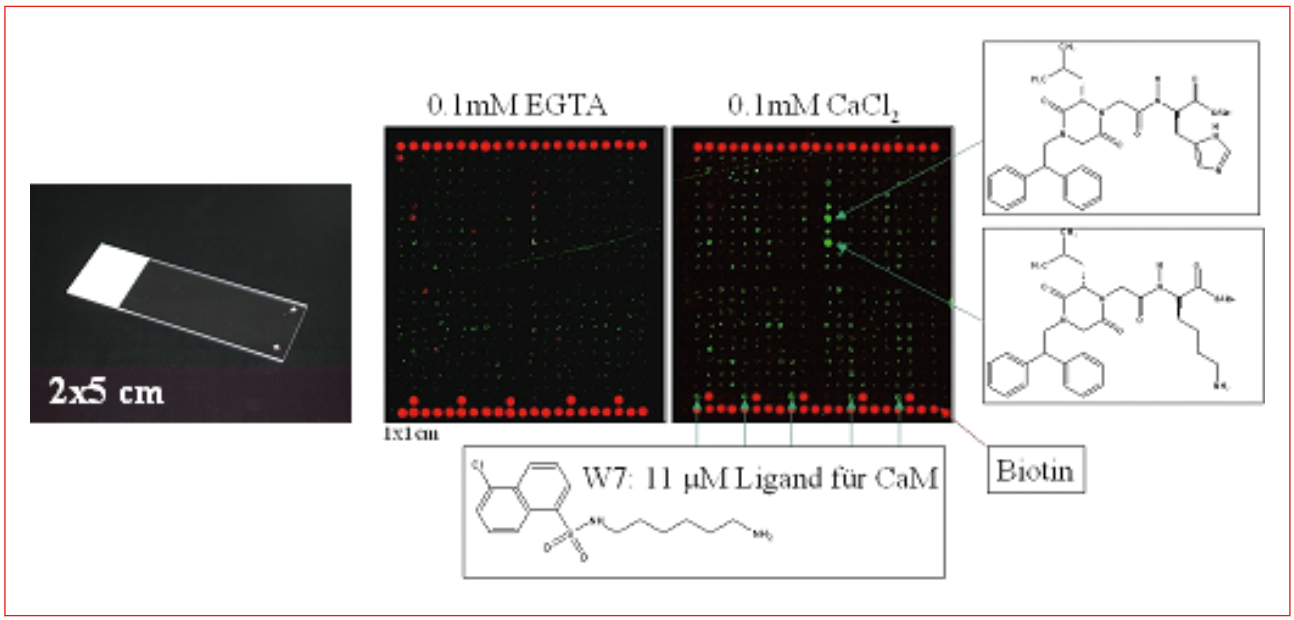
Our methods were initially developed for peptide synthesis and screening to identify peptide ligands of protein targets. They were primarily applied to studies of protein-protein interactions and immunology. Supported through funding by the BMBF (Lead Project “The Drug Discovery Machine” under the leadership of Evotec AG) and the German National Genome Research Net (NGFN), we started successfully to extend our synthetic methodologies to other types of drug-like synthetic molecules. The repertoire of synthetic reactions is now also complemented by gentle enzymatic transformations dedicated to more demanding natural-product-like structures.



Principle synthetic approach: For the stepwise chemical assembly (A) of our compound libraries we exclusively apply solid-phase synthesis and carry out the coupling of small building blocks (monomers) as well as mixtures of these (pools). The starting monomers are anchored to the solid support either via a stable linker to yield covalent attached products or via a cleavable linker to allow release of the product into solution. Coupling efficiencies are optimized with a series of model syntheses to yield crude products of >80% purity as judged by HPLC and mass spectroscopy (B). In this way, molecular libraries of several thousand and up to several billion compounds can be quickly synthesized for screening in biological assays. The figure exemplifies the stepwise assembly of a diketopiperazine by coupling an amino acid (1), chloroacetic acid (2), an amino acid methyl ester (3), chloracetic acid (4), and an amine (5) followed by in situ *cyclization.*



An example for a key biochemical transformation which allows to extend to many other follow-up syntheses. Our expertise in biotransformation of small molecules profits in part from work of the previous research group of "Microbial Transformation" at the Centre (head Prof. Kieslich) which provided us a large collection of microbe strains and fermentation protocols in the form of a bio/data bank. During our work in recent years on the genetic engineering of enzyme substrate specificities (Zielinski et al. 2006), we have obtained substantial expertise in biotransformations involving two different families of enzymes, aryl-hydroxylating dioxygenases and sucrose-utilizing glycosyltransferases.

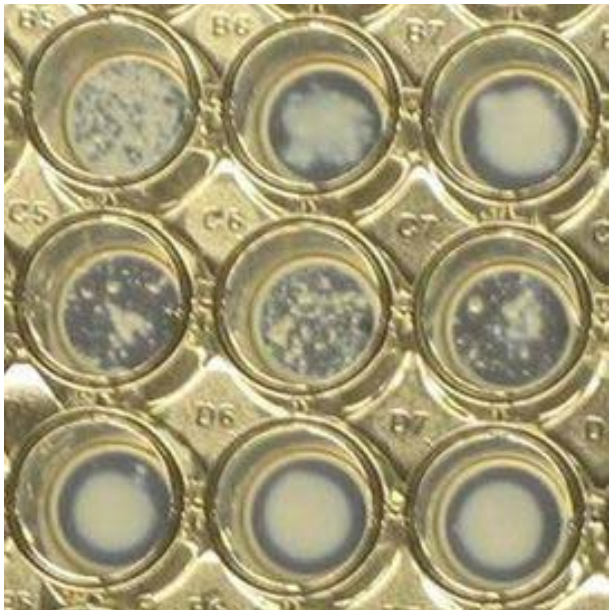


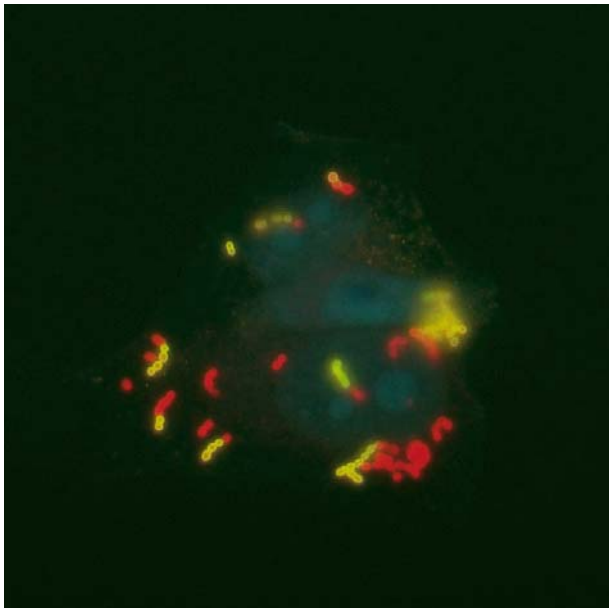
A microarray of diketopiperazines prepared by new SC^2-process on a glass microscope slide is probed with calmodulin (CaM) in the absence (EGTA, left) and presence of calcium ions yielding two new ligands for CaM. W7 is a known small molecule ligand for CaM; biotin serves as control and is detected by addition of labelled streptavidin to the sample. – The synthesis of the compounds follows essentially standard SPOT synthesis protocols except that a special, acid sensitive amino-cellulose membrane is used. Individual spots are separated post-synthesis with the help of a punching device and the resulting discs are dissolved in a strong acid. The solutions of the cellulose with the compounds still covalently attached are then printed and adsorbed onto the target planar surfaces, usually glass microscope slides. We therefore call this process "spotting compound-support conjugates": SC^2. One standard cellulose spot yields 0.5 ml of stock solution from which picoliter aliquots are used to print up to 10^8 microarray copies.

Currently, more than 90,000 compounds in different formats are archived and applied in screening campaigns. The HZI compound archive contains

- over 100 natural products from the GBF myxobacterial metabolite collection
- 7,000 small organics purchased from EMC Microcollections (Tübingen)
- 17,000 small organics from the ChemBioNet collection
- 40,000 small natural product hybrids (home-made "recombinations" of natural product fragments)
- over 25,000 cellulose conjugated small molecules (diketopiperazines, triazines)

We have successfully built the required infrastructure and methodologies in the two first years of our new research direction towards chemical biology of infectious diseases. During that time, already very promising small molecules with potential anti-tumor (p27 stabilizing proteasome inhibitors), antiviral (proteasome inhibitors) and anti-infective (interferon enhancer) activity were newly discovered from our compound archive. Additionally, promising synthetic adjuvants based on derivatives of MALP-2, a macrophage activating lipopeptide discovered at the Centre by P. Mühlradt, as well as other lipid structures have been prepared for vaccination research. We aim to continue supporting this research with our chemical synthesis expertise.





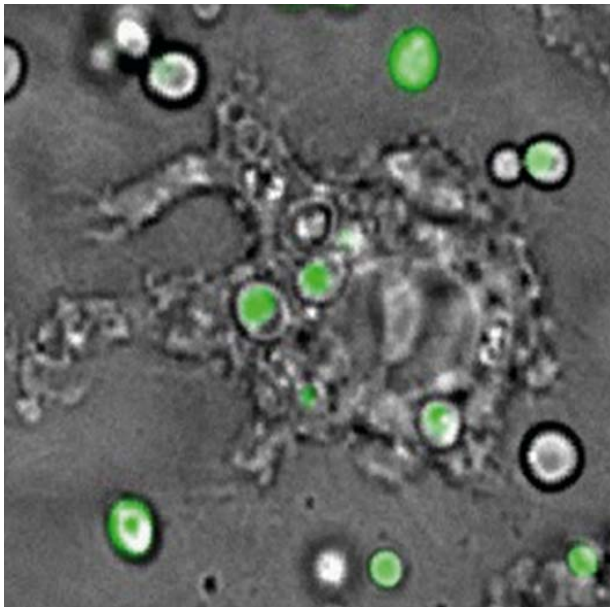
Examples for bioassays developed and used in the department. Left: Bacterial isolates of Staphylococcus epidermidis *strain 29002 from colonized central venous catheters are cultivated in microtiter plate wells and treated with compounds. Lower row: regular biofilm in 96-well microtiter plate, upper two rows show effects on biofilm architecture caused by synthetic peptides (in collaboration with W. Bautsch, Klinikum Braunschweig); Right: Assay for invasion of HEp2-cells by the bacterium* Streptococcus pyogenes. *The differential staining procedure shows internal streptococci in red, and external in yellow. The compound screen looks for changes in the ratio of red to yellow bacteria. Together with G. S. Chhatwal (Dept. Microbial Pathogenesis). Photo (le): HZI; Photo (ri): HZI, Rohde*

Identification of molecular targets of anti-infectives

Fungal infections are among the most important nocosomial (hospital) infections, in particular, in immuno-compromised patients, and new targets for therapeutic intervention have to be identified urgently. That is why we focus the in-depth mechanistic analysis on compounds targeting *Candida albicans* as a representative pathogen, or macrophages, as representatives of the innate immune system.

At the molecular level, recognition of *C. albicans* by macrophages stimulates intracellular signal transduction cascades resulting in the expression of genes, such as of nitric oxide (NO) synthase or of various cytokines in the macrophages and of stress protecting genes in *C. albicans* – stress from starvation, osmosis and oxidation. We established assays to monitor nitric oxide production, induction of cytokines (TNFα, IL-6) and the phagocytotic activity of the macrophage.

We could show that these activities are indeed influenced by selected chemical compounds. Although a reduced phagocytotic activity of macrophages after incubation with the actin-interfering compound cytochalasin was expected, the stimulation of nitric oxide and cytokine production by some natural compounds, which were described to affect the dynamics of microtubuli, was not described before. Additionally, we could show that otherwise non-cytotoxic compounds, such as the kinase inhibitor purvalanol, are able to suppress cytokine or nitric oxide production. For the elucidation of the underlying molecular mechanisms, we established 2D-gel electrophoresis for proteome analysis, with particular consideration of protein phosphorylation events.



Fluorescently labelled Candida albicans *(green cells) meet a macrophage (cell line RAW 264.7) and are eliminated by phagocytosis.* *Photo: HZI, Rohde*

In *C. albicans* we observed a strong influence of the genetic background on the susceptibility to different fungicides. Three out of five wild-type strains proved to be resistant to compounds which were previously identified through their activity on *Hansenula anomala,* also known as *Pichia anomala.* These were described to cause intracellular glycerol accumulation. Thus, we have established miniaturized assays for the determination of metabolites and enzyme activities involved in glycerol metabolism. As additional tools, we study various *C. albicans* mutants, in which genes related to stress protection were inactivated.We also exploit the comprehensive collection of viable single-gene mutants from the surrogate strain *S. cerevisiae*. Differential antifungal susceptibility profiles of mutant versus corresponding wild-type strains will highlight pathways affected by the fungicides. First results confirm stress response pathways as potential targets. These investigations will be complemented by comparative gene expression analysis of resistant and susceptible strains. We also observed the capability of strains to adapt to stress conditions and compound treatment. By applying a systems biology approach, we will analyse the dynamic behaviour of the intracellular signalling networks. Mathematical models may then reveal key reactions which are perturbed by the small molecules.

Acknowledgements We thank Prof. Ken Timmis, previous head of the Division of Microbiology, for his initial involvement in and support of the Chemical Pipeline. We are grateful to all our co-workers as listed in the respective project reports.

Ronald Frank born 1948, studied chemistry at the University of Hamburg; Dipl.-Chem. 1974; PhD at Institute of Organic Chemistry, Hamburg; Dr. rer. nat. in Chemistry, Biochemistry and Microbiology, 1979; PostDoc at GBF, research group DNA-Synthesis, 1980-1984; since 1985, senior scientist at the HZI; since 2003 Head of Department of Chemical Biology; co-founder of AIMS Scientific Products, 1998. Coordinator of the Chemical Pipeline.

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 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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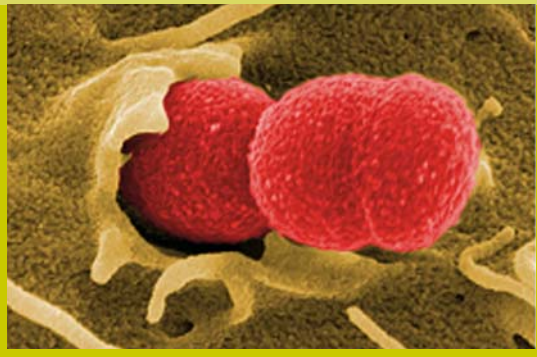
FOCUS RESEARCH REVIEWS SPECIAL FEATURES



Photos: left: Invasion of Streptococcus pyogenes *serotype M3 into a human epithelial cell (HEp-2)* | *centre and right: Two of the strains of Myxobacteria discovered by Prof. Reichenbach and his co-workers:* Nannocystis exedens *ssp.* pulla *(ce) and* Chondromyces robustus *(ri).* | *Photos: HZI, Rohde (le)* | *Reichenbach (ce & ri)*

SCIENTIFIC REPORTS FACTS AND FIGURES

Bacterial Pathogenesis: Insights into a New World Discovered by High Resolution Field Emission Scanning Electron Microscopy (FESEM)

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Pathogens have evolved a wide variety of strategies to adhere to and invade the host cells. Rearrangements of the actin cytoskeleton are often involved in the zipper-like, or trigger-like, uptake processes. Other pathogens co-opt existing endocytotic pathways of the eucaryotic host cell, or they trigger host cell responses for their own advantage by injection of proteins through a secretion system. Most of these pathways have been identified and studied with light microscopic methods like phase-contrast, immunofluorescence and confocal microscopy. Especially high resolution confocal microscopy has pushed forward considerably our knowledge of the cross-talk between pathogen and host cells.

One drawback of confocal images is that they only depict labelled colourized components and structures in a dark background and are not able to image the surrounding reference space around the labelled structures at the same time. Especially for this purpose scanning electron microscopy (SEM) has been shown to be a potent tool to considerably extend observations made by other approaches.

Only SEM studies could demonstrate convincingly the morphological basis of, for example the trigger- and zipper-like entry mechanisms of pathogens. The introduction of field emission scanning electron microscopes (FESEMs) – a further step forward in resolution power – allowed for the first time the labelling of proteinaceous pathogenicity factors of interest with gold-particles which are then detectable on the ultrastructural level of bacteria at magnifications up to 200,000 times. Modern FESEMs are often equipped with a cryo-unit which, in combination with high-pressure freezing, allows one to image the morphology of specimens in a vitrified status to overcome the possible introduction of artefacts by aldehyde fixation and dehydration in conventional imaging.

The Electron Microscope Platform at the Helmholtz Centre for Infection Research (HZI) is equipped with a Zeiss field emission scanning electron microscope (FESEM) DSM982 Gemini with an installed EDX analysis system for elemental analysis and an attached cryo-stage for cryo-FESEM work. The FESEM facility is widely used by nearly all research groups throughout the HZI. The main task is to develop and perform techniques for ultrastructural and morphological analysis and immuno-localization of proteins. FESEM techniques are used especially to visualize the adherence to, and invasion of, host cells by a wide range of pathogens. Preparation protocols have been customized to fulfill the wide requests of different researchers on the campus.

In addition, new methodologies have been developed to immuno-localize pathogenicity factors on the bacterial cell surface, or the interface between bacterial and host cell membranes, using FESEM. Furthermore, gold-particles coated with isolated proteinaceous pathogenicity factors have been proven to be a useful tool for studying the crosstalk between pathogen and host cell. Furthermore, the platform is equipped with two transmission electron microscopes (TEM) which are used to support findings by FESEM studies especially for immuno-localization of pathogenicity factors inside bacteria or host cells and for following the intracellular trafficking of pathogens.

Historical background of Scanning Electron Microscopes (SEM) At the same time the first transmission electron microscope was nearing completion by Knoll and von Ardenne in Berlin-Lichterfeld in the 1930s, a scanning electron microscope (SEM) prototype was also constructed. Unfortunately, the resolution of this first SEM was no better than that of a light microscope. Over the next 30 years, some refinements and improvements were incorporated into the SEM by Zworykin at RCA Laboratories in the United States and McMullan and Oatley at Cambridge University in England. Nevertheless, it was not until the mid-1960s that the first commercially available SEM was sold, the Cambridge Mark Stereoscan.

Since then, several companies have been involved in the development of modern SEMs, such as Zeiss, Philips (nowadays FEI), Cambridge, Hitachi, and Jeol. Zeiss introduced the first digitally controlled SEM in 1985, the DSM950. Another innovation highlight was the introduction of a field emission emitter as the electron beam source which pushed the resolution power of a field emission scanning electron microscope (FESEM) with a Schottky cathode to around 1 nm at an acceleration voltage of 15 kV or to 4 nm at only 0.1 kV.

Working principle of a SEM The electron source and the lenses in the SEM are comparable to a transmission electron microscope (TEM). However, instead of forming an image by passing through a thin sample onto a phosphorescent screen, the lenses in a SEM are used to generate a demagnified, focused spot of electrons that is scanned, in most cases, over the surface of the electrically conductive specimens. The impact of the electrons on the sample generates a variety of different signals, including low energy secondary electrons (SE-electrons) from the uppermost layers of the specimen. These SE-electrons are detected by an Everhart-Thornley SE-detector or inlens SE-detector.

How is the image formed? For each point where the electron beam strikes the sample and generates SE-electrons, a corresponding pixel is displayed on the viewing monitor. The brightness of the pixel is directly proportional to the number of emitted SE-electrons of the corresponding sample surface. Since the electron beam is focussed as a very fine beam and scanned over the sample, the numerous pixels start to form a continous-tone image composed of many density levels or shades of gray. This shading in the image is similar to a conventional black and white photograph, in which light and dark areas give the impression of depth. This conveys the three-dimensionality of SEM images.

The resolution of a SEM is mainly limited by the achievable spot size of the beam on the specimen surface. A convenient magnification for imaging with wolfram cathode-equipped SEMs is around 5000x. SEMs with an electron source with a higher brightness, such as the Lanthanhexaborid (LaB6) cathode, were then introduced. This pushed the resolution of a SEM down to around 5 nm at 20 kV with a magnification up to 20000-30000x.

These magnifications were useful to study interactions of pathogens with host cells, but these SEMs could not, for example, resolve gold labelling of pathogenicity factors on the bacterial surface. Only the introduction of field emitter cathodes (like the Schottky emitter) in the early 1980s extended the resolution further down to around 1 nm at 15 kV, resulting in a routine magnification for such FESEMs in the range of 10000-100000x. Resolving a gold-particle down to 5 nm in size in labelling studies was now achievable.

Even more important is the high resolution power at lower kV values between 1-5 kV of around 2 nm at 5 kV and 4 nm at 1 kV which, as a consequence, allowed for the first time a real surface imaging of the specimens. Fig. 1 illustrates the differences in resolution of an identical specimen when imaged with a thermionic Lanthanhexaborid (LaB_6) cathode (Fig. 1A) and a Schottky field emitter cathode (Fig. 1B). The image of *Escherichia coli* EPEC bacteria with the LaB_6 cathode revealed – imaged at an acceleration voltage of 10 kV – a blurred picture of the bacterial surface with no distinct structural details visible, whereas the image taken with a field emitter shows clear distinct patterns on the bacterial surface demonstrating the excellent resolution even at 1 kV.

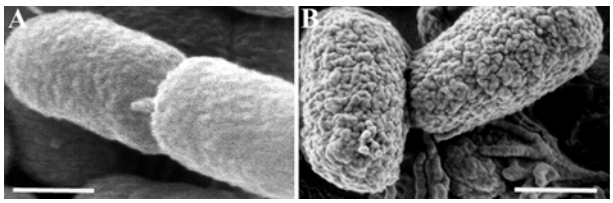

Fig. 1. Comparison of the resolution power. A lanthanhexaborid (LaB_6) equipped conventional SEM is unable to resolve distinct protein patters on the surface of Escherichia coli *EPEC at 10 kV(A) whereas a field emission SEM depicts these patterns even with at a ten times lower acceleration voltage of 1 kV (B).*

Imaging of host pathogen interactions A prerequisite for imaging samples in a FESEM is the excellent preservation of the ultrastructure by aldehyde fixation and dehydration of the samples with organic solvents, like acetone and ethanol. For biological samples a critical-point drying with liquid CO2 must be performed before sputter coating with gold for electric conductivity of the specimen. When imaging specimens are prepared in such a way in a FESEM,

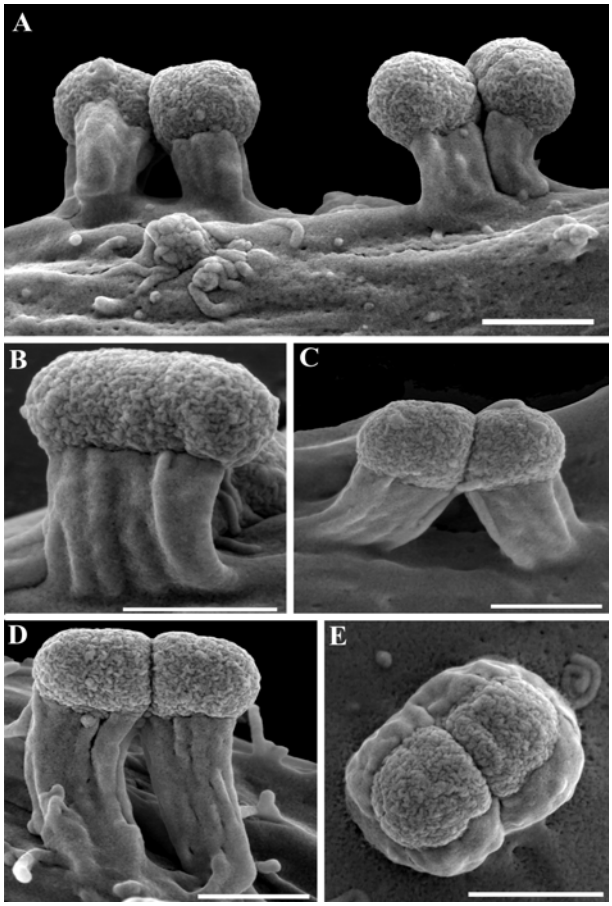

Fig. 2. Pedestal formation of E. coli *EPEC on fibroblast cells. Images show the pedestal formation process at different observation angles, A-D side views, E top view. Bars represent 0,5 μm.*

crystal clear images can be obtained, as depicted in Fig. 2. Shown is the formation of actin pedestals triggered by *E. coli* EPEC at high resolution and imaged from different viewing angles by tilting the object stage in the FESEM. For example, Fig.2B and D represent side views, whereas Fig. 2E is a top view.

Another aspect of viewing such images should also be mentioned. These images give quite an aesthethic view of what nature has created, demonstrating that science, as seen through a high resolution FESEM and with the operator's photographic eye, is an exploration of natural beauty and of the mechanisms which have created it.

These images give a very convincing view of the actin pedestal formation process, but the question lingers whether these FESEM studies extend our view when compared with, for example, confocal images of the pedestal formation process. Support and extension of earlier findings, due to the higher resolution power of a FESEM compared to light microscopic techniques, is demonstrated in Fig. 3 and Fig 4. In Fig. 3 the entry process of *Streptococcus pyogenes* is depicted as seen by a Z-series in a confocal microscope (Zeiss LSM510 meta, 10 succesive sections are shown). The host cell actin was stained with phalloidin-Alexa green, whereas invading streptococci chains were stained with an anti-Streptococcus antibody and a secondary antibody coupled to Alexa-red.

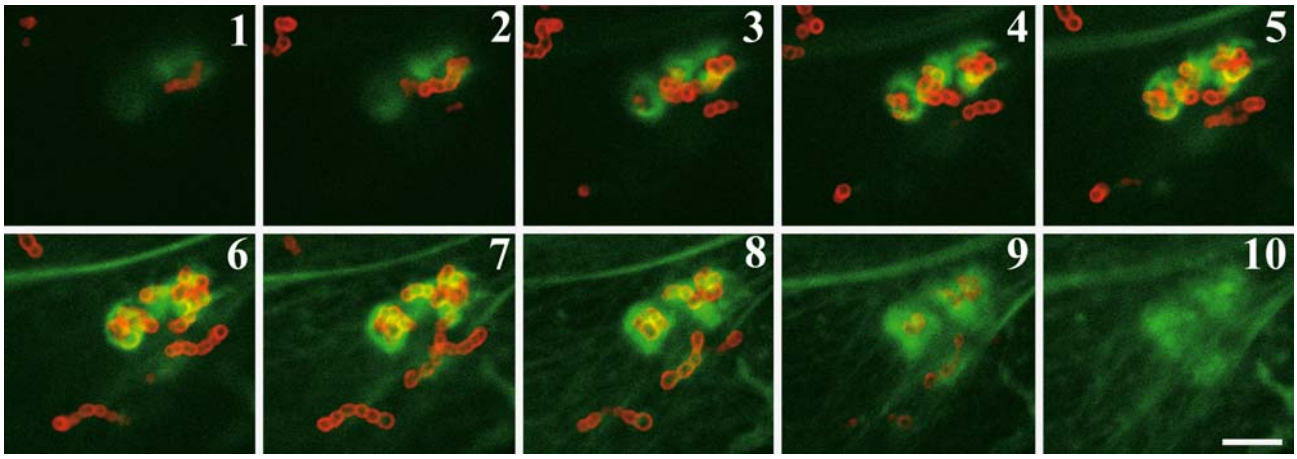

Fig. 3. Confocal Z-series images of invading Streptococcus pyogenes *into human endothelial cell (HUVEC). Invading streptococci (red) are surrounded by host cell actin (green) at the side of entry. Bar represents 2,5 μm.*

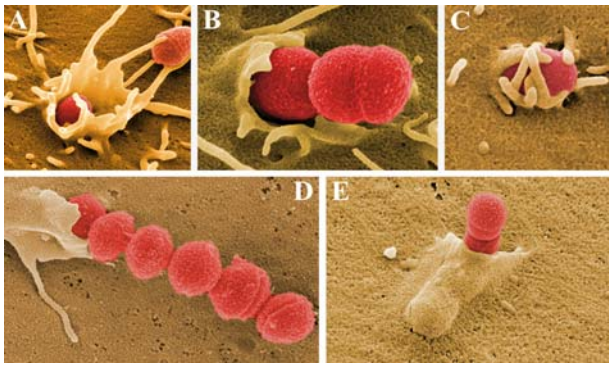

Fig. 4. Multiple invasion mechanisms of S. pyogenes *clinical isolates. A and C depict invasion of serotype M1 and B of serotype M3 streptococci into human epithelial cell (HEp-2), whereas in D and E the invasion into human endothelial cells (HUVEC) by serotype M3 streptococci is shown.*

The confocal Z-series shows that actin rearrangements (green) are involved in the uptake process of the bacteria because a cloud of actin can be seen around the invading streptococci. However, these images give no morphological details of what the actin rearrangement looks like. Do we find a zipper-like invasion (the invading streptococci are very closely covered with the host cell membrane), or a trigger invasion mechanism with extended membrane-ruffling at the attachment side of the streptococci, or are some other invasion mechanisms involved in the uptake process?

If one compares these images with the FESEM images shown in Fig.4 it is obvious that a wide variety of different actin rearrangements can be easily distinguished when the invasion process of different clinical streptococcal isolates is viewed by FESEM. Invasion processes can start with either extended membrane-ruffles in which the invading streptococci reside (Fig. 4A), with only minor actin assemblies around the bacteria (Fig. 4B), or when only a very intimate contact with the host cell microvilli on the surface is visible (Fig. 4C). The streptococci shown in the confocal images in Fig. 3 invade by a mechanism which can be described as zipper-like (Fig. 4D and E). However, this fact was not deducible from the confocal images. The confocal images give only a colour picture of the components involved in the uptake process in an otherwise dark background. They, therefore, are unable to image the surrounding reference space to give more detailed information.

Another example of a newly detected invasion mechanism of Group A streptococci is depicted in Fig. 5. Applying high resolution FESEM it was demonstrated that streptococci carrying streptococcal fibronectin-binding protein I (SfbI) after binding to fibronectin, trigger a signalling pathway that results in co-opting of caveolae, components of a eucaryotic endocytotic pathways, for their own uptake and benefit. The aggregated caveolae (Fig. 5A, arrow heads) start to fuse and form large invaginations in the host cell membrane (Fig. 5B) through which streptococci can invade the cell (Fig. 5C). After caveolae-mediated uptake streptococci reside in caveosomes which do not fuse with the lysosomes of the host cell, therefore ensuring survival and persistence.

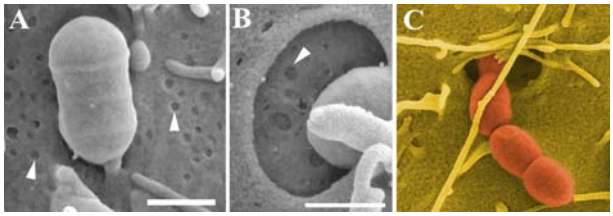

Fig. 5. Fibronectin- SfbI protein mediated invasion by co-opting host cell caveolae. Caveolae (arrow heads) aggregate around adherent streptococci (A) and fuse to form large invaginations (B). Through these invaginations streptococci are internalised (C) and reside inside the host cell in caveosomes. Bars represent 0.5 μm.

Detection of a novel sheated structure of the type IV secretion system of *Helicobacter pylori* *H. pylori* is the causative agent of different gastroduodenal diseases and has a role in the development of gastric cancer. Bacteria of the type 1 Hp strains possess a cag pathogenicity island which codes for a type IV secretion system. This was shown to be involved in the infection of the cag protein into the host cell with subsequent interleukin-8 secretion by the cell.

Antibodies against two proteins of the secretion system labelled two distinctly different parts of the bacteria (Fig. 6). Antibody HP0527 was made against a virB10 homologous protein and HP0532 was made against a virB7 homologous protein of *Agrobacterium tumifaciens,* in which the type IV secretion system was first described and characterized. HP0527 labelled a structure which seemed to be facing away from the bacterium as a kind of an appendage (Fig. 6A), whereas HP0532 labelled certain areas on the bacterial surface (Fig.6B).

More ultrastructural details were not obtainable from the confocal microscopic studies. When *H. pylori* was examined at high magnification (up to 100.000 times) in the FESEM filamentous-like surface organelles were easily detectable, which extended mostly from one pole of the bacterium (Fig. 7A). The organelles consisted of a central rigid needle (arrow) which is focally and, when in contact with host cells, totally covered by a proteinaceous structure (arrow heads).

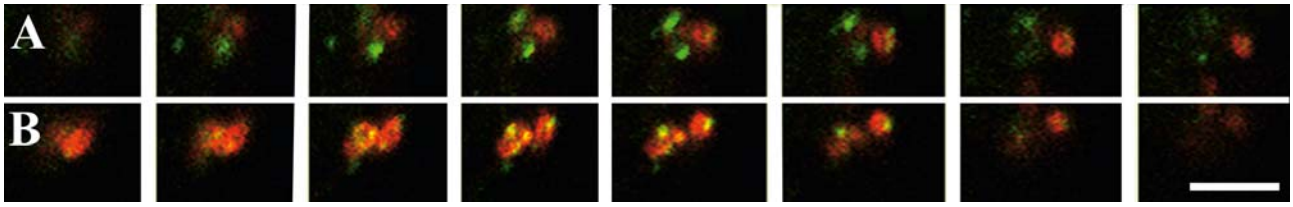

Fig. 6. Confocal Z-series images of two labelled proteins of the type IV secretion system of Helicobacter pylori. *In A the antibody HP0527/Vir B10 labelled a protein on a filamentous-like structure of the bacterium whereas antibody HP0532/Vir B7 labelled distinct areas on the bacterial surface (B). Bar represents 2* μm.

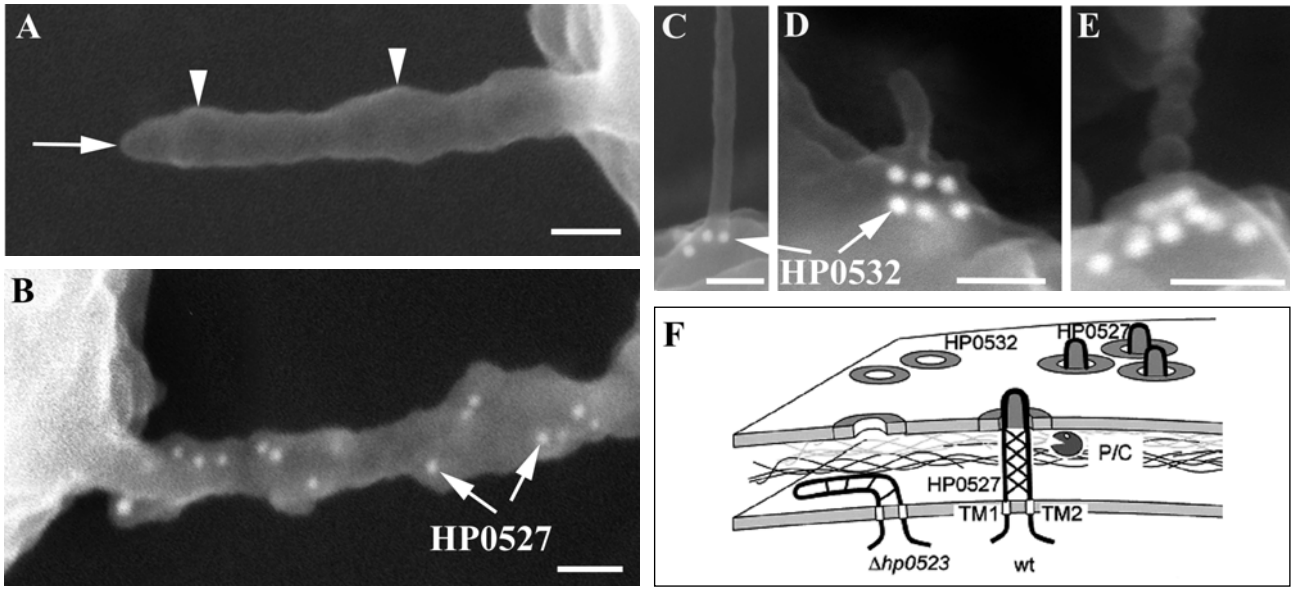

Fig. 7. FESEM analysis of filamentous-like structure and immuno-label of two different proteins. The image depicted in A reveals that this organelle consists of a central rigid needle (arrow) which is partially or totally covered by protein (arrow heads). The protein is detectable with the HP0527/Vir B10 antibody coupled to protein A-coated gold-particles (B, white dots, arrows) alongside the needle. In contrast, antibody HP0532/Vir B7 detects a protein on the base of the filamentous organelle in the bacterial cell wall (C-E); F depicts a schematic drawing of the HP0527 secretion and assembly. Bars represent 100 nm.

The advent of the high-resolution annular "in-lens" secondary electron detector, which is built in the case of the Zeiss Gemini above the Gemini objective lens, produces high contrast images. This allows for the detection of gold-particles down two 2 nm even in the FESEM and enabled researchers to transfer immuno-localization studies from the post-embedding labelling on ultrathin sections for TEM applications towards Immuno-FESEM labelling studies. This has the tremendous advantage that the labelled protein can be imaged in the surrounding ultrastructural details.

Applying the two antibodies in Immuno-FESEM showed that HP0527/Vir B10 protein is located alongside the needle (Fig. 7B) and HP0532/Vir B7 is located only on the base of the filamentous organelles (Fig. 7C-E). Needles which were only partially covered with protein exhibited no label for both antibodies. Furthermore, for the Vir B10 homologeous protein, a significant increase in the amount of protein could be demonstrated when *H. pylori* were in contact with AGS host cells. These labelling studies together with the underlying visible ultrastructure of the bacteria enabled the deduction of a scheme of the HP0527 secretion and assembly process (Fig. 7F).

Electron microscopy has long been thought of as providing only a static picture. With time, this kind of slight misconception has been changing gradually towards an approach which includes also some kinetic, dynamic data of processes following a triggered process; for example, by pulse-chase experiments, or in combination with immunogold labelling studies of specimens which have been fixed at different time points after initiating a certain event.

As mentioned earlier SfbI-carrying streptococci bind via the bridging molecule fibronectin to α5ß1 integrins. For integrins it is known that they start to trigger a signalling pathway only after clustering of the integrin molecules has occurred on the cell surface. When SfbI protein was coated onto gold-particles with 15 nm in size and these complexes were incubated together with human endothelial HUVEC cells, the onset of integrin clustering could be followed by FESEM in a kinetic manner. In Fig. 8A it is demonstrated that the gold complexes existed only for single or double SfbI-gold-particle complexes. When applied to HUVEC cells, a more or less random distribution of the SfbI-gold complexes (white dots, arrows) was detectable after 15 minutes (Fig. 8B). After 45 minutes it was obvious that the SfbI-gold complexes were aggregated and a few caveolae (arrow heads) could be detected in the near vicinity (Fig. 8C). After

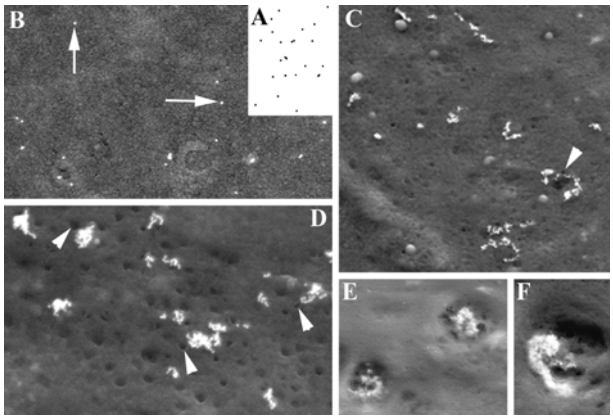

Fig. 8. Indirect visualization of integrin-clustering on the surface of human endothelial cells (HUVEC). SfbI-coated gold-particles (A) bind to α5β1 integrins via fibronectin. After 15 min a random distribution is detectable on the cell surface (B, arrows). After 45 min the gold-particles start to aggregate (C) and a few caveolae (arrow head) can be identified around the gold aggregates. After 1 h larger SfbI-gold aggregates appear together with numerous caveolae (D, arrow heads). Then the formation of large invaginations is triggered and after 2 h most SfbI-gold complexes are found inside the invaginations (E and F).

one hour, these aggregates were even more pronounced and a high number of caveolae were present around them (Fig. 8D). The formation of large invaginations was detectable after two hours and most of the SfbI-gold complexes were found in these invaginations, whereas the surrounding HUVEC cell membrane was nearly devoid of any SfbI-gold (Fig. 8E and F).

These studies unequivocally demonstrated the clustering of the integrin molecules - not only by indirect visualization via SfbI and fibronectin and gold-particles -, but showed that the aggregation of host cell caveolae, triggered by a signal of clustered integrins, also aggregate around the SfbI-gold complexes with subsequent up-take into the cell.

Cryo-FESEM studies of capsule and binding of the extracellular matrix Conventionally prepared specimens for FESEM have the intrinsic problem that dehydration has to be performed.This results in the introduction of, in some cases, artefacts into the samples. To overcome this problem cyro techniques have been introduced especially for TEM,

such as high-pressure freezing, freeze etching or freeze substitution. Only a very few facilities have been dedicated to cryo studies with FESEM. During the past few years more and more applications have been described in the literature and the potential in this method is beginning to be appreciated mostly by microbiologists and cell biologists.

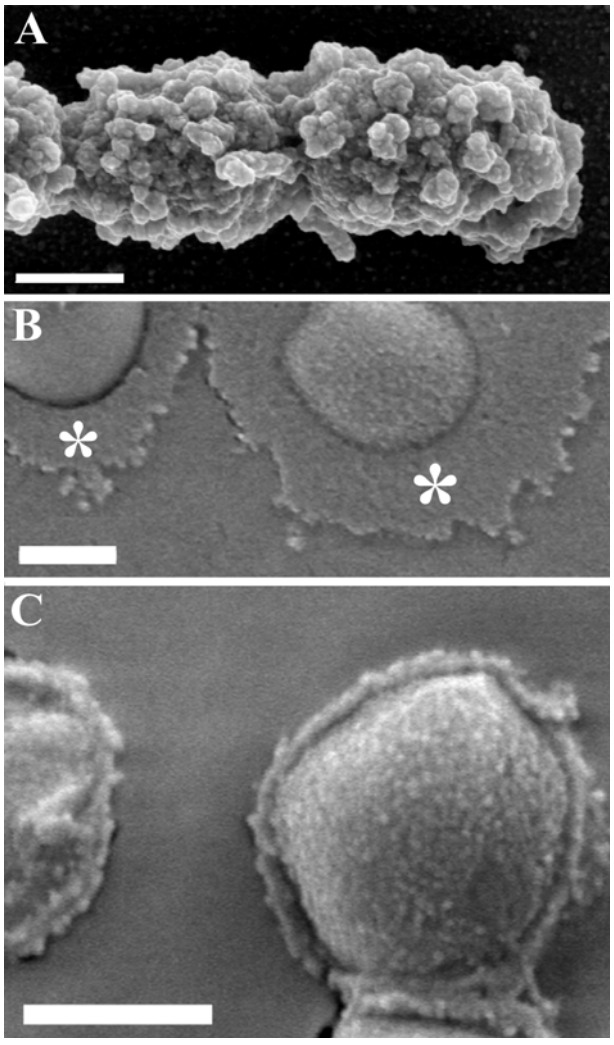

Fig. 9. Comparison of conventional and cryo prepared pneumococcal capsules. In A the collapse of the capsule after dehydration and critical-point drying is visible. After cryo preparation a well preserved capsular structure is retained for extracellular pneumococci (B, star) whereas isolated intracellular bacteria show a loss of capsule (C) demonstrating phase-variation of pneumococci during internalisation. Bars represent 0.5 μm in A and 0.25 μm in B and C.

The capsular polysaccharide of *Streptococcus pneumoniae* is an important virulence factor and protects against phagocytosis. Since the bacterial capsule is a highly hydrated structure it is very hard to preserve these delicate structures for observation in an electron microscope. The introduction of capsule stabilizing agents, like lysine in combination with ruthenium-red, allowed visualization of the capsule in the TEM. However, when such treated specimens were prepared for FESEM the collapse of the capsular structure due to dehydration artefacts was easily visible (Fig. 9A). This problem could be overcome by freezing the same sample in liquid nitrogen, with subsequent freeze-fracturing and freeze-etching. The FESEM image depicts a very well preserved capsule of *S. pneumoniae.* (Fig. 9B, stars). In addition, this method allowed a direct measurement of the size of the capsule of different clinical isolates. Cryo-FESEM also demonstrated that invading pneumococci lose their capsule during uptake since intracellular bacteria exhibit no capsular structure anymore (Fig. 9C). This phenomenon is known as phase-variation.

Another example of cryo-FESEM is depicted in Fig. 10. For *S. pyogenes* strains it was shown by studies with radiolabelled collagen IV that they are able to recruit collagen type IV via surface-bound fibronectin. Furthermore, recombinant SfbI-protein was also able to bind collagen via fibronectin. Cryo-FESEM was applied to study the matrix assembly potential of two different *S. gordonii* strains, the wild-type strain and a strain expressing the fibronectin-binding repeat region of the *S. pyogenes* SfbI-protein.

The wild-type strain exhibited only the Gram-positive cell wall after freeze-fracturing and freeze-etching (Fig. 10A), whereas the SfbI-protein expressing strain was covered with a dense layer of fibronectin and collagen (Fig. 10B, stars). Interestingly, not only the cell surface of an individual bacterium was covered, but the matrix assembly around adjacent bacteria was also detectable, which resulted in the formation of aggregates (Fig. 10B, large image).

By comparison, the wild-type strain formed a straight line of bacteria (Fig. 10A, large image). The binding of collagen was demonstrated by immuno-labelling with collagen antibodies on the TEM level. The dark dots represent bound collagen on the bacterial surface (Fig. 10C). Furthermore, collagen recruiting streptococci were also able to colonize collagen fibres (Fig. 10D).

Therefore, by a direct visualization of the matrix assembly process and by imaging of the binding to collagen fibres, FESEM has contributed to unraveling a novel aggregation and colonization mechanism for streptococci.

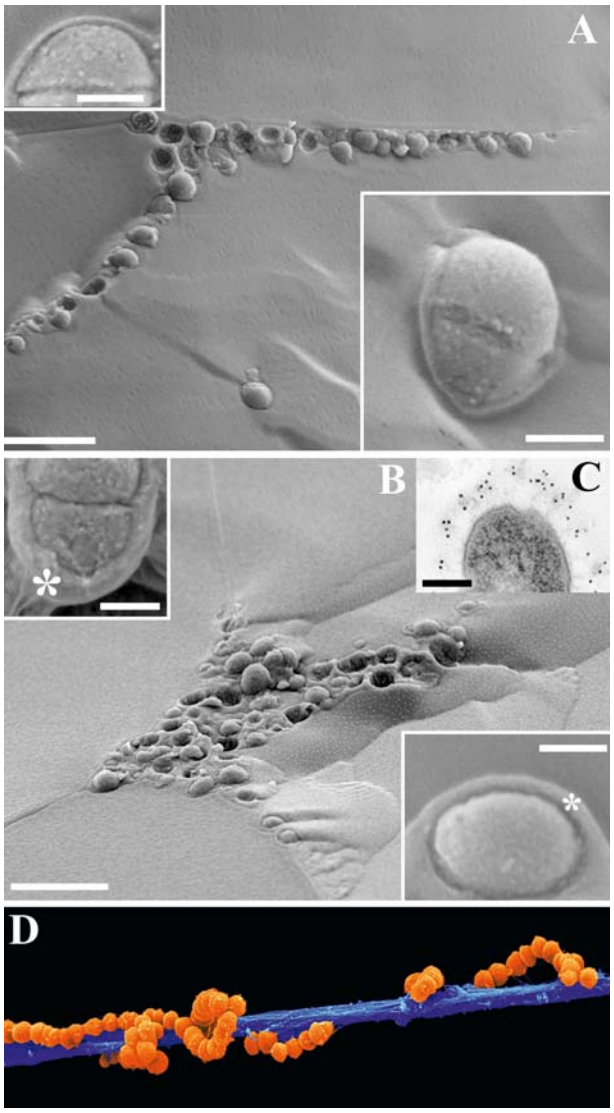

Fig. 10. Matrix assembly of SfbI expressing S. gordonii *strains. Cryo FESEM was applied to demonstrate the assembly of collagen in the presence of fibronectin on the bacterial surface. Freeze-fractured* S. gordonii *exhibit the Gram-positive cell wall (A) whereas SfbI-expressing* S. gordonii *show a dense layer of collagen around a single bacterium (B, stars) and between adjacent bacteria resulting in the formation of large aggregates, thus preventing phagocytosis by neutrophils. In C the presence of collagen on the bacterial surface is demonstrated by immuno-labelling with anti-collagen antibodies and protein A-gold on an ultrathin section (TEM image). D depicts the binding capability of SfbI-expressing* S. gordonii *to collagen fibres. Bars represent 5 μm in the large images of A and B and 0.5 μm in the inserts, 0.5 μm in C.*

Conclusion and perspectives The use of electron microscopy has declined considerably over the past decades despite the incredible ability of a wide array of EM methods to provide information usually not available by other approaches. In this aspect it seems that the loss of interest in the use of electron microscopy began as new technology emerged for light microscopic techniques, especially videomicroscopy and high resolution confocal microscopy. A short glance in cell biology or microbiology orientated journals undoubtedly reveals that EM image plates have been more and more replaced by colourful confocal image plates. Furthermore, the methods for immunofluorescence have been constantly improved such as fluorescence resonance energy transfer (FRET) to identify biochemical reactions in living cells or 2-photon confocal microscopy. Although some of the light microscopic technology has been pushed to achieve resolutions of around 100 nm electron microscopy remains to be the only technique with sufficient high resolution to combine sensitive protein detection with detailed information of the underlying substructures of bacteria or eukaryotic cellular compartments.

With the advent of high resolution FESEMs electron microscopy has been revitalized and some new electron microscopic centres have been established. The few examples in this article of high resolution FESEM analysis of host–pathogen interactions have demonstrated convincingly that scanning electron microscopy is a very powerful tool in unravelling such interactions. To develop a comprehensive view of the host-pathogen interactions future electron microscopic studies must stand side by side with complementary light microscopic, genetic, biochemical and molecular approaches to unravel the still unknown secrets of pathogenic bacteria and their interplay with the host cell. New developments in respect to electron microscopic preparation methods like high-pressure freezing, freeze-substitution and cryo-electron tomography and the newly invented transmission and scanning electron microscopes will open up new fields of insights into the world of host-pathogen interactions.

All photos: HZI, Rohde

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Natural Products: An Indispensible Source of New Drugs

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Three decades ago, we started a research project on natural products from bacteria at the Helmholtz Centre for Infection Research, formerly German Centre for Biotechnology, GBF. From the beginning, this was a close cooperation between the Departments of Microbiology, later renamed Biology of Natural Products, and Chemistry of Natural Products, but also involved other departments, *e.g.,* Instrumental Analysis and the Fermentation Service of the centre.

Soon, industry partners became interested as well and tested our compounds for medical and agricultual applications. In the course of the years we gained more and more experience, and the project expanded considerably. In the end, around 600 compounds had been isolated and characterized, belonging to about 100 basic structures. Many of those compounds were entirely new and often had interesting and unusual mechanisms of action. This is reflected in the more than 1,000 publications by laboratories all over the world, which are synthesizing our compounds, using them in studies on mechanisms of action or as tools in biochemical research, or investigating the genetics of their production. Three compounds were extensively tested for application (sorangicin, melithiazol, soraphen), and one became a clinical candidate as an anticancer drug (epothilone).

Why did we embark on such a programme? Natural products – normally they are secondary metabolites – play a highly important role in medicinal and, somewhat less so, in agricultural chemistry. Of the 877 new drugs of low molecular weight introduced between 1981 and 2002 worldwide, 50 to 60% were natural products or derivatives thereof, or were synthesized using natural products as leads. This contribution increases even further in certain fields of application, *e.g.*, to 78% among antibacterials, or to 74% among anticancer drugs. The high yield of bioactive substances among natural products may be explained by the function those compounds have for their producers: They are selected for interaction with biomolecules. Although in many cases we do not know their exact role in the life of the producing organisms, their selective advantage may be deduced from the complex genetic apparatus required for their biosynthesis – which would not be retained without a selective pressure –, and by the fact that among the structural variants produced by an organism the main compounds are almost always the most potent ones.

The specific biological activity of natural products is due to their chemical diversity and steric complexity, which is far beyond that normally seen in synthetic compounds. In order to produce a well-defined, unique effect, the drug must fit certain regions of the target molecule – virtually always a protein – in a key-lock mechanism. A particular steric structure of the drug and a defined pattern of reactive groups on it are a prerequisite for such a close interaction. This is because the target proteins themselves have a precise steric structure, which sets high standards for the configuration of a selectively interacting drug met by only very few compounds.

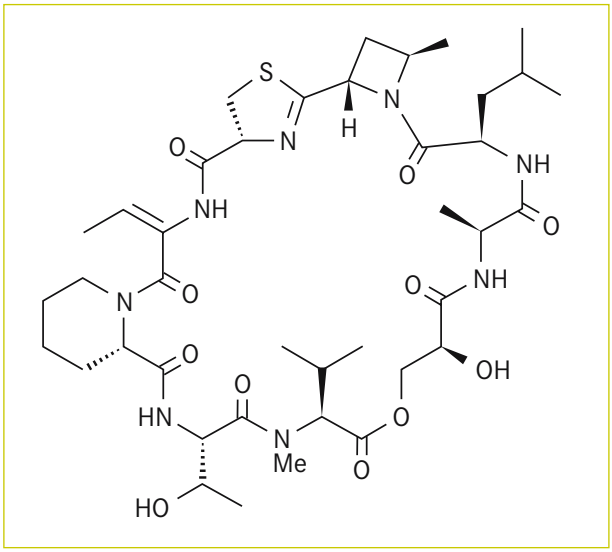


Fig. 1. The elucidation of the mechanism of action of a new compound is by no means trivial and may easily take years to achieve. According to NCI data, the cytotoxic vioprolids, which only act on eukaryotic cells, have a mechanism of action not known from any other drug. So far, it defied all efforts of elucidation.

The enormous pool of chemically diverse natural products offers a good chance to discover such a compound. Amazingly, and in spite of the restrictions just mentioned, sometimes compounds of entirely different chemical structure produce the same specific effect. Thus, for example, rifampicin and sorangicin both block specifically eubacterial RNA polymerase by binding to the ß subunit. In fact, there even is substantial cross-resistance between the two. Similarly, paclitaxel and epothilone both bind to the ß subunit of tubulin thereby stabilizing microtubuli.

When interacting compounds have been discovered, their binding to the target protein can be determined and their interactions explained. But the hope to design new drugs *de novo* on the basis of information about the target did not materialize. As a rule, the interactions of drug and protein are too subtle, and the comparatively huge target molecule too complex to reliably identify sensitive regions.

Incidentally, a further advantage of screening natural products is the possibility of discovering new targets nobody had in mind before. This was the case with several of our myxobacterial compounds, *e.g.*, soraphen, ambruticin, and myxothiazol.

A paradox: Active on targets in the human body Another interesting aspect is that natural products may act on proteins for which they have not been designed because their producers are never confronted with them, *e.g.*, proteins involved in human metabolic diseases. The explanation is that the evolution of proteins does not rely primarily on variation of amino acid sequences in the polypeptide chains. Such variation would be astronomical since a middle-sized protein of 250 amino acids in length has $2x10^{325}$ possible sequences, most of which would, however, have many energetically equivalent conformational forms and thus would be useless for a protein with a defined purpose. Rather, the tens of thousands of different proteins present in the human body, for example, are estimated to be composed of only 600 to 800 distinct domains; that is, compact, mostly globular sections of proteins, 50 to 350 amino acids long, with a defined folding pattern and a stable conformation.

New proteins appear to arise during evolution mainly by reshuffling and modification of domains. A protein may consist of one to a dozen different domains, which usually have different functions in the protein. And a certain domain may appear in various proteins with quite different tasks. Many of those domains have been around for a very long time and are present in quite diverse organisms, even bacteria. It is obvious that natural products may act on proteins in different organisms and of different use, if those proteins are composed of structurally similar domains.

After what has been explained before, the importance of natural products in drug discovery should be sufficiently clear: There is a large pool of structurally highly diverse compounds with a defined stereochemistry that have been selected for interaction with biological material and are directly available for screening. So far, no other strategy is able to supply such a body of chemical material and knowledge. Yet, after a peak interest in natural products in the 1970s and 80s, industry reduced research in this field more and more. This may be the main reason why the annual number of new chemical leads worldwide has gone down from an average of 30 to 17.

Why is it that industry lost interest in natural products? There are several reasons. One is: too expensive and too slow. Natural products usually appear in extracts as complex mixtures, which are not well tolerated by many of the sophisticated modern screening tests. And the supply of additional material may become difficult.

However in past decades much progress has been made in analytical and preparative chemistry as well as in the synthesis of even very difficult molecules. In addition, the amounts of substance needed for screening and structure elucidation have been reduced dramatically. Also, identification of the active compound in a mixture, and making sure that it is not already known (deconvolution) is now much facilitated by modern equipment.

Another reason for the decline of interest in natural products is the competition of resources. In the past, industry invested enormous sums into the development of combinatorial synthesis and libraries. But while this approach may have the charm of being intellectually attractive to chemists and indeed provide huge numbers of compounds, the success story for pharmacologists is still poor. This may change with time by the introduction of more sophisticated chemistry and by making use of structural elements derived from decomposed natural products. Of course, chemical modification of natural lead structures has always been done. For example, ixabepilone (Bristol-Myers Squibb) and ZK-EPO (Schering) have been selected from more than 300 semisynthetic and synthetic variants, respectively, of epothilone. This is necessary, because the extensive

interaction of a drug with its target molecule is only one requirement for a good drug: Side effects, stability in the body, penetration into organs and cells, elimination and other pharmacokinetic and pharmacodynamic properties are equally essential.

As far as the costs for natural products research are concerned, a look on the expenses for the development of new drugs is revealing. The cost of the anti-HIV drug, Fuzeon, is estimated to have amounted to more than € 600 million without promotion. More than € 300 million went, however, into clinical trials, and only about € 6 million into research.

Clearly, the cost of finding a good new lead is marginal in comparison to drug testing and the process of production development.

Still, another reason for the scepticism toward natural products is restrictions on collecting biological material in many parts of the world, imposed by the 1992 Rio Convention on Biological Diversity, which makes it necessary to negociate treaties with various governments, who often put forward unrealistic claims.

Producers of natural products This brings the question of producers of natural products to attention. Currently, more than 150,000 natural products may be known, many of them structurally related, however. Most of them have been discovered in plants, which have been a source of remedies for human ailments since time immemorial. More recently, ethnopharmacology has exploited the knowledge of medicinal plants used by natives, but systematic studies of the chemical repertory of plants have also been performed in many places for many years.

The study of secondary metabolites of microorganisms had to wait until general microbiology had advanced sufficiently in the 1930s and 40s. Very few earlier applications of microbial secondary metabolites are known, *e.g.* of ergot alkaloids of secale cornutum used to stimulate uterine contraction during child birth, or, more exotic, feeding fly-agaric (*Amanita muscaria*) to Swedish and Finnish warriors to stimulate aggression (also, to kill flies in fly traps).

But today, microbial secondary products have become a very important source, especially of antibacterials and antifungals. Microbial substances have the advantage that their production is independent of climate, season, and locality, as it can be done in large bioreactors of up to 400m^3 content.

In the past 30 years, marine animals, mostly plankton feeders, like sponges, corals, and tunicates, supplied many interesting compounds. Quite a few of them are closely related to microbial products, and it is very likely that most of them originate from the microbial food of those animals.

Myxobacteria – newly dicovered producers of secondary metabolites In contrast to plants, where many families are rich in secondary metabolites, production of such compounds is restricted to very few groups of microorganisms. At the time when we started our program, in 1975, apart from a small group of molds, only three groups of bacteria were known to be good at synthesizing secondary metabolites: the actinomycetes, by far the richest source (more than 10,000 compounds), the bacilli, and the pseudomonads. Since those groups had already been known as good producers for 35 years, it became ever harder to find new structures with them.

We therefore decided first to look for a new source of secondary metabolites, and by good luck almost at once succeeded when we came across the myxobacteria. Having worked with those organisms before, we had the experience to isolate and cultivate them, so we were able to obtain their products without much delay. Not to forget: the organization and equipment of the GBF were perfect for this type of work.

Myxobacteria are eubacteria living in soil and belonging, with about 50 species, to the Deltaproteobacteria class. Their rod-shaped cells move by gliding, or creeping, and produce coherent, somewhat slimy swarm colonies that spread on the culture plate. They are social bacteria with highly developed communication systems. When living conditions become unfavorable, hundreds of thousands of cells aggregate, pile up, and produce fruiting bodies. Those may be simple, slimy, globular masses (Myxococcus), but also may become little treelets with a stalk and sporangioles on top *(e.g., Stigmatella, Chondromyces)*. Myxobacterial fruiting bodies usually are 0.1 to 1mm in size, and often can be recognized with the naked eye. Inside the maturing fruiting body, the vegetative cells convert into desiccation-resistant myxospores. In this form the bacteria may survive 5 to 15 years of drought at room temperature. Myxobacteria have the largest genomes known from bacteria. The genome of one strain of *Sorangium cellulosum,* a cellulose degrader, which has been fully sequenced at the GBF and in the institute of Prof. Pühler in Bielefeld, is 13.04 Mbp long, about three times the size of the *E. coli* genome.

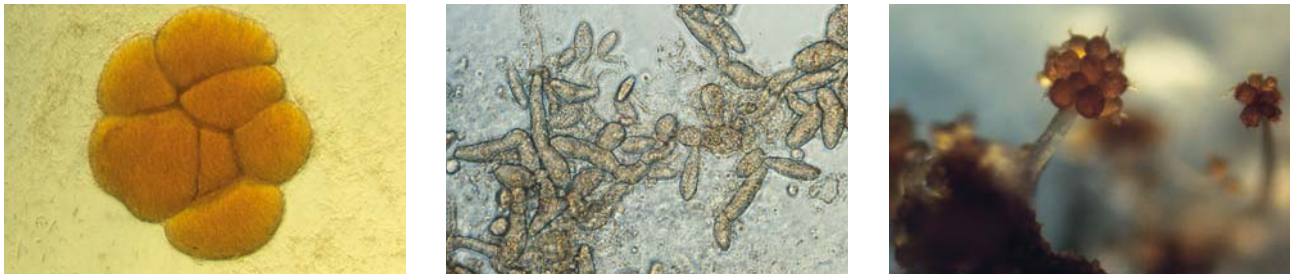


Fig. 2. To keep up our screening, it was necessary to isolate continuously new strains. This resulted in the end in a collection of 7000 strains of myxobacteria, the largest ever brought together and available worldwide. It is now deposited at the German Collection of Microorganisms (DSMZ). As a side-effect, we discovered 9 new species, 5 new genera, and 2 new families, the largest addition to myxobacterial taxonomy in the past 80 years. Examples are (fruiting bodies of) a) Byssovorax cruenta*, a cellulose-degrader; b)* Nannocystis exedens *ssp.* pulla*; and c)* Chondromyces robustus. *Photos: Reichenbach*

Why do myxobacteria synthesize so many secondary metabolites? We can only speculate about this. One reason may be that myxobacterial swarms are fixed to the spot in their habitats and thus are forced to defend the place. Also, by killing and enzymatically lyzing other organisms they may provide themselves with essential nutrients. Further, since most myxobacteria live by decomposing other bacteria, they are constantly exposed to foreign DNA and may pick up pieces useful for biosynthesis of secondary metabolites. Thus, the epothilone gene cluster appears to contain sections that come from somewhere else. As the analysis of the metagenome in soil suggests that there are many thousands of still undescribed bacterial species – there would be a huge reservoir of genes indeed. But not all myxobacteria are equally rich in secondary metabolites. Genera, we found to be good producers are *Sorangium, Polyangium, Chondromyces, Myxococcus, Cystobacter, Archangium,* and *Stigmatella.* Also, the origin of strains may play a certain role. Strains isolated from samples coming from cold and temperate climates are often inferior to strains from warm environments. Many strains synthesize several, up to 7, chemically distinct substances with different mechanisms of action.

What kinds of compounds did we find in myxobacteria? Only two of our compounds were already known from other bacteria: althiomycin – from a streptomycete – and pyrrolnitrin – from pseudomonads. Several others are structurally related to known bacterial metabolites – archazolid, tartrolon, thiangazol, ratiadon, saframycin –, or to compounds isolated from marine animals - chondramide, apicularen, saframycin –, which is especially remarkable because only very few myxobacteria have been found in marine habitats.

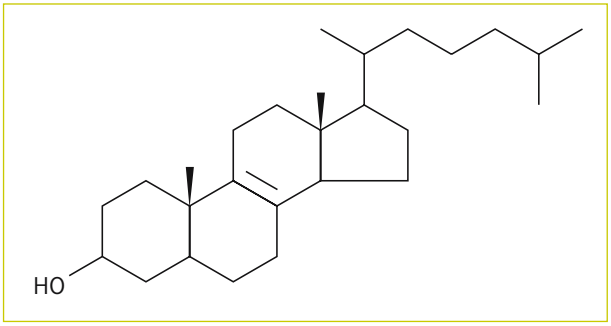


Fig. 3. Myxobacteria also synthesize very unusual primary metabolites, e.g.*, steroids. While steroids are typical constituents of eukaryotic cells, there is only one other bacterium known which synthesizes those compounds.*

But many myxobacterial compounds are entirely new. Mostly, they are polyketides or peptides – often with very unusual amino acids – and are synthesized on multienzyme complexes. In some cases – myxovirescin, epothilone, tubulysin – the polyketide synthases contain polypeptide synthetase modules. Some structures look rather exotic; *e.g.*, thiangazole with three thiazole rings and one oxazole in sequence, resembling tantazole from a marine cyanobacterium, or tartrolon B, one of only four known boron containing natural products.

Concerning application, the mechanism of action of a compound is of cardinal importance. Many of our myxobacterial substances show biological activity, which is, of course, a consequence of our screening methods. Initially, we exclusively used cultures of bacteria, yeasts, molds, and animal

cells as indicator systems. Only later, when sophisticated HPLC equipment became available, could we analyze culture extracts directly. We then discovered products without activity in our biological test systems. This approach is only meaningful, if the new compounds are subsequently introduced into elaborate and highly specialized screening tests as applied in the pharmaceutical industry.

Perhaps our most fascinating discovery is the five myxobacterial compounds that interact with the cytoskleleton of eukaryotic cells, an extremely rare mechanism among microbial substances. Rhizopodin has a dramatic effect on cell morphology: Animal cells, such as mouse fibroblasts, increase in size, round up, and sprout long, branched, runner-like extensions. The reason for this is a decay of the (fibrillar) F-actin that is responsible for shape, motility, and separation of daughter cells during cell division – by way of a contractile actin ring. The rhizopodin effect is irreversible: The cells become multinucleated, but continue to grow and survive for several weeks.

The chondramides have the opposite effect: They stimulate actin polymerization and stabilize the F-actin, which, too, results in large, multinucleated cells. The chondramide effect is identical with that of phalloidin, one of the toxins of the death-cap *(Amanita phalloides)*. In fact, chondramide prevents the binding of phalloidin to F-actin, *i.e.*, it has the same binding site.

More relevant as potential anticancer drugs are compounds that act on the tubulin cytoskeleton, because they block mitosis and induce apoptosis, *i.e.*, the controlled cell death. There are two types. Disorazol causes disappearance of microtubules from cells at concentrations of less than 100pM, and a depolymerization of microtubules *in vitro* at µM concentrations. A similar effect is observed with tubulysin, remotely related with dolastatin 10, which was originally isolated from a sea hare, *Dolabella auricularia*, but, in fact, is produced by the marine cyanobacterium, *Symploca* spec., the food of the mollusc. The mechanism of action of tubulysin was studied, and very similar morphological changes of tubulin were found as for dolastatin. The biosynthesis of tubulysin in *Pyxidicoccus fallax* (formerly *Angiococcus disciformis*) is performed by a non-ribosomal peptide synthetase/polyketide synthase hybrid, but requires additional modifying enzymes.

Epothilone The most important contribution of myxobacteria so far is epothilone, which, like paclitaxel, promotes tubulin polymerization and stabilizes microtubuli. The compound blocks the cell cycle in the G2/M phase, *i.e.*, before chromosome separation during nuclear division. It induces apoptosis, but may be less toxic than the substances mentioned above because it leaves the microtubuli in non-dividing cells intact, although it abolishes their dynamicity. Microtubuli, however, are essential for intracellular transport processes.

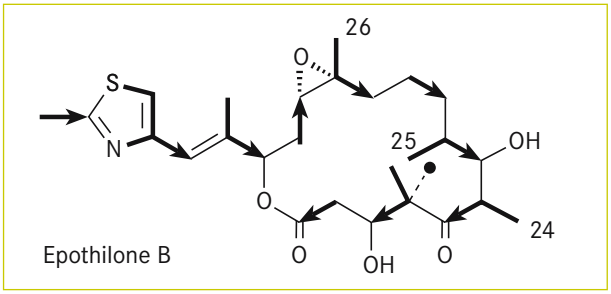


Fig. 4. Epothilone B is biosynthesized from acetate, propionate, cysteine, and a single methyl group, as indicated by the arrows.

Epothilone is especially valuable because it is immune to cellular export mechanisms and also to several mutations in ß tubulin that confer resistance to paclitaxel, so that it is still active on multi-drug resistant (MDR) cancer cells. It therefore may help patients who no longer respond to other cytotoxic drugs.

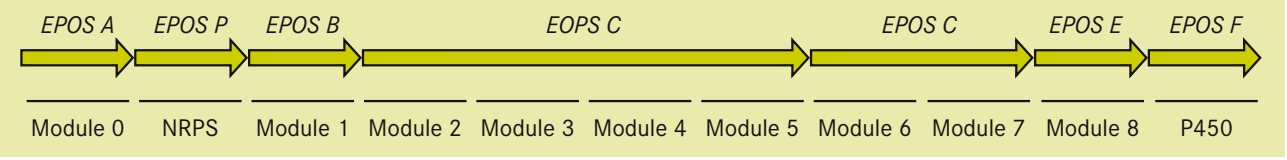
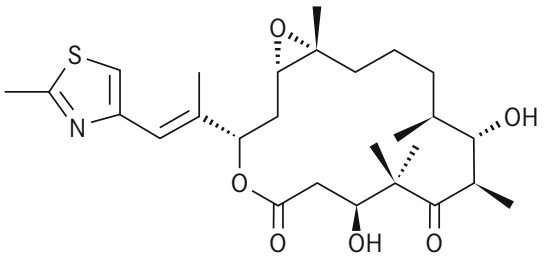
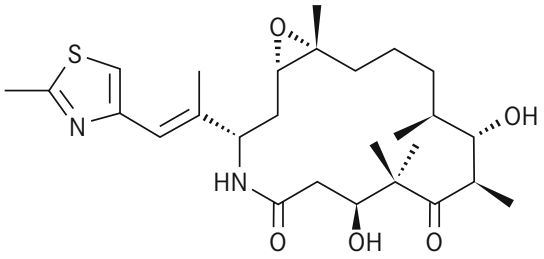


Fig. 5. As is typical for microbial secondary metabolites, the genes for epothilone biosynthesis are in a cluster consisting of 56,000 base pairs (fully sequenced, and patented, by Novartis: Molnar et al., ChemBiol 7:97, 2000). Note that the polyketide synthase contains a non-ribosomal peptide synthetase module, EPOS P.

The discovery of the mechanism of action of epothilone in 1995 set off tremendous activities in research institutes and the pharmaceutical industry worldwide. Since then, more than 500 articles have appeared on syntheses, analogs, structure-activity relationships, biosynthesis, genetics, biochemical effects, and preclinical and clinical results. Several clinical candidates have been promoted by Bristol-Myers Squibb, our partner (ixabepilone), Novartis (patupilone and methylthio epothilone), Schering (ZK-EPO), and Kosan/ Hoffmann-La Roche (KOS-1584). Ixabepilone has by now completed phase III of clinical evaluation and probably will be presented to the FDA for approval in the near future.



Epothilone B **1b** (EPO-906)



Ixabepilone **23** (BMS-247550)

Fig. 6. In ixabepilone, the clinical candidate of Bristol-Myers Squibb, the lactone group (the oxygen in the ring) is replaced by a lactam in order to make the compound more resistant to the attack of esterases in the human body.

So, what can be learnt from this whole story? When one tries to develop a new field of research, it is naive to expect a quick success. Methods have to be developed, which is time consuming and not very gratifying because there is not much to publish. One unavoidably makes mistakes, misses interesting possibilities of application, does not find suitable partners at the right time, has to convince critical decission makers, etc. Yet, with patience, tenacity, and some luck, ultimately results may be obtained that justify the effort and perhaps induce other investigators to continue this kind of research.

Prof. Reichenbach Born in Karlsruhe 1936. Studied biology, chemistry and geography at the universities of Karlsruhe, Kiel, München, and Freiburg. State examination (Freiburg 1961), doctor's degree (Karlsruhe 1965). Postdoctoral fellow 1966-1968 (Minneapolis, Morgantown). Research assistant 1968-1975 (University of Freiburg, Prof. Drews), habilitation 1971 (General Microbiology, Freiburg). Since 1975 head of the Departments of Microbiology (1975-1984), of Microbial Secondary Metabolites (1984-1994) and of Biology of Natural Products (since 1994) at the HZI in Braunschweig. Apl. Professor at the Technical University of Braunschweig (1976). Retired in 2001.

FOCUS RESEARCH REVIEWS SPECIAL FEATURES

Photos: left: Matthias Weide, RG Upstream Processing, during his laboratory work | centre: Peggy Riese is preparing for a PCR-experiment | right: Elena Reinhard during the preparation of an ELISA experiment using a laboratory robot
Photos: HZI, Bierstedt

SCIENTIFIC REPORTS FACTS AND FIGURES

INFECTION AND IMMUNITY

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

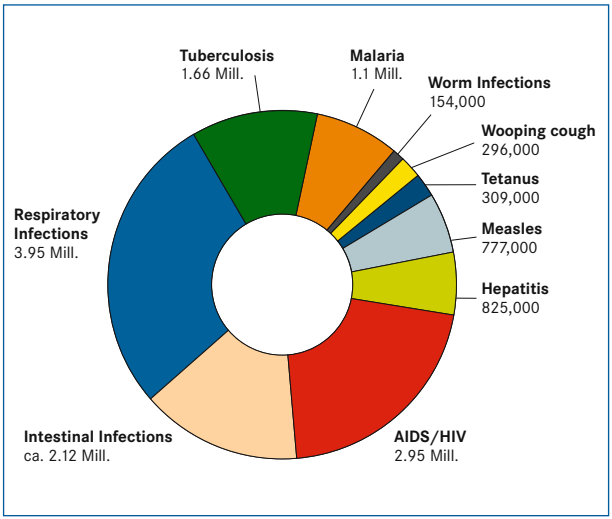
Infections cause one third of all disease-related death cases worldwide. Even though improved hygiene and the availability of antibiotics and vaccines have lead to a continuous decline in the outbreak of such diseases in recent decades, we are confronted with the fact that many infectious diseases are 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 started to become a global threat once again.

In industrial countries infectious diseases have acquired a new, but onerous impact due to modern high-tech medication: transplant patients or those under intensive care are very 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 focusses 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 for establishing new diagnostic and effective therapuetic 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 genes, combined with knowledge about the interactions of microbial genes with host cellular genes, is an excellent basis for the directed design of chemotherapeutic strategies against microbial pathogenes. Functional genome analysis also provides insight 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 undermined by certain microorganisms, resulting in latent or chronic infections, are still only barely understood.

The research programme “Infection and Immunity” of the Helmholtz Centre for Infection Research covers 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 drugs and strategies to prevent and treat 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 a 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 prevent and treat 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

Microorganisms

TOPIC SPEAKER | **Prof. Dr. G. Singh Chhatwal | Division of Microbiology | gsc@helmholtz-hzi.de**

Microbial infections were, and still are, a serious hazard for human health. In spite of the availability of a large number of anti-infectives, the mortality and morbidity due to microbial diseases remain high in developing, as well as industrialized countries. Prerequisite to developing novel combat strategies is a complete understanding of the processes by which pathogenic micro-organisms cause diseases and the identification of the microbial factors involved. Therefore, in this subject area, the objective is to study the biology and the molecular biology of microorganisms with the goal being to identify and characterize virulence factors, to elucidate the structure-function relationships of these factors and to analyse cellular networks and microbial communications.

This topic will employ different genetic, molecular and structural approaches on virulence factors of a number of relevant micro-organisms. The current choice of pathogens, streptococci, pneumococci, *P. aeruginosa*, Yersenia and influenza virus has been made on the basis that they stand for specific biological features and that sufficient information and expertise has been accumulated.

Group A streptococci cause a wide spectrum of diseases in humans, including invasive diseases and sequelae, such as rheumatic fever. Pneumococci are responsible for life-threatening diseases, such as pneumonia and meningitis. Oral streptococci, besides their association with dental diseases, have gained a lot of health relevance in the last few years because of their ability to cause diseases like septicemia and endocarditis. *Pseudomonas aeruginosa* is the most dominant bacterial pathogen causing chronic lung infection in cystic fibrosis patients.

One of the common strategies employed by pathogenic microorganisms is the diversity of the strains. This topic therefore also deals with the sequence analysis and subsequent comparisons to understand why certain strains cause diseases, whereas other highly related strains do not. Also, the knowledge of the function of genetic pathways, for example, those implicated in essential metabolic pathways, can be used to identify molecular targets for the design of novel antimicrobial drugs.

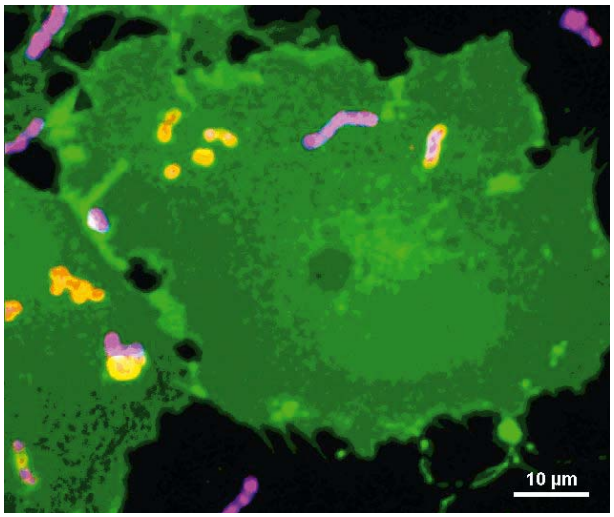
To achieve these objectives, this topic constitutes four different projects. These projects, although different in expertise, show a high degree of synergy and bring together joint efforts to reach the goals of this topic. The topic includes the following projects:

Identification and characterization of virulence factors of group A, C and G streptococci, oral streptococci, and pneumococci. Emphasis is on the factors that are responsible for different disease manifestations. For epidemiological analysis of strepto-coccal diseases a custom-designed DNA array is being used. The interaction of the identified bacterial factors with the host cells is another approach to elucidate disease mechanisms and to define intervention targets.

The treatment of chronic microbial infections such as lung infections caused by *Pseudomonas aeruginosa* with usual therapeutic agents has not yielded favourable outcomes because of biofilm formation. The project therefore deals with the molecular mecha-nisms of biofilm formation and is generating information about the evolution and adaptation mechanisms of *P. aeruginosa* to its habitat. This information will lead to the development of novel antimicrobial treatment regimes.

Cell-to-cell communication, also called “quorum sensing”, is an important pathogenic process that leads to biofilm formation. Understanding and interfering with microbial communication therefore opens up new opportunities for fighting infectious diseases and developing potential alternatives to antibiotics. This project deals with the elucidation of mechanisms of biofilm formation and identification of the involved microbial factors by using *Streptococcus mutans* as a model organism. Search for inhibitors of biofilm formation is also one of the goals of this project.

Microorganisms express an arsenal of virulence factors that enable them to establish infection in the host and evade immune defence. In order to understand how these factors interfere with the host cell processes, a structure biological approach is employed in this project. The focus in the last two years has been on the type III secretion system of certain Gram-negative pathogens and the virulence factors of the influenza virus. This analysis should help to understand underlying molecular mechanisms of pathogenicity, which is a prerequisite to design new control strategies.



Invasion of streptococci in endothelial cells. Endothelial cells were transfected with GFP Rac1 in (green) and then infected with streptococci. After one hour the intracellular and extracellular streptococci were differentially stained. Extracellular streptococci are shown in magenta, and intracellular bacteria are stained reddish-yellow. *Photo: HZI, Rohde*

01.1 Structural Analysis of Virulence Factors

PROJECT LEADER | **Prof. Dr. Dirk Heinz | Division of Structural Biology | dih@helmholtz-hzi.de**

PROJECT MEMBERS | Isa Astner | Karsten Bruns | Carina Büttner | Dr. Susanne Freese | Dr. Joop van den Heuvel | Dr. Hartmut Niemann | Dr. Alok Sharma | Dr. Wolf-Dieter Schubert | Jörg Schulze | Dr. Victor Wray

Bacteria, viruses and other microbial pathogens infect the host by means of a limited arsenal of virulence factors. They specifically interfere with the host cell processes, for instance, to enable the pathogen to spread inside the host, or escape from the immune reaction. The aim of this project is the structural analysis of microbial virulence proteins using x-ray crystallography, or nuclear resonance spectroscopy (NMR), to learn more about the varied mechanisms of the pathogens, and develop from this knowledge new strategies to ward off the pathogens.

Another project deals with the structural analysis of important metabolic enzymes.

Type-III-secretion systems: Infections by the needle Gram-negative bacteria, such as the plague causing pathogen *Yersinia pestis,* possess a so-called type-III secretion system (T3SS) by means of which a certain class of virulence factors, known as type-III effectors, can be injected into the host cell with a fine molecular needle.

To make it possible for the effectors to get into the host cell, they need to be kept in a translocation competent conformation by so-called chaperones inside the bacteria cell. The structure of SycT (Fig. 1), the chaperone for the *Yersinia* protease YopT, indicates that SycT forms a 2:1-complex with partly folded YopT by utilizing two prominent hydrophobic areas.

The human-pathogen bacterium EPEC equally translocates the membrane receptor Tir via a T3SS, which permits the anchorage of bacteria on intestinal cells. Tir, in its phosphorylated state, is recognized by the host protein Nck, which activates further processes. The structure of the SH2 domain of Nck with a Tir-phosphopeptide illustrates this host-pathogen interaction for the first time at atomic resolution.

Virulence factors of the influenza virus Influenza A virus (IAV) is one of the most common pathogens threatening humans and animals, with the potential to cause disastrous pandemics. It is evident from at least three pandemics, the most serious outbreak being the “Spanish flu” (1918–1919), which claimed 20–50 million lives worldwide. The novel 87-amino acid protein PB1-F2, first reported in 2001, has been found to contribute to the pathogenicity of IAV by disrupting mitochondria. Recently, we have reported the first structure and unusual oligomerization of this pre-apoptotic protein and have proposed a model for mitochondrial interaction. Our findings should help to understand the underlying molecular mechanism of pathogenicity in highly infectious IAV strains.

ALAS, a key enzyme of heme biosynthesis Tetrapyrrols, such as heme, belong to the biologically most important pigments and play a fundamental role in life on earth by acting as co-factors in numerous proteins and enzymes. The biosynthesis of the macrocyclic molecules requires about a dozen enzymatic reactions.

The first step is being catalyzed by 5-aminolevulinic acid synthase (ALAS), whose dysfunction in humans can lead to serious anemias. The first crystal structure of an ALAS (Fig. 2), does not only explain the catalytic mechanism of the enzyme at atomic resolution, it also rationalizes the structural consequences of mutations in the enzyme leading to the known human diseases.

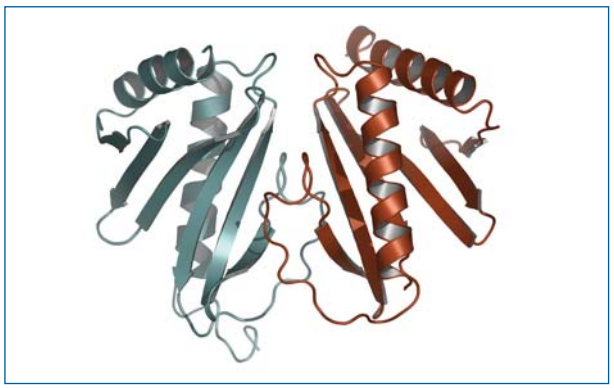


Fig.1. The structure of SycT, the chaperone for the Yersinia *protease YopT*

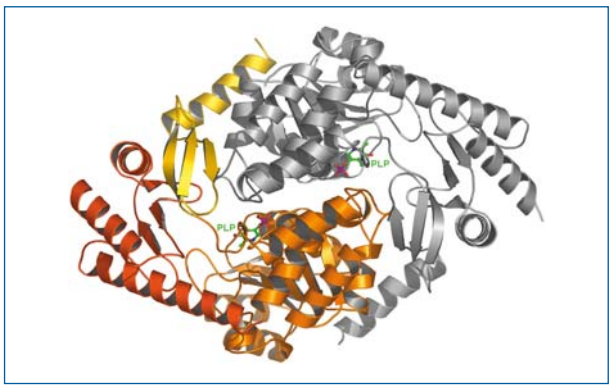


Fig.2. The first crystal structure of 5-aminolevulinic acid synthase

01.2 Pathogenesis of Chronic *Pseudomonas aeruginosa* Infections

PROJECT LEADER | **Priv.-Doz. Dr. Susanne Häußler | Helmholtz University Junior Research Group Chronic Pseudomonas Diseases | sus@helmholtz-hzi.de**

PROJECT MEMBERS | Florian Bredenbruch | Andree Meissner | Caroline Zaoui | Yusuf Nalca | Vanessa Jensen | Mathias Müsken

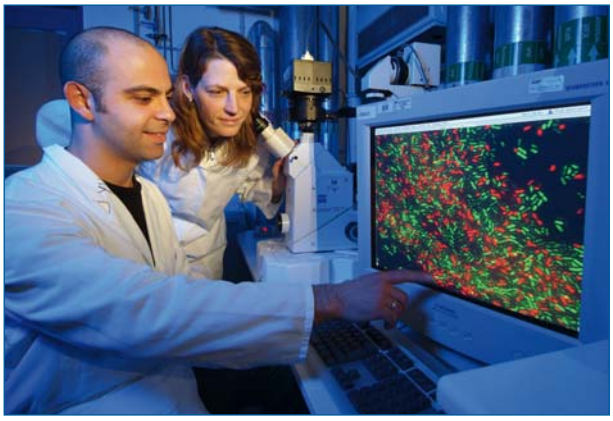
The diagnostic and therapeutic strategies that have served us so well in the treatment of acute bacterial diseases have not yielded favourable outcomes when applied to chronic infections where bacteria grow in matrix-enclosed sessile biofilm communities. Although every single cell is able to induce a 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 cooperation.

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

Small Colony Variants We have previously demonstrated that an adherent subgroup of so-called small colony variants (SCVs) is selected in the CF lung. The switch to an auto-aggregative biofilm forming SCV phenotype of *P. aeruginosa* seems to be linked to the expression of the "Chaperone Usher Pathway" (CupA) gene cluster. This gene cluster encodes for bacterial fimbriae and is regulated via the modulation of the newly identified bacterial signal molecule, cyclic di-GMP, in an antagonizing way. Future work will concentrate on the identification of *cupA* expression in clinical isolates and in *P. aeruginosa* within explanted CF lungs.

We furthermore aim to identify mutations that underlie the diversity of *P. aeruginosa* populations grown in biofilms. Very recently, a microarray hybridisation-based method has been established to map mutations within the *Helicobacter pylori* genome. Together with the company Affymetrix, we have started to design a similar custom-made array for *P. aeruginosa,* in order to identify mutations that evolve during *in vitro* biofilm growth and during the course of chronic *P. aeruginosa* infections. The knowledge about the genotypes that are specifically selected at different stages of the disease, should support our knowledge about the evolution and adaptation mechanisms of *P. aeruginosa* to its habitat. It might aid in the development of new antimicrobial treatment regimes for the clinical management of chronically infected CF patients.

Cooperation is supported by communication Apart from two homoserine lactones, *P. aeruginosa* produces a third intercellular signal that is referred to as the *Pseudomonas* quinolone signal (PQS). It is involved in cell density dependent virulence factor regulation - also known as quorum sensing (QS) - and the establishment of biofilms. We have dissected the biosynthetic pathway of PQS and demonstrated that PQS plays a significant role in bacterial iron homeostasis. Nevertheless, there seem to be PQS specific effects that are independent of the PQS effect on *P. aeruginosa* iron homeostasis. The detailed elucidation of the links between quorum sensing, the PQS regulon and genes involved in iron acquisition in *P. aeruginosa* will be a challenging task for the future.



Yusuf Nalca and Dr. Susanne Häußler discussing on fluorescence microscopic images of Pseudomonas aeruginosa *bacteria grown within biofilms.* *Photo: HZI, Bierstedt*

01.3 Virulence Factors of Streptococci and Pneumococci

PROJECT LEADER | **Prof. Dr. G. Singh Chhatwal | Department of Microbial Pathogenicity and Vaccine Research | gsc@helmholtz-hzi.de**

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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 are also emerging as important human pathogens and have recently been associated with rheumatic fever. Despite the availability of antibiotics, the mortality and morbidity due to these infections remains very high. Identification, molecular characterization and structure function analysis of their pathogenicity factors are a prerequisite to the design and development of novel control strategies.

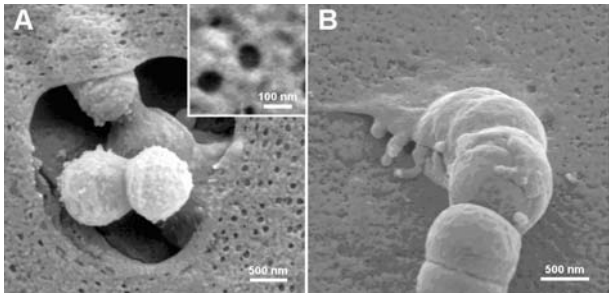
Streptococcal invasion and invasive diseases Invasion of streptococci in host tissue and cells is an important step in pathogenesis. We have now elucidated the mechanisms of invasion and intracellular survival. The internalization of streptococci takes place by fibronectin-mediated caveosome formation and by fibronectin-independent formation of membrane ruffles. Intracellular survival of streptococci leads to persistence and plays a key role in recurrent infections. The epidemiology of invasive streptococcal disease in Europe was studied using a custom-design DNA array with 227 genes – potentially – encoding virulence factors. The analysis underscores the complexity of the disease.

Animal pathogenics in humans Group C (GCS) and G streptococci (GGS) usually reside as commensals in both human and animals, but are also capable of causing severe infections. While group A streptococcus (GAS) causes rheumatic fever (ARF) and rheumatic heart disease (RHD), recent evidence has emerged linking GGS with RHD. As the aggregation of collagen type IV correlates with GAS strains of high rheumatogenic potential, we investigated whether GCS and GGS isolates have similar activity and whether there is a difference between animal and human isolates. Both have the ability to bind human type IV collagen, but only human isolates were able to aggregate the collagen on their surface. We have identified a peptide involved in collagen aggregation, which is present in certain surface proteins of GAS, GCS and GGS – it might represent an important diagnostic marker for rheumatogenic streptococci.

Virulence factors of alpha-hemolytic streptococci These streptococci are emerging as a serious health hazard due to their role in septicemia and endocarditis. Molecular events that make these bacteria highly pathogenic in niches other than the oral cavity are not fully understood. The aim is to describe new virulence mechanisms in alpha-hemolytic streptococci and the distribution of the new and the already known virulence factors within this group.

Therefore, we established a strain collection in cooperation with the university hospital in Leipzig. The DNA-microarray has been produced and we have identified a protein in the Anginosus-group that is similar to collagen binding protein FOG for GGS. PCR has identified an unknown protein that resembles M-proteins only partially and may be the product of a mosaic gene containing pneumococcal material.

Proteomic analysis of pneumococci Pneumococci reside as harmless commensals in the respiratory tract but also cause serious diseases. We used proteomic analysis in order to identify factors involved in conversion of commensals to pathogens. The proteomic analysis included 2,000 protein spots, out of which 240 different proteins were identified. These data were used for a comparative proteomic analysis between a virulent strain and an isogenic mutant. Through this analysis, two differential regulated proteins were detected, out of which one protein was identified as ABC-glutamin transporter.



Invasion of streptococci in human cells. The electromicrograph shows the accumulation of caveolae during the internalisation (inset A) and the formation of so called cavesomes during SfbI protein-mediated invasion (A). In contrast, he internalisation of streptococci belonging to serotype M3, which do not express SfbI protein, leads to formation of membrane ruffles (B)

Photos: HZI, Rohde

01.4 Microbial Communication

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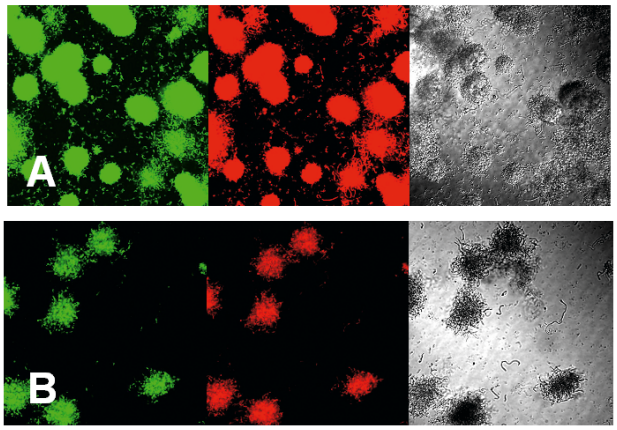
PROJECT MEMBERS | Dr. Helena Sztajer | Dr. Brigitte Kunze | Dr. Ramiro Vilchez-Vargas | Agnes Bodor | Johannes Leonhäuser | Ina Buchholz

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 sulfur 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 unraveling the environmental biology of this important marine group. We have discovered a great diversity of novel quorum sensing signaling molecules, the regulatory role of which is the subject of future functional genomics investigations.



Biofilm of Streptococcus mutans *UA 159 grown without (A) and with (B) Ambruticin, a compound isolated from Myxobacteria. Confocal laser scanning image of anaerobic biofilm stained with the bacterial live/dead fluorescence dyes.*
Photo: HZI

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 characterizing their mechanism of action.

Pathogenesis

TOPIC SPEAKER | **Prof. Dr. Werner Müller | Department of Experimental Immunology | wmu@helmholtz-hzi.de**

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.

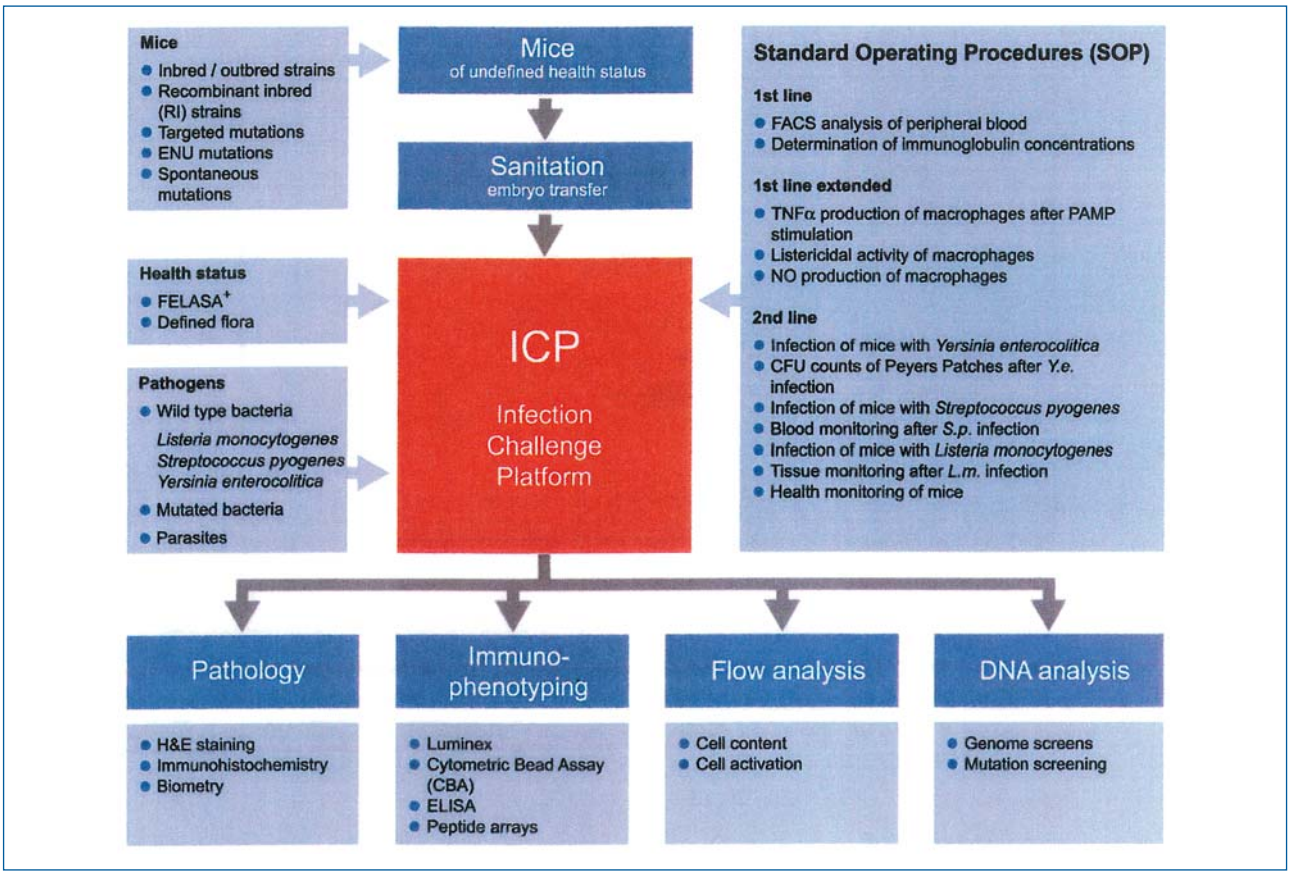
We approach the analysis of pathogenesis at various levels, starting with analysis of alterations on the protein network using proteomics, a task performed by the research group of Lothar Jäntsch. We analyse the signalling cascades induced by pathogens in cell lines that are genetically altered by either the specific knock-down of genes using RNAi technology, or by inactivating single genes by gene targeting. The groups of Stradal and 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, Andreas Lengeling, 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 groups of Lengeling and Müller perform 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.

Finally, the group 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.



The Infection Challenge Platform was established by the Department of Experimental Immunology in close collaboration with the junior research group Infection Genetics headed by Andreas Lengeling. This specialized laboratory allows the analysis of mechanisms of infections in vivo. *Graphic: HZI*

02.1 Molecular Mechanisms of Host-Cell / Pathogen Interactions

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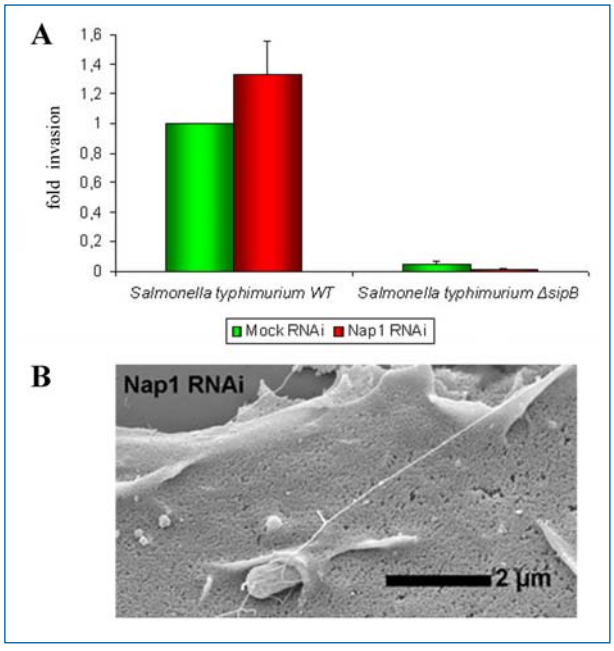
PROJECT MEMBERS | Dr. Frank P. Lai | Dr. Markus Ladwein | Tanja Bosse | Malgorzata Szczodrak | Jennifer Block

This project aims to characterise the precise molecular mechanisms driving actin reorganisation during the interaction of different pathogens with their hosts. Recent work in our, and many other laboratories has highlighted actin as a prime target for different pathogens to manipulate host cell behaviour for their own needs. This is not surprising, given the essential function of actin reorganisation in most types of cellular motility processes, including migration, phagocytosis or membrane traffic.

N-WASP Actin polymerisation is catalysed by protein complexes enhancing actin filament nucleation. A prominent example of this is the Arp2/3-complex, which is activated, for example, by proteins of the Scar/WAVE and WASP families. In previous years, members of the group contributed to work demonstrating the essential function of the WASP family protein N-WASP in different types of host-pathogen interaction. These include the intracellular actin-based movement of *Shigella flexneri* and the motility of pathogenic *E. coli* (EPEC and EHEC) on the surface of intestinal epithelial cells. Using cell lines genetically depleted of N-WASP, we were also able to demonstrate more recently an important contribution of N-WASP/Arp2/3-complex-driven actin assembly to the efficiency of growth factor internalisation via clathrin-mediated endocytosis. This physiological function explains why evolutionarily distinct pathogens such as vaccinia virus and pathogenic *E. coli* have independently evolved to exploit N-WASP's biochemical activities.

Invasion-pathways The actin regulators discussed above can plug into signalling pathways leading to activation of small GTPases of the Rho-family, with Cdc42 and Rac1 signalling to WASP and WAVE family proteins, respectively. Although genetic removal of Cdc42 did not abrogate cell migration and protrusion formation, this GTPase has appeared critical for the actin rearrangements accompanying InlB-dependent host cell invasion of *Listeria monocytogenes. In vivo,* this pathway allows bacterial entry into cells lacking E-cadherin, the expression of which is restricted to epithelial tissues. Listerial InlB directly interacts with the hepatocyte growth factor (HGF) receptor c-Met, eliciting actin-dependent cell surface rearrangements, which culminate in the dragging of the bacteria into the host cell cytoplasm. In this pathway, both Cdc42 - but not N-WASP - and the phospholipid kinase PI3-Kinase contribute to activation of Rac1, whose interaction with WAVE-complex is essential for InlB-induced invasion.

Membrane ruffling Gram-negative bacteria such as *Salmonella typhimurium* or *Shigella flexneri* can also induce their own phagocytosis into normally non-phagocytic host cells. As opposed to *Listeria*, bacterial effector proteins capable of cytoskeletal manipulation are directly injected into the host-cell cytoplasm using a type III secretion system. *Salmonella* invasion is normally attributed to dramatic local cell surface alterations, so-called ruffles, thought to function in engulfment of the bacteria. Nevertheless, we have recently established that neither Cdc42, nor its interaction partner N-WASP, or the WAVE-complex, are essential for *Salmonella* internalisation, although the latter is absolutely required for membrane ruffling. Consistent with this, the presence of local ruffles is not a prerequisite to *Salmonella* entry. Identification and characterisation of the actin regulators essential for *Salmonella* and *Shigella* invasion are currently a subject of intense investigation.



(A) Invasion of wildtype (WT) Salmonella typhimurium *is increased rather than decreased in WAVE-complex-defective cells (cells stably suppressed for WAVE-complex expression by RNA interference targeting Nap1). ΔsipB is a type III-secretion-defective* Salmonella *strain employed as negative control. (B) Scanning electron micrograph showing entry of wildtype* S. typhimurium *into Nap1 siRNA-treated cell (Nap1 RNAi). Note the absence of prominent ruffles known to accompany bacterial entry in the presence of WAVE-complex (collaboration with Dr. Manfred Rohde, HZI).* *Photo: HZI, Rohde*

02.2 Identification and Characterization of Bacterial Virulence Factors

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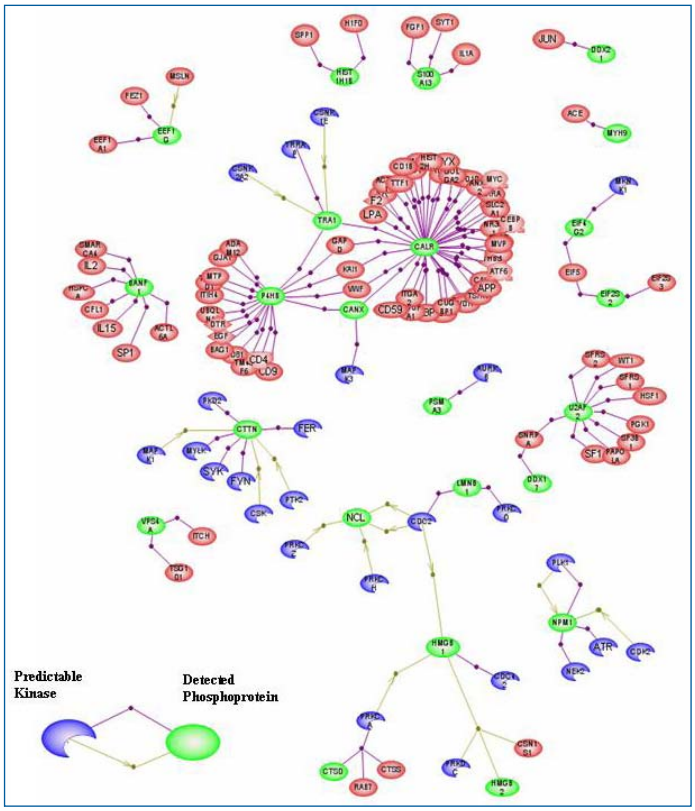
PROJECT MEMBERS | Dr. Uwe Kärst | Dr. Josef Wissing | Tobias Reinl | Roman Fischer | Claudia Hundertmark | Sebastian König | Zofia Magnowska

The research group functionally characterizes surface proteins from *Listeria monocytogenes.* This Gram-positive, facultative intracellular human pathogen can cause food-borne infections like meningo-encephalitis, meningitis and prenatal infections, especially in immunocompromised patients. All virulence factors that have been identified so far exhibit two common features: they are under the (in)direct positive or negative control of the transcriptional regulator PrfA, or belong to the recently detected VirR regulon. And they are part of the secreted or surface protein fraction from *Listeria.*

Identification and functional characterization of new bacterial virulence factors The research activities of this project comprise comparative proteome analyses between the pathogenic EGD-e wild type strain, different *prfA* mutants and the non-pathogenic *L. innocua* species with a particular focus on extracellular and surface proteomes. For example, lipoprotein anchoring in bacteria is mediated by the prolipoprotein diacylglyceryl transferase (Lgt). Deletion of the lgt gene impairs intracellular growth of the bacteria in different eukaryotic cell lines and leads to an increased release of lipoproteins into the culture supernatant, facilitating the identification of PrfA-dependent lipoproteins. In contrast to earlier studies in *E. coli,* we demonstrated that lipidation by Lgt is not a prerequisite for activity of the lipoprotein-specific signal peptidase II (Lsp) in *Listeria.* Furthermore, the transmembrane protein Lmo1695 – a member of the VirR-dependent virulence regulon – was demonstrated to be essential for lysinylation of (di)phosphatidylglycerol. It mediates resistance against antimicrobial peptides and significantly contributes to listerial pathogenicity *in vitro* and *in vivo.*

Signal transduction analyses of infected host cells Proteome research has gained importance in cell biology studies and has begun to play a decisive role in the characterization of complex relations between pathogens and host cells. Current research focuses on visualization-aided exploration and data mining of peptide profiles and time-resolved characterizations of host cell signaling pathways for the bacterial invasion. C-Met and E-cadherin signalling served as model pathways for establishing quantitative phosphoproteome analyses based on IMAC, iTRAQ™ and LC-MS/MS. Two bacterial surface proteins were found to be essential and sufficient for invasion of epithelial cells: Internalin B (InlB) interacts with the growth factor receptor tyrosine kinase c-Met (HGFR), whereas Internalin A (InlA) binds to E-cadherin. Both virulence factors subvert host cell signaling, resulting in the coordinated reorganization of the actin cytoskeleton that enables the bacteria to invade the cell.

In 2006, we combined state-of-the-art technologies and began studying, quantitatively and systematically, the regulation of phosphorylation sites directly at protein kinases. Our workflow currently permits the purification of about 30% of the human kinome and gives access to known and novel regulatory phosphorylation sites. Comparative analyses of total cell lysates subsequent to the extracellular presentation of various recombinant InlA and InlB constructs to different epithelial cell lines define modulated phosphorylation events at a minute scale. These studies clearly aim at the time-resolved analysis of differentially phosphorylated proteins and already yielded hypotheses about signaling pathways associated with the process of bacterial invasion.



Identification of kinases involved in InlA-mediated E-cadherin signaling by the combination of quantitative phosphoproteome analyses and reverse network engineering. *Graphic: HZI*

02.3 Signalling to Acting Dynamics

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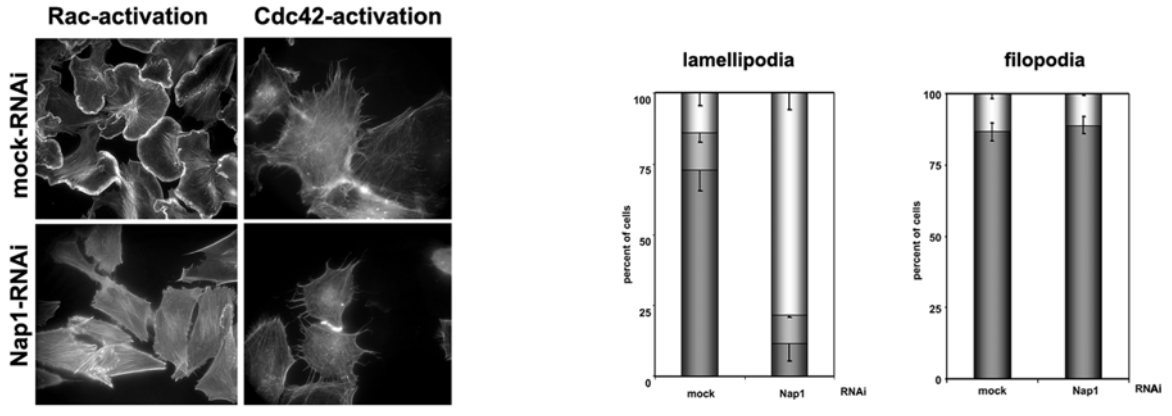
PROJECT MEMBERS | Jan Hänisch | Kai Städing | Stefanie Weiß

Numerous cellular functions and processes depend on dynamic changes of the actin cytoskeleton, including morphogenetic movements during embryonic development, chemotactic movement of cells of the immune system and migration of fibroblasts during wound healing. A large number of actin binding proteins have been described, which regulate the dynamic reorganisation of the actin cytoskeleton. However, the exact signalling pathways and principles for the spatial and temporal control of 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, members of which act downstream of small GTPases, key signalling switches 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 Cdc42-induced filopodia. The discovery that similar dynamic changes of the actin cytoskeleton play an essential role in host-pathogen interactions has had, and still has, an enormous impact on this field.

Lamellipodia and WAVE WAVE proteins, prominent members of the WASP/Scar family, play an important role in 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 and activate WAVE, which led us to search for the missing links between Rac1 and WAVE proteins. We were able to establish the ability of at least three proteins, Nck associated protein 1(Nap1), Specifically Rac associated protein 1(Sra-1) and the Abl interacting protein family (Abi proteins) to link Rac to WAVE proteins. The resulting protein complex, the ubiquitous WAVE complex, is now well established as being essential to the formation of lamellipodia downstream of Rac.

Filopodia, beyond WAVE- and Arp2/3 complex When targeting the expression of WAVE-complex subunits by RNA interference (RNAi), we found that this protein assembly is not required for the transduction of Cdc42 signals to filopodia formation, while WAVE-dependent lamellipodia formation was abolished. This finding contrasted markedly to earlier models of filopodia formation that proposed a requirement for lamellipodial actin to feed filopodia formation. To finally clarify the putative requirement for any NPF, or the Arp2/3 complex itself, we then suppressed the expression of Arp2/3 complex by RNAi and found that Cdc42-induced filopodia formation is not affected, while many other cellular processes are impaired. Therefore, a different actin polymerisation machinery appears to be needed for filopodia formation, the identification of which is currently being pursued. In the course of these studies, we have already identified an important contribution of novel F-actin bundling proteins to efficient filopodia protrusion, such as EGF receptor substrate 8 (Eps8), insulin receptor substrate p53 (Irsp53) and vasodilator stimulated phosphoprotein (VASP).



Nap1 RNAi leads to suppression of WAVE-complex activity and consequently to a loss of Rac-induced lamellipodia (left panel and graph). In contrast, Cdc42-induced filopodia formation is not affected (right panel and graph). Legend to graphs: dark grey represent cells with and light grey cells without lamellipodia or filopodia. Photos: HZI

02.4 Genetic Mechanisms of Infection Susceptibility and Macrophage Functions

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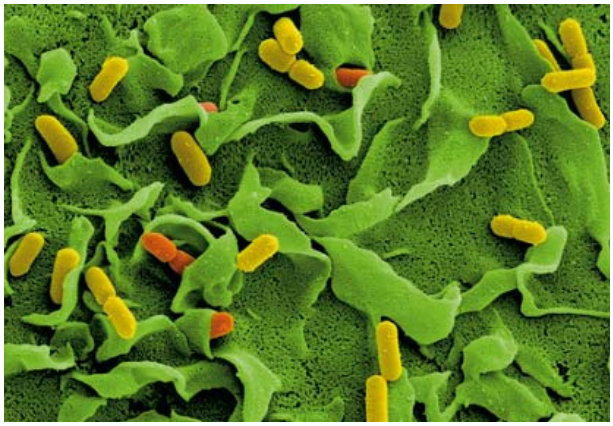
PROJECT MEMBERS | Dr. Jens Böse | Dr. Bastian Pasche | Phillip Hahn | Meike Küting

The focus of our group is the investigation of genetic susceptibility to infection and the development of new mouse models for human infectious diseases. Susceptibility to infections is determined by complex interactions between environmental and host genetic factors. Very often, multiple genes influence predisposition to infectious diseases by acting at different stages of the infection process. This makes it difficult to identify host susceptibility genes in genetically heterogeneous human populations. Our laboratory takes advantage of the mouse as a model organism to understand basic processes of immune defence and inflammation. Because the immune system of mice is in many aspects very similar to the human immune system, it is possible to compare the function of corresponding – orthologous – genes in both species.

Genetic basis of infection susceptibility in mice By using different mutant and inbred strains of mice we are trying to identify genes that are associated with susceptibility, or resistance, to different pathogens, *e.g.* Streptococci, Listeria, influenza and filaria. To accomplish this, we are analysing the immune status of infected mice under standardized conditions. These activities are part of the HZI "Infection Challenge Platform" (ICP), which provides an infrastructure for standardized phenotyping of mouse mutants under defined environmental conditions. In collaboration with Prof. Claude Libert (University of Ghent), we demonstrated that SPRET/Ei mice are resistant to LPS-induced septic shock and to infection with *Listeria monocytogenes*, due to a defect in the production of the cytokine interferon-β. This finding helps to better understand molecular processes involved in the establishment of septic shock.

In a second project, our group is interested in the understanding of macrophage functions. Macrophages are important effector cells of the innate immune system and provide a first line of defence against many microorganisms. They are essential for the control of common bacterial infections. Using mouse genetics, we aim to identify factors that can modulate macrophage effector functions and that are important for innate host immune responses. Recently, we could show that the jumonji C-domain containing protein Jmjd4b is crucial for the regulation of inflammatory responses that are mediated by macrophages.

Development of a new mouse model for human listeriosis In collaboration with the Division of Structural Biology, we recently established a new mouse model for human listeriosis. Listeriosis is caused by the intracellular, Gram-positive bacterium *Listeria monocytogenes* and is associated with the development of sepsis and meningitis in immunocompromised patients. So far, no mouse model of listeriosis was available by employing the natural – oral – route of infection in mice. By introducing structure-derived modifications into the listerial virulence factor internalin A, the pathogen was adapted to the mouse, leading to an enhanced binding to the murine E-cadherin receptor. The new mouse infection model now allows investigations of *Listeria* host colonization, as well as an *in vivo* analysis of mucosal immune responses that are initiated at the natural site of infection.



Invasion of Listeria monocytogenes *EGD in human dendritic cells* *Photo: HZI, Rohde*

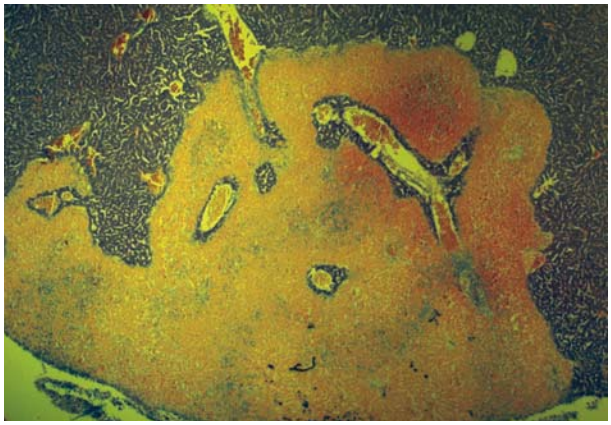
02.5 The Pathogenesis of *Streptococcus pyogenes* in the Mouse Model

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

PROJECT MEMBERS | Dr. Oliver Goldmann | Dr. Maren von Köckritz-Blickwede | Torsten Loof

Streptococcus pyogenes is a prevalent human pathogen capable of causing a variety of diseases, ranging from very mild infections – *e.g.* pharyngitis – to diseases with high mortality rates – *e.g.* streptococcal toxic shock syndrome. *S. pyogenes* has re-emerged as a public health hazard, due to the increased incidence of rapidly progressive severe infections observed worldwide since the mid-1980s. Current control measures for treatment or prophylaxis of streptococcal diseases rely on chemotherapy. However, there is a consensus that, in the longer term, vaccination might constitute the most effective strategy to diminish this global disease burden. An important consideration for the design and implementation of vaccines is an understanding of the contribution of host genetics to disease susceptibility. In the particular case of *S. pyogenes,* several studies have shown that individuals within a single human population vary genetically in their susceptibility to infection with this pathogen. Thus, different individuals will develop infections of differing severities after an encounter with the same streptococcal strain.

As a model for human variation, inbred mouse strains have been critical to our understanding of the role of host genetics in the susceptibility to *S. pyogenes* infection. Thus, different strains of mice differ markedly in their susceptibilities to *S. pyogenes.* While some strains of mice – *e.g.* BALB/c – are very resistant to this pathogen, being able to clear and survive the infection, other strains – *e.g.* C3H/HeN – are much more susceptible, allowing progressive bacterial multiplication, development of sepsis and death. In our studies, we have assessed whether susceptible mice can become more resistant to *S. pyogenes* infection after vaccination. Our results show that vaccination endows both resistant and susceptible mice with an equal capacity to control *S. pyogenes* infection, clearly indicating that resistance to *S. pyogenes* can largely be achieved in the innately susceptible hosts by vaccination. Therefore, the implementation of a functional streptococcal vaccine may significantly contribute to the reduction or elimination of severe cases of invasive streptococcal disease.



Liver damage caused by S. pyogenes *infection in the mouse model.* *Photo: HZI*

Natural killer cells – a therapeutic target for streptococcal septic shock? The resurgence of streptococcal toxic shock has renewed interest in understanding the development of this clinical manifestation and in the development of more effective therapeutic treatments. The development of these new therapeutic approaches is not an easy task, due to the complexity of the immunological defences and the feed-back mechanisms of the inflammatory cascade. One of the goals of our studies is the identification of target cell populations involved in the development of streptococcal septic shock using a mouse model of infection that resembles the development of this condition in humans. Our results show that natural killer (NK) cells significantly contributed to the progression of *S. pyogenes*-induced septic shock by amplifying the inflammatory response. Depletion of NK cells significantly improved the survival of mice after bacterial inoculation and delayed the development of pathology and organ failure. This is a clear example of how the identification of host cell populations critically involved in the development of septic shock during streptococcal infection could be highly valuable for future clinical applications.

02.6 Systems Genetics of Infection and Immunity

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

PROJECT MEMBERS | Dr. Dunja Bruder | Dr. Lothar Gröbe | Dr. Robert Geffers

This project aims to identify complex genetic networks that determine the susceptibility to infections, or regulate the immune system. Comprehensive phenotypic studies are performed in large mouse families that differ in their genetic background. In this way, many phenotypic traits as well as gene expression patterns can be associated with genetic variation, and the regulatory interactions determined. This approach is called "systems genetics." We are applying a systems genetics approach in mice to understand the basic molecular mechanisms in two medically highly relevant areas: infection of the mammalian host with influenza virus and the regulation of the immune system.

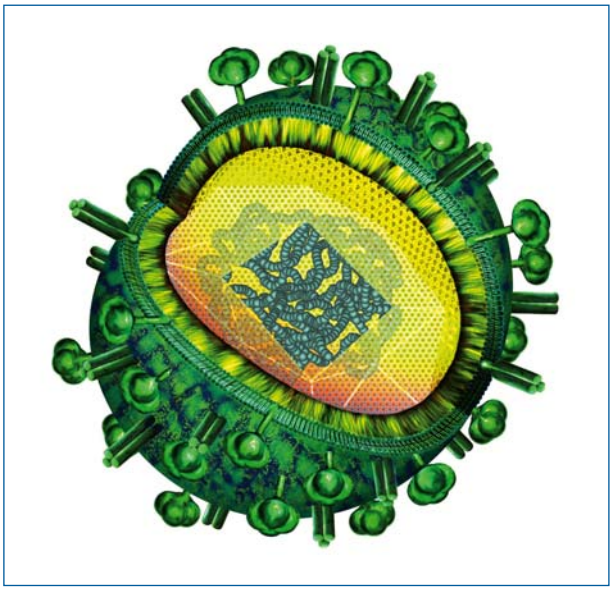
Host susceptibility to infections with influenza virus
Every year, influenza infections cause about 500 million severe cases of disease worldwide and 500,000 cases in Germany. About 8,000 people die each year from influenza infections in Germany. The value of the mouse model has been well demonstrated for recent and historical (1918) influenza A/H1N1 subtypes, as well as the recent high pathogenicity bird influenza subtype A/H5N1.

In our research group, we are analysing factors of host susceptibility to infections with influenza virus by infecting different mouse recombinant inbred strains. The pathology, course of disease and immune response is being studied, and whole genome expression analysis of the lungs performed. These studies 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 remaining host tissue is left intact, a complex interaction of effector T cells and regulatory T cells is needed.

We are analysing, at a genome-wide level, the expression profiles in T cells from different recombinant inbred mouse strains and associate gene expression levels with genotypes. In this way, single regulatory interactions can be described as quantitative traits. In addition, differences in expression profiles of whole sets of target genes that are controlled by the same genomic locus can be identified as groups of co-regulated genes. In this systematic approach, regulatory interactions, master regulatory gene loci, and regulatory networks can be identified and interaction networks can be modelled.

A world-wide network for complex genetics Our activities are highly integrated into various networks of scientific experts in systems genetics: The German Network for Systems Genetics (GeNeSys) is coordinated by the HZI, and the complex trait consortium (CTC) represents a world-wide scientific network. Both consortia aim to collect all the data related to complex trait studies in mice into a single public database, GeneNetwork, which has been created at the University Heath Science Centre at Memphis, Tennessee.



Model of an influenza virus *Graphic: HZI, Miessen*

02.7 Biology of the Immune Defence

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The genomes of mouse and man have been deciphered and the analyses have shown that both genomes are similar enough in order to use the mouse as a model to decipher the functions of genes. Within the Department of Experimental Immunology we are developing new methods to generate mouse mutants that allow the dissection of mechanisms leading to disease in man. We are generating mouse mutants with predefined genetic modifications of any gene of interest. As soon as such mutants are available they must be very thoroughly analysed in order to understand the functions of the genes. For this, the Department of Experimental Immunology, in collaboration with the Junior Research Group "Infection Genetics", established an Infection Challenge Platform that allows us to follow well-defined infection processes.

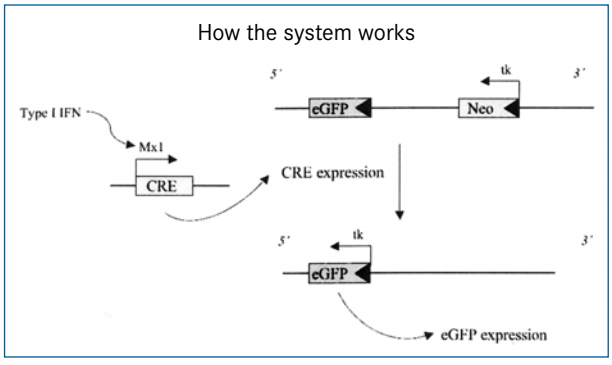
A model for inflammatory bowel disease of man We developed one mouse mutant that lacks one cytokine named Interleukin-10. It develops inflammatory bowel disease in a conventional animal facility. If this mutant is transferred to the Infection Challenge Platform, the normal gut flora is replaced by a well-defined gut flora. Under these conditions, the mice do not develop the disease. This demonstrates that both the environment we live in and the genes we inherited determine the chance of development of an inflammatory bowel. With the infection platform, we are now adding back bacteria or parasites in order to induce an inflammatory bowel disease again. We try to define components of a normal flora that could lead to an inflammatory bowel disease in a vulnerable individual.

Understanding the cytokine network In addition to the generation of single gene targeted mutants we use more sophisticated methods that allow us to switch genes on or of in vivo by a method called "conditional gene targeting." We applied this technology to the Interleukin-10 gene. The Interleukin-10 gene is present in all cells of our body. If we specifically inactivate the gene in T-Lymphocytes, such mutants will develop inflammatory bowel disease just like the complete Interleukin-10 deficient mouse mutant. Apparently, Interleukin-10 – produced by T-Lymphocytes – is very important to control the immune system in the gut, thereby preventing inflammatory bowel disease.

If the Interleukin-10 gene is inactivated in macrophages, the picture changes. These mutants do not develop in inflammatory bowel disease, but become susceptible to a lethal septic shock. When produced by macrophages, Interleukin-10 can prevent this in our model. In cases where the Interleukin-10 is eliminated in T cells, the mice are still protected from septic shock. T cell derived Interleukin-10 is therefore dispensable under these conditions.

Our results demonstrate that the conditional gene targeting of gene-encoding components of the cytokine network will lead to new insights into the cytokine network. It is very important to know which cell type produces a certain cytokine at a given time, especially for computer models that attempt to simulate the cytokine network.

Searching for small molecules The conditional gene targeting method allows the development of cell-based assay systems to analyse one particular signalling event within the cytokine network. This allows the screening of new small compounds, which are able to modify this signalling event, and represent potentially new lead compounds for future drugs. We established a cell-based system that allows the analysis of the type I interferon signalling events and established a cell line that starts to produce a green fluorescent protein after treatment of the cells with type I interferon. We used the natural compound library of our centre and identified small molecules that are able to enhance, or suppress, type I Interferon responses.



The system to analyse the Typ I Interferon signalling system. The cell line contains a transgene that encodes a recombinase under the control of the Mx1 promotor that is switched on by Type I Interferon. The recombinase in turn activates a target gene that will start to express a green fluorescence protein. The cell starts to glow. Graphic: HZI.

Inflammation and Immunity

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

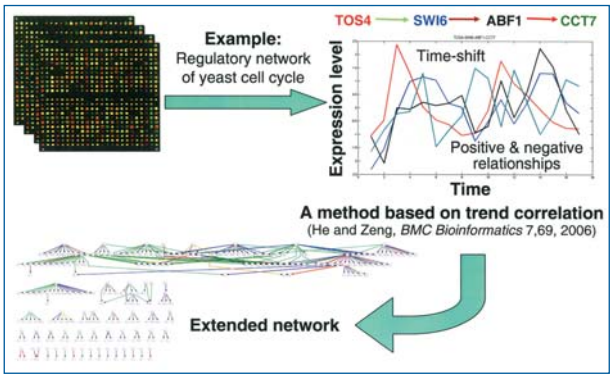
Innate defense activation and inflammation are among the first reactions of the immune system to infection. These events, in turn, are essential to initiate the specific and often long-lasting adaptive immunity through B and T cells, a prerequisite for the clearance of pathogens. The reactions are initiated 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 controlling cell-to-cell interactions. These interactions orchestrate both the rapid innate defense as well as the adaptive immunity. Dysregulation can lead to severe negative outcomes, such as toxic shock and autoimmunity.

The topic “Inflammation and Immunity” tries to identify key processes and responsible molecules in this context to obtain a basic understanding of the normal processes and to elucidate reactions which lead to pathogenic conditions. Along the way, molecular targets for pharmaceutical development are identified as well as approaches for therapies and vaccinations.

As a response to viral and also to bacterial infections the Interferon system is one of the focuses of this topic. The same is true for the T-cell response with particular respect to the decision on tolerance, anergy, autoimmunity or defense. A third focus concerns intracellular signalling, the expression of individual genes and their contribution to regulatory networks.

While most approaches use the mouse as a model organism, human individuals are also analysed or used as sources for cells. Cell cultures are employed when the complexity of the whole organism is too high for analysis. Increasingly, methods are used and further developed that allow transferring *in vitro* manipulated cells into mice to follow their functionality.

New approaches are aimed at a mathematical description of inter- and intra-cellular processes as parts of complex networks. Bioinformatics is used to describe the experimental approaches towards the regulation of the interferon system and the biology of regulatory T cells.



From time-series data to regulatory network by reverse engineering Graphic: HZI.

03.1 Structural Analysis of the Innate Immune System

PROJECT LEADER | **Dr. Wolf-Dieter Schubert | Research Group Molecular Host-Pathogen Interactions | wds@helmholtz-hzi.de**

PROJECT MEMBERS | Gregor Hagelüken | Thomas Wollert | Maike Bublitz

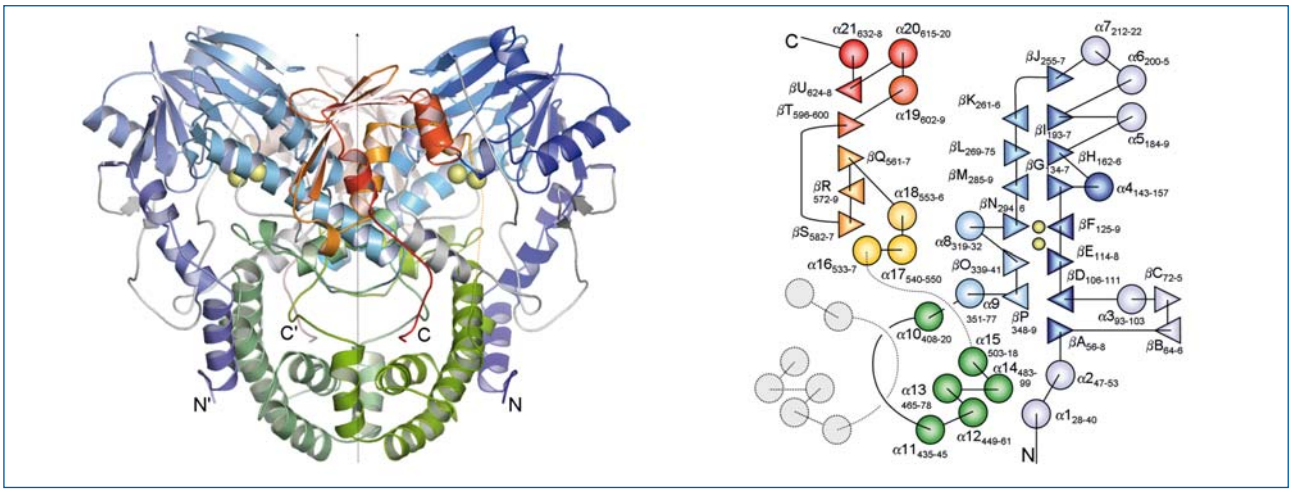
In the group "Molecular Host-Pathogen Interactions" (MHPI) in the Division of Structural Biology (SB), we are concentrating on the molecular details of general human defense mechanisms against invading pathogens (innate immunity), as well as the molecular strategies of pathogens in infecting humans.

***Pseudomonas aeruginosa* – an allrounder** *Pseudomonas aeruginosa* is a bacterium that is both a widely distributed inhabitant of aqueous or moist natural habitats, as well as a dangerous pathogen that causes chronic and acute infections in humans (*e.g.* in cystic fibrosis). *P. aeruginosa* is remarkable in its resistance to many common sanitary products. As a result, this mostly harmless bacterium has developed into one of the most frequently transmitted pathogens in otherwise sterile environments, such as hospitals. On the other hand, *P. aeruginosa* is a frequent inhabitant of sewage plants and is especially prevalent in polluted waters.

One reason among many for the adaptability of this bacterium results from the fact, that *P. aeruginosa* is able to degrade primary alkylsulfates, a family of compounds frequently found in the described environments. A prominent member of this family is a detergent, known as sodium dodecyl sulfate (SDS) or sodium laurylsulfate (SLS) - a major constituent of shampoos, toothpastes und other foaming personal hygiene products. The pathogen is able to absorb the fragments of SDS, metabolizing them, if the need arises, as its sole source of sulfate and carbon.

The structure of SdsA We have investigated this property of *P. aeruginosa* and found that the bacterium produces and secretes an enzyme, SdsA, specifically for this purpose. SdsA binds SDS (and related compounds) and degrades it to the constituent sulfate group and dodecanol moiety. To understand this process at the atomic level, we produced this enzyme in large amounts, crystallized it and solved its three-dimensional structure by X-ray crystallography. The enzyme SdsA consists of two intertwined polypeptides. Each polypeptide runs continuously from the amino terminus to the carboxy-terminal end creating numerous coils, α-helices or β-strands. These structural elements create three distinct domains indicated by distinct colors: The N-terminal blue region defines the catalytic domain, followed by the green dimerization domain and an orange-colored substrate binding domain. The latter domain recognizes and recruits suitable molecules from the surrounding medium passing them to the catalytic domain by means of a hydrophobic slide. The catalytic domain, in turn, binds two positively charged zinc ions. These zinc ions appear to polarize a suitably placed water molecule, inducing it to nucleophilically attack and degrade a molecule of SDS positioned opposite.

We have also attempted to identify potential inhibitors of SdsA that would bind to the enzyme without being degraded. Specifically adding traces of such inhibitors to detergents and personal hygiene products would inactivate SdsA and remove SDS as a source of energy to *P. aeruginosa,* eliminating the pathogen. 1-Decansulfonic acid is such an inhibitor. It is similar in size and shape to SDS, but is not cleaved by SdsA.



Two schematic representations of SdsA from Pseudomonas aeruginosa. *Graphic: HZI*

03.2 Signal Transduction and Gene Regulation

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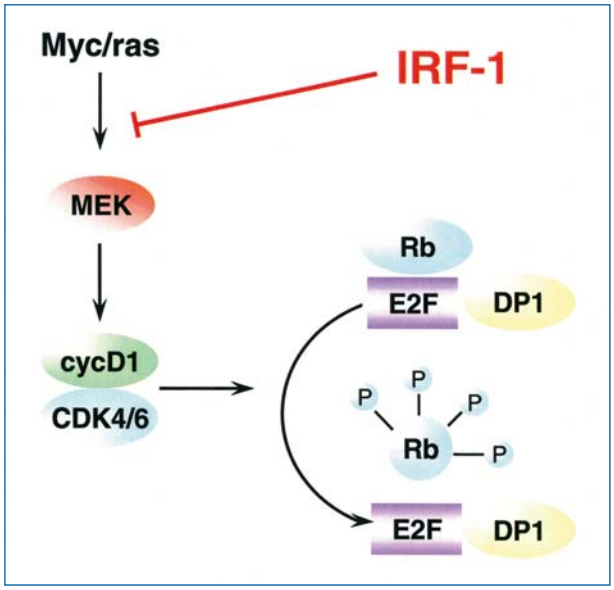
An attack by pathogens induces numerous activities in host cells, including innate immune responses and inflammation. One of our studies focuses on the role of interferons as a key defence mechanism produced after infection. Interferons protect cells from viral infection. They stimulate innate and adaptive immune responses, and, influence cell growth and survival. In several interferon-related research subjects, we have characterised the Interferon Regulatory factor-1 (IRF-1) and its effects on cell growth and transformation. Our aim is to understand the signalling network which leads to IRF-1 mediated effects.

Gene regulation by IRF1 IRF-1 is a transcription factor that is induced by a number of cytokines, viruses and interferons. As a transcriptional activator, IRF-1 mediates all its activities by activating target genes which, in turn, cause proliferation inhibition, reversion of oncogenic transformation and modulation of immune responses. One of the most striking effects of IRF-1 is its ability to revert the transformed phenotype of different tumor cell types towards an untransformed phenotype. Gene expression studies showed that 60% of all genes deregulated by oncogenic transformation are reverted to levels of normal cells, indicating that IRF-1 interferes with a master regulator of oncogenic transformation.

Regulation of cell cycle Another phenotype of IRF-1 expression in transformed cells is their accumulation in the G1 phase of the cell cycle. We found that this is mediated by the repression of cyclin D1, a gene which is upregulated in many neoplastic cells as a consequence of MAPK activation. IRF-1 represses the upregulation of cyclin D1 by inhibition of the MAPK pathway activation. This inhibition of cyclin D1 expression by IRF-1 is sufficient to revert the oncogenic phenotype to normal growth behaviour and to prevent tumour formation.

We are now assessing the role of other genes regulated by IRF-1 in cell cycle control, tumor suppression and infection defences.



IRF-1 prevents induction of cyclin D1 stimulation by inhibition of the MAPK pathway. Mitogens and oncogenic signals induce the MAPK pathway (MEK). This leads to a stimulation of cyclin D1 (cycD1) transcription. Cyclin D1 regulates the cyclin dependent kinases (CDK) 4/6 to phosphorylate and inactivate pRB. Repression of pRB leads to cell cycle progression.
Graphic: HZI

03.3 Epigenetic Principles of Gene Regulation

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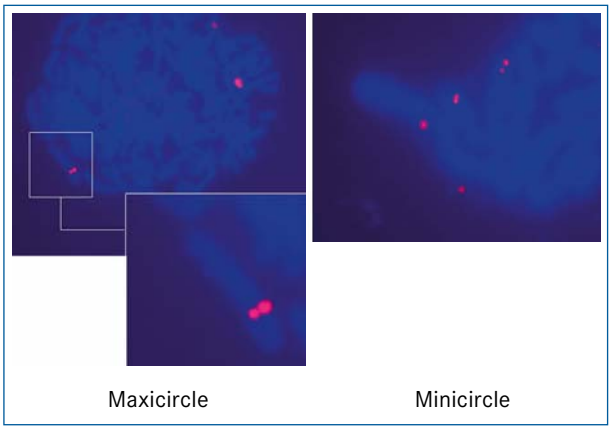
Our studies focus on the functional organization and differential regulation of *loci* relevant for host-pathogen interactions. These are, in particular, the type I interferon gene *loci* of humans and mice. Recent indications that inflammatory responses are not only suppressed by type I interferons, but also tuned by central cross-interacting modulators, such as YY1/YY2 and PARP-1, has triggered detailed investigations of the regulatory networks acting in the context of nuclear architecture. The active elements mediating these functions are being classified and utilized for the design of vectors with novel properties. Knowledge derived from these studies is the basis for a rational generation of transgenic animals and of cell lines with a variety of potential applications.

SIDD Analysis of Interferon(I) Induction Principles
Nuclear architecture is determined by the dynamic association of scaffold/matrix attachment regions (S/MARs) with the nuclear skeleton. S/MARs separate functional genomic regions in a cell-type specific manner. Using new concepts, novel applications of the SIDD algorithm have revealed the basics of remote gene control because the elements with regulatory potential – so called 'DNAse I hypersensitive sites' – have imprints in a SIDD profile. This approach led to the clear-cut demonstration that, over-all, the interferon-ß genes in humans (huIFNs) and mice (muIFNs) obey widely different regulatory principles.



Status of plasmid- and minicircular episomes: FISH analyses. For the episomal plasmids we find single, chromosome-associated signals and, in about 40% of all cells, also intense doublets that cover corresponding positions on both chromosome indicating eventual integration events. All minicircle preparations show signals throughout the metaphase spread and copy numbers between 5 and 15 but no indication of integration. *Graphic: HZI, Rohde*

Nonviral Episomes Nonviral Episomes could be developed due to the inclusion of a huIFN-ß associated S/MAR element in a circular vector. We could successfully introduce "mini-circles" hat are devoid of prokaryotic vector parts and -origins. Deletion of these sequences by site-specific recombination not only increases the cloning capacity and permits establishment of the vector in the absence of selection pressure, but also eliminates the targets for cellular defense mechanisms. These minicircles hold great promise for multiple applications in nondestructive transgenesis, including biotechnological expression systems and novel gene therapy regimens.

Cassette Exchange (RMCE) In RMCE, the genomic address consists of a set of two heterospecific recombinase target sites, permitting the exchange of the intervening sequence for the gene of interest (GOI) as part of a similar cassette. This process locks the GOI in place. Presently, the scope of RMCE-applications is extended to "multiplexing" principles, which either permit the simultaneous, directed targeting of several pre-defined genomic sites, or the elaboration of chromatin domains in a successive fashion. To avoid any cross-interaction, multiplexing has to be based on FRT variants that differ in at least four spacer positions. Seven new mutants with a maximum mutual discrimination have been synthesized and are under current scrutiny.

Halo-FISH techniques All of the above studies are supported by FISH and Halo-FISH techniques, which are well suited to visualize and classify independently regulated genomic regions, to elucidate the status of episomes and to characterize the functional status of (retro)viral integration sites. These results have led to the demonstration that a common feature of retroviral integration events is their placement *adjacent* to a strong S/MAR.

Halo-FISH demonstrated that this mechanism is valid for functional human endogenous retroviruses (HERVs) and also for SIV. Our studies have been extended to aspects of lentiviral persistence and the susceptibility to lentivirus infection.

03.4 Cellular Models for Infection

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Mouse models – established from transgenesis, or via adoptive transfer of cells – are being successfully used for diverse purposes to elucidate specific questions in infection and immunity. Furthermore, cell lines generated thereof are valuable tools for studying individual processes on a less complex level. In this project we are establishing new model cell lines and trangenic mice.

Mice with tightly controlled and predictable gene expression and a new generation of retroviral producer cell lines In the establishment of new mouse models we are focusing on tightly controlled transgene expression. For this purpose both transcriptional and genetic control systems were implemented in murine embryonic stem cells. Transgenic mice are currently being generated that allow the precise regulation of transgene expression in defined tissues.

For the establishment of transgenic mice, but also for defined genetic manipulation of cell lines, the project relies on recombinase mediated cassette exchange (RMCE) combined with a stringent selection regimen for isolating recombined cells. Embryonic stem cell lines were tagged with Flp recombinase target sites (FRT) for the establishment of transgenic mouse lines with predictable expression properties.

Accordingly, we applied this strategy to the development of a new generation of recombinant retrovirus producer cell lines. Based on HEK293 cells, we developed a retroviral producer cell line in which a chromosomal hot spot is used for targeted integration of a therapeutic vector. This cell line, called Flp293A, supports a highly efficient production of recombinant retroviruses ($2x10^7$ ip/10^6 cells) and is currently being evaluated for the production of therapeutic viruses.

Conditional immortalization Despite the successful development of methods for the constitutive immortalization of cells, it is clear that immortalized cell lines that express genes to overcome the limited proliferation of primary cells do not necessarily represent the properties of the cells from which they were derived. To generate cell lines that more precisely mirror the properties of the primary parental cells, we have developed a method for conditional immortalization. For this purpose, we used a tightly controlled expression system that allows both strictly Dox dependent expression, and also transduction, of the cassettes in a single step. Thereby, cell proliferation can be strictly controlled. Viral transduction allows the implementation of the system in various cell types including resting cells.

Endothelial cells Since endothelial cells represent a major barrier for pathogens and play a pivotal role in infection processes and subsequent inflammation, we focus in this project on endothelial cells and cell lines. The difference between blood and lymph endothelial cells is investigated by analysis of phenotypic properties, cell surface markers and gene expression profiles. We have begun to characterize antigen uptake and processing and the reactivity of inflammatory mediators for both cell types.

Cells and mice from the above described models are used to understand the interactions between cells, implant materials and bacteria. Measurement of cell growth, cell physiology, and RNA expression profiling were carried out and subjected to bioinformatic analysis. We identified cell proliferation, inflammatory events and sterol metabolic pathways as a major target of ions that are released from metallic stents.

03.5 Development and Function of T Cells

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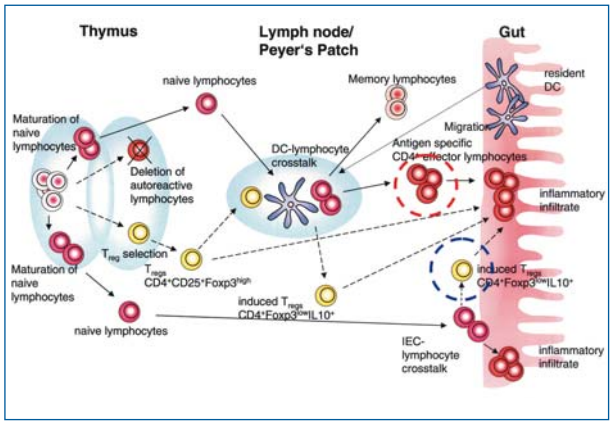
At the centre of our research interest stands the analysis of specific mechanisms for the induction of tolerance and the regulation of pathogen-specific immune responses through functional genomics. It is our aim to develop novel and highly effective therapeutic approaches for diseases with immunological dysfunction as apparent in chronic inflammation and infection.

Mucosal immunity of the intestine Our research focus here is on basic mechanisms of mucosal immune regulation and its therapeutic manipulation in chronic inflammation. To this end, we have established new transgenic mouse models for intestinal inflammation and have studied the modulation of the mucosal micro-environment by human pathogens and probiotics.

We have established a transgenic mouse model to gain better insight into basic mechanisms of T-cell mediated immune responses, mucosal dysregulation and peripheral tolerance induction in intestinal disease. It is based on the specific expression of a model antigen – HA of influenza virus A – in epithelial cells of the gut. Based on this mouse model we could recently unravel some of the missing pieces in the elucidation of the immune pathogenesis of inflammatory bowel disease. Our mouse model is meanwhile applied by several national and international cooperating partners to improve our understanding of the complex interactions between infection, inflammation and tumourgenesis in the intestinal tract.



How are pathogen-specific immune responses regulated in the intestine? The immune system of the mucosa of the gut has the difficult task to be tolerant towards a broad spectrum of nutritional components and at the same time to provide a crucial barrier against potential pathogens (bacteria, viruses, fungi, and parasites). To ensure this a tightly regulated network of epithelial cells, antigen-presenting cells, and effector cells is required. Over the last years we could unravel basic mechanisms of T-cell mediated immune responses, mucosal dysregulation and peripheral tolerance induction in intestinal diseases and could show that especially local immune responses orchestrated by regulatory T cells are required for the maintenance of mucosal homeostasis. Graphic: HZI

Regulatory T cells Regulatory T cells (Tregs) play a central role in the maintenance of tolerance. A lack of these cells can lead to overwhelming autoaggressive immune responses associated with tissue damage. In severe cases, this may result in clinical overt diseases, like Multiples Sclerosis, Rheumatic Disease, or Type-I-Diabetes. In addition, the outcome of immune responses in the context of infections has been shown to be controlled by regulatory CD4+ T cells. For a better understanding of Treg function in the prevention of autoreactive immune responses, we generated extensive transcriptional profiles of different subsets of murine and human regulatory T cells. Based on these studies, we were able to identify several new activation independent marker genes (Nrp1 and GPR83) of Tregs. The diagnostic relevance of Nrp1 as a molecular marker has now been confirmed in several high ranking studies by other groups in clinical trials. Moreover, we have studied innovative new ways to study candidate genes through retroviral overexpression in naive T cells and subsequent analysis *in vivo.*

In a recent research project, together with Prof. Christoph Klein of the MHH, on primary immunodeficiencies leading to infection, we were able to identify Gfi1 as the key transcription factor required for the maturation and activation of dendritic cells. The project has also led us to the molecular key – adaptor molecule p14 – for understanding a new primary immunodeficiency based on microarray studies on patient samples from an affected family.

For the molecular characterization of Tregs in clinical studies we have also developed a custom array that contains 350 Treg-associated genes. Based on this designer array, we identified new molecular pathways in human Tregs. We are performing studies with this new technology with clinical partners in the fields of infection, inflammation, and cancer. It is our aim to generate “Signalling-Networks” of human Tregs as a basis for their therapeutic manipulation in patients.

03.6 Mucosal Immunity and Inflammation

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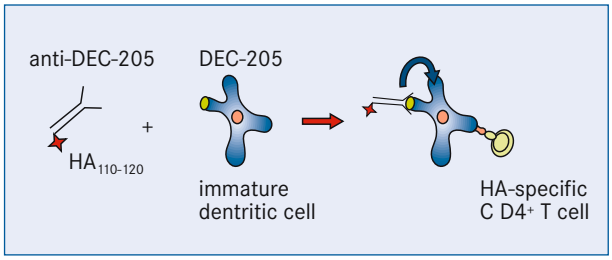
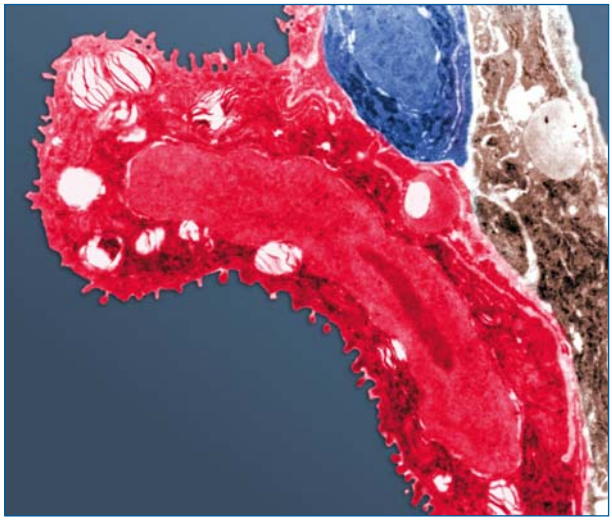
PROJECT MEMBERS | Dr. Marcus Gereke | Milena Tosiek

Utilizing a transgenic mouse model for T-cell mediated lung disease, our group focuses on the characterization of the complex interaction of different immune-cell populations under autoimmune conditions. These studies will be extended to mice infected with Influenza A virus. The aim is to improve our understanding of basic mechanisms underlying autoimmune disease and peripheral tolerance induction. In this context we also aim at improving therapeutic approaches to interfere with auto-reactive T-cell responses that are based on the induction of regulatory T cells by antigen targeting of immature dendritic cells.



Targeting antigen to immature dendritic cells.

Immune regulation in the lung In the past, we have established a transgenic mouse model that is based on transgenic expression of influenza hemagglutinin in the lung mucosa. Breeding of these mice with mice carrying T cells specifically recognizing this protein leads to severe pulmonary inflammation. Initial studies revealed that chronic antigen stimulation in the lung leads to the induction of regulatory T cells which counteract uncontrolled disease progression. Currently, we are working on the mechanisms that underlie tolerance induction. Comprehensive molecular and functional characterization of epithelial cells that do express the disease-causing antigen in the lung mucosa revealed that these cells are actively involved in the regulation of T-cell-mediated immunity in the lung. At present, we are extending our analyses to other potentially important immune cells to get a better understanding of the complex mechanisms that underlie dysregulations within the mucosal immune system of the lung. A further aspect that we will focus on is whether influenza infection can cause the loss of immunological tolerance to self-antigen in the lung, and thereby, lead to the induction of autoimmunity.



Shown here is an activated T lymphocyte (blue) on its way through the lung tissue (brown) making contact with a Type II alveolar epithelial cell (red). Photo: HZI

Mouse model for autoimmune hepatitis We have established a new mouse model that is based on the deletion of the gene encoding the “autoimmune regulator” AIRE. This transcription factor controls the expression of tissue- specific antigens in medullary thymic epithelial cells and is involved in the deletion of self-antigen specific T cells in the thymus. Lack of it leads to autoimmune diseases in humans and mice. A first characterization of AIRE-deficient mice revealed that the gene defect leads to the establishment of autoimmune hepatitis. Molecular analyses indicate that also in the liver peripheral tolerance mechanisms are active, which lead to the expansion of regulatory T cells at the site of inflammation. Since to date there are only a few suitable mouse models for autoimmune hepatitis available, further studies on AIRE-deficient mice may shed more light on the molecular and cellular mechanisms of such disease.

Therapeutic intervention in type I diabetes In a further transgenic mouse model for type-I diabetes, we tested a new approach for therapeutic immune intervention. It is based on targeting a pancreas-specific protein antigen via a specific antibody to immature dendritic cells. Stimulation of auto-aggressive T cells by immature dendritic cells leads to the induction of pancreas-specific regulatory T cells, which prevent disease development in diabetes-prone mice. At present, we are conducting a BMBF-funded study concerning the safety of this therapeutic approach in the context of infection. Our long-term aim is a broad characterization and optimization of this attempt with respect to possible applications for these therapeutic antibodies in clinical trials.

03.7 Immune Effectors: Molecules, Cells and Mechanisms

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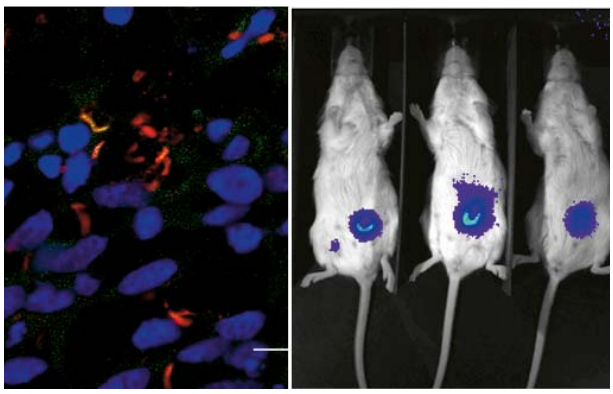
Upon infection, pathogenic bacteria target particular organs and cells of the host. For instance, after the intravenous infection of mice, *Listeria monocytogenes*, a facultative anaerobe and facultative intracellular bacterium, is quickly removed from the blood stream by macrophages. In the spleen, mainly macrophages found in the marginal zone and characterized by the marker ERTR-9 are infected, to some extent dendritic cells and neutrophils. The infected ERTR-9 macrophages are most likely also the cells that start to produce cytokines and may represent the primary producers of the Type I interferons responsible for the apoptosis induction of T cells and immune escape mechanism of Listeria. The cell specificity observed for Listeria may be due to the engagement of the receptors expressed on the target cells. ERTR-9 is a C-type lectin. Accordingly, administration of mannan significantly decreased the number of Listeria in such cells. Similarly, the integrin Mac1 binds activated complement component C3. Listeria are known to activate C3. Infection of C3-deficient mice significantly decreased the infection of Mac1 expressing cells. Thus, we were able to define some receptors responsible for the specificity of Listeria towards its target cells.

Tumour homing bacteria – delivery vehicles for therapeutic molecules? One peculiar "target organ" that many bacteria invade, are solid tumours. This is believed to be due to the particular micro-environment that a tumour can provide. The insufficient blood supply of fast growing tumours results in areas of low oxygen pressure and necrotic areas. Both properties provide favourable growth conditions for such bacteria. We have used several strains of Salmonella, *Escherichia coli* and *Shigella flexneri* to investigate the physiological status of tumour homing bacteria with the intension of using them as delivery vehicles for therapeutic molecules. First, we noticed that the bacteria induced necrosis formation early during their invasion. Thus, not only the limited blood supply but also the bacteria themselves were responsible for the death of many tumour cells. On the other hand, the bacteria attract leukocytes, like neutrophils and macrophages, to the area of infection. Probably, such cells are responsible for the containment of the bacteria to the necrotic areas and the rim of viable cells that is formed around them. Interestingly, despite the use of invasive bacteria, all microorganisms are found extracellularly. This is most likely due to capsules and other structures that are formed by the bacteria and which exhibit properties of biofilms.

L-arabinose induced promotor Many of the recombinant bacterial carriers delivering vaccines or therapeutics exhibit the problem of instability. This is due to the metabolic burden that recombinant proteins represent for them. We have developed a new strategy to avoid such problems. We have used a bacterial promoter (P_{BAD}) that can be induced by the sugar L-arabinose. We injected bacteria that carry firefly luciferase as a reporter gene under the control of P_{BAD} into mice bearing a solid tumour. Bacteria were enriched in the tumour as expected. Interestingly, upon administration of L-arabinose to such mice, we could detect strong transient expression of luciferase.

The P_{BAD} promoter is tightly controlled. Therefore, we were able to clone and induce suicide genes within this system. This adds important safety features to our bacterial delivery vehicles. We now intend to expand this tumour-specific inducible system to express toxins and other therapeutic molecules within a tumour.



Tumours that were colonized with Salmonella typhimurium *carrying inducible fire fly luciferase were analysed by immunohistology (left). Red are the bacteria stained with a specific antibody. Blue are the nuclei stained with DAPI. Displayed is a necrotic area where only nuclei are found and no intact cells. Induction of luciferase in tumour colonizing bacteria revealed by* in vivo *imaging (right). Luciferase was induced and the mice were analysed after injection of luciferin by non-invasive imaging on an IVIS 100 6 hours after induction.*

Photos: HZI, Westphal, Lößner

03.8 Imaging Cellular Dynamics of Immunological Processes

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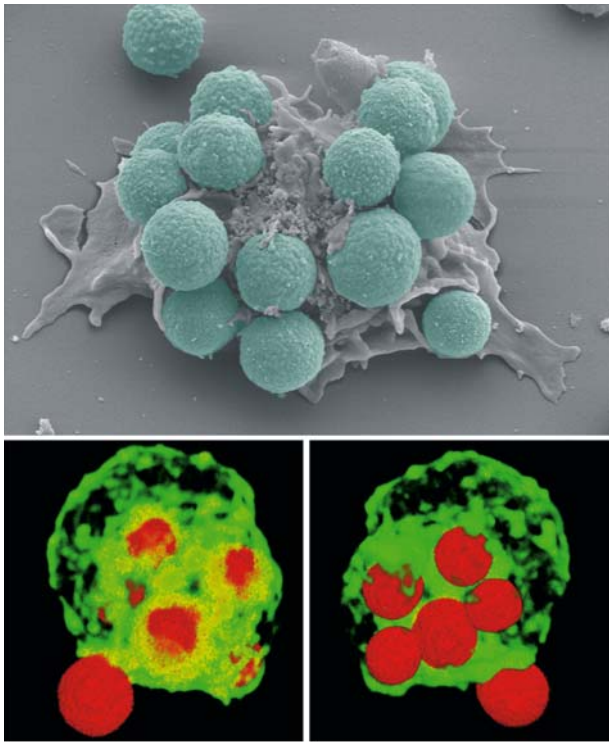
Immunity can broadly be divided into a humoral – mediated by soluble factors such as antibodies or complement – and a cellular arm – mediated by whole cells, *e.g.* T cells, B cells, dendritic cells and neutrophil granulocytes. While humoral immunity is only indirectly observable by looking at its effects, cells can be directly visualized while being "at work". Two examples will highlight why this is an important task.

Why observation of immune cells is important Because antigen presenting cells (DC) are at the beginning of every new cellular immune response, they reside in the periphery of the body where they take up invading pathogens and transport them to draining lymph nodes to present them to T cells. Although central to cellular immunity, this transportation, especially its *in vivo* dynamics, is not well understood.

There are numerous situations, where a defect in the generation of immune responses might be explained by disturbed DC migration. In the case of immunotherapy of cancer, which now tries to make use of DC as carriers of tumour antigens, a major unsolved problem is the optimal way of DC application to the patient without disturbing their inherent migration potential. Therefore, being able to visualize normal and defective DC migration *in vivo* would provide a useful tool to get insights into this basic process, to optimize protocols for vaccination programmes, or to understand disease processes.



A dendritic cell engaging antigen-specific T cells during the process of T-cell activation. The "spaghetti" structures around are fibres of artificial extracellular matrix consisting of 3-D collagen fibres. *Photos: HZI, Narang, Rohde, Gunzer*

Another aspect of cellular immunity, which is currently being studied intensively, is the physical interaction of T cells with different antigen-presenting cells (APC) during antigen presentation. While most of the work underlying current theories for T-APC interaction has been performed *in vitro*, nowadays imaging in explanted lymphatic tissue and tissues in living animals has shed light on the very dynamic migration processes going on in real lymphatic tissue. Such studies will lead to new thinking on how T-cell activation is achieved *in vivo* and what goes wrong in the case of disease or lethal infection.

What we do in the junior research group Seeing is believing – and understanding. To this end, we visualize the dynamics of cellular immunity while it is taking place by the use of state of the art microscopy techniques. This approach enables us to get comprehensive insights into the biophysical dynamics underlying cellular immune processes. As a simplified *in vitro* system we make use of artificial 3-D extracellular matrices as environment for the study of cellular dynamics. This system provides a major tool for generating and testing working hypotheses. We could demonstrate, that phagocytosis of human pathogenic fungi by murine and human phagocytes is strongly influenced by the environment and this is also dependent on the type of fungus. Parallel to these efforts, we have established imaging within explanted lymph nodes, as well as lymph nodes in living mice, by using time-lapse confocal and 2-photon microscopy. The latter technique is able to generate high-resolution images deep within vital tissue. To get a complete picture, imaging must be established both at sites of immune induction – e.g. lymph-nodes – as well as immune intervention. Therefore, the analysis of innate and adaptive immune responses against an infection with the pathogenic fungus *Aspergillus fumigatus* will also be studied at the primary infection site, the lung. With our work we hope to get a better understanding of immune processes as they evolve, how they successfully function and when, or why, they fail.

03.9 Bioinformatics of Cellular Networks

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Vertical integration of molecular networks Phenotypical characteristics of cells often arise from interactions between genes, proteins and metabolites. Here, we constructed an integrated molecular network (IMN) of *Escherichia coli* from metabolic reactions, metabolite-protein interactions (MPI) and transcriptional regulation data. We studied three fundamental aspects of cellular processes: feedback regulation of gene expression, network motifs and global organization. Intriguingly, we found that feedback regulation of gene expression in *E. coli* is exclusively mediated by MPIs. Sixty-nine such feedback loops (FBLs) were identified.

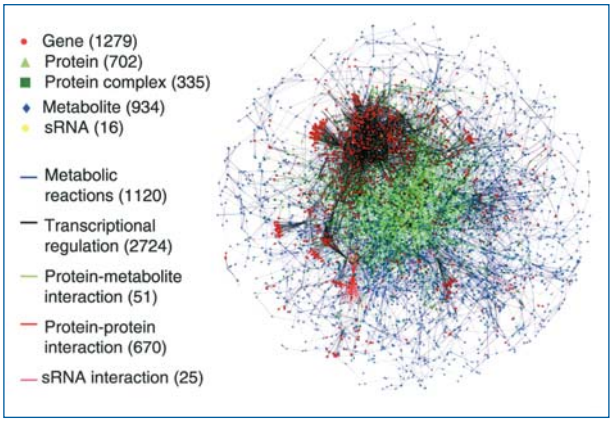
Further analysis of IMN revealed a bow-tie connectivity structure spanning three molecular levels in a nested way. Network motifs are considered a fundamental characteristic and the building blocks of biological systems. We detected thirteen three-node network motifs comprising five composite motifs, which included at least two different types of interactions and we analyzed their significance in the bow-tie. About 75% of them are interconnected, thereby forming the backbone of the giant strong component (GSC). This study demonstrated the necessity and usefulness of a vertical integration of molecular networks and of its global analysis for better understanding cellular processes and their regulation.

Modularity analysis of IMN We have used modularity as a parameter of clustering and performed modular analysis on an integrated molecular network of *E. coli.* It was found that clustering this complex network significantly grouped together genes of known similar function in well-defined physiologically related modules. Identifying network motifs and correlating them to the modules of highly connected nodes may define their potential functional role. Distribution analysis of these motifs within, and between, the various functional modules supported the fact that these motifs represent basic patterns of regulation and the organization of genes into modules.

Functional linkage between genes and/or proteins To help infer functional linkages between genes from expression data, we have developed a new method called trend correlation (TC). This entails calculating a maximal local alignment of change trend score by dynamic programming and a change trend correlation coefficient between the maximal matched change levels of each gene pair. This new method considers time shifts and inverted relationships.

The TC method is demonstrated with data from yeast cell cycle and is compared to the local clustering (LC) method and the widely used Pearson correlation coefficient (PCC) based clustering method. The biological significance of the gene pairs is examined with several large-scale yeast databases. A significant number of the gene pairs only inferred by the TC method are process-identity, or function-similarity, pairs, or have well-documented biological interactions, including 443 known protein interactions and some known cell cycle related regulatory interactions. Furthermore, for a p-value threshold of 1E-5, the percentage of process-identity and function-similarity gene pairs among the shared part of the three methods reaches 60.2% and 55.6% respectively, building a good basis for further experimental and functional study. The TC method can significantly augment the current major methods to infer functional linkages and biological networks, and is especially useful when time-series data are utilized.

The methods mentioned above and their modified versions are currently used to study the responsive networks of T cells to chronic simian immunodeficiency virus (SIV) infection. More advanced methods are also developed for investigating the maturing process of dentritic cells and infection of Listeria monocytogenes in cooperation with other groups.



Interactome: E. coli

Prevention and Therapy

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

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

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 aim, 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 a novel group of thermophylic and mesothermophilic myxobacteria was evaluated for their biosynthetic potential. New isolates were also examined for their suitability as hosts for the expression of polyketide synthase gene clusters. The studies performed to elucidate the mechanism of action of the antifungal benzolactone Cruentaren A demonstrated that this compound specifically inhibits the mitochondrial F0F1 ATPase.

On the other hand, 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 have recently established the full 3D structure of the natural macrolide antibiotics archazolid, etnangien and chivosazol, and the novel anti-tuberculosis lead, thuggacin. New preparative methods were developed, which allow effective and convergent synthesis of key bioactive synthons, such as chiral amines or stereogenic polyketides. These methods and a detailed understanding of their molecular architecture enabled total syntheses of these pharmaceutical leads and provided the basis for the development of simplified analogues with improved chemical and/or biological properties.

The project "Chemical Biology of Infectious Diseases" has established the required infrastructure and methodologies for a systematic search of novel anti-infectives, based on combinatorial chemical synthesis and high-throughput screening. Very promising small molecules with anti-tumour, antiviral and anti-infective activities were discovered from existing chemical compounds archives, which include more than 90,000 structures. In addition, compounds were synthesized with activity as adjuvants, or bacterial biofilm inhibitors, as well as for studies on "Quorum-Sensing".

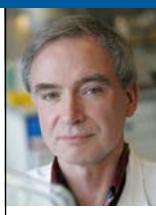
Candida albicans is one of the most important pathogens associated with nosocomial fungal infections, particularly in immunocompromised patients. Therefore, in the "Identification of Molecular Targets of Anti-infectives" project, an *in vitro* infection model of macrophages by *C. albicans* was selected for mechanistic investigations. Fungicides, for which the molecular mechanism of action is still unknown, and immunostimulatory or immunoinhibitory molecules were selected as test compounds. The selected

fungicides were expected to interfere with the fungal stress response network. A *C. albicans* mutant was identified showing increased sensitivity respect to the parental strain. Preliminary results indicate the production of cytokines as a direct macrophage response to the exposure to some of the selected myxobacterial secondary metabolites.

The “Antigen Delivery Systems and Vaccines” project is focused on the development of tools and strategies to optimize the delivery of vaccine antigens, particularly by the mucosal route. The p17 protein of HIV-1 is a structural protein essential in the viral life cycle. Intranasal vaccination with p17 and a synthetic agonist of the Toll-like receptor 2/6 (MALP-2) triggered strong immune responses at systemic and mucosal levels. Functional characterization studies demonstrated that MALP-2 promotes activation and maturation of B cells, which in turn is critical for the stimulation of T cell responses. Additional work led to the identification of a novel T cell subpopulation with immune modulatory functions in nasal associated lymphoid tissues. These cells maintain a local tolerogenic milieu that can be reverted through stimulation of Toll-like receptor signalling cascades. Finally, a new SopB-mediated immune escape mechanism was identified in *Salmonella* spp., which can be subverted to improve the efficacy of vaccines based on live attenuated bacterial carriers.

The “Therapeutic Cellular Vaccines” project is geared to developing strategies to break the immune escape mechanisms operating in persistent infections. 3D high-resolution imaging techniques were established to study the interactions between immune cells and target cells.. Antigen presenting cells were modified using adenoviral vectors encoding antigens and immunomodulatory molecules to improve their antigen presentation capacities. To perform functional studies on the modified cells, a transgenic murine model was established, which uses influenza hemagglutinin as an autoantigen and surrogate tumour antigen. In a second experimental model, activation of the interferon regulatory factor-1 led to the inhibition of tumour growth. To facilitate translation of basic research into cell therapies, cGMP-compliant production schemes for adenoviral vectors and modified dendritic cells were established using a closed integrated bag system.

Marita Sylla observing the concentration of a microbial extract by rotary evaporation during the drug discovery process.
Photo: HZI, Bierstedt



04.1 Microbial Diversity and Natural Products

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

PROJECT MEMBERS | Dr. Klaus Gerth | Dr. Rolf Jansen | Dr. Brigitte Kunze | Dr. Herbert Irschik | Dr. Gabriella Molinari | Heinrich Steinmetz

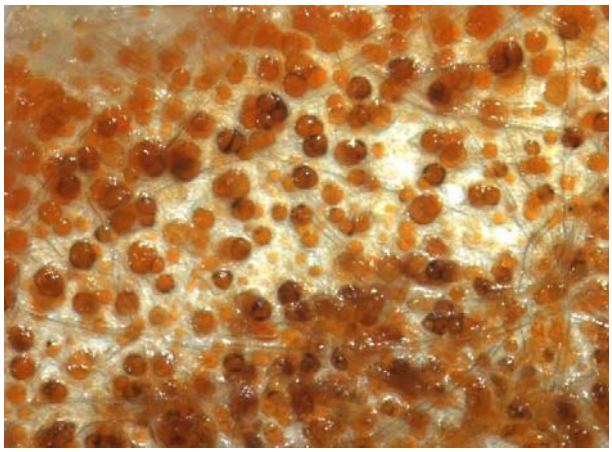
Microbial infections are responsible for 25% of all deaths worldwide. The battle against microbial resistant pathogens demands new and better drugs, which are becoming more difficult to find after decades of hard work on discovery programmes. However, natural products and their derivatives, continue to be rich sources for lead discoveries. They represent more than 60% of the current drugs and exhibit a high chemical diversity. The microbial world represents 90% of all biological diversity and less than 1% has been explored. There is clearly a wealth of diversity still to be investigated. Our strategies involve the isolation and cultivation of new microorganisms in combination with the development of new screens to find new natural products with new mechanisms of action.

Powerful new isolates We count on a large and increasing collection of microbial extracts obtained from Myxobacteria, from other gliding bacteria, and from microorganisms isolated in unexplored and/or rare environments. Extracts showing powerful biological activities in several parallel screens are continuously characterized. New screens were implemented, aimed at discovering new activities, *e.g.* neuraminidase inhibitors or against biofilms. Chemical characterization of selected active extracts is being performed to determine whether or not the observed bioactivities are generated by metabolites with new chemical structures.

A novel group of Myxobacteria - the thermophylic and mesothermophilic Myxobacteria - was isolated, characterized and its biosynthetic potential investigated. Several new isolates were examined in cooperation with Prof. Dr. Rolf Müller, Saarbrücken, for their suitability as hosts for heterologous expression of polyketide synthase gene clusters.

Hundreds of strains of other gliding bacteria, such as *Flexibacter* and *Lysobacter*, were also screened. Of these, 50 and 70 % respectively, produced bioactive metabolites. Novel metabolites were identified and are now being characterized. The complete structure elucidation of Catacandin, a compound produced by lysobacteria, is under investigation.

Cruentaren A The mechanism of action from Cruentaren A was investigated. This strong antifungal and cytotoxic benzolactone is produced by the myxobacterium *Byssovorax cruenta.* Studies to unravel the molecular target showed that Cruentaren A specifically inhibited the F_0F_1 ATPase activity in mitochondria preparations of both the yeast *S. cerevisiae* and beef heart with IC_{50} values of 15-30 nM. In contrast, other members of the structurally closely related benzolactone enamide class, such as Apicularen A and salicylihalamides, were shown to be specific inhibitors of the V-ATPase.



Fruiting bodies of Sorangium cellulosum. *Photo: HZI, Gerth*

04.2 Medicinal Chemistry of Anti-Infectives

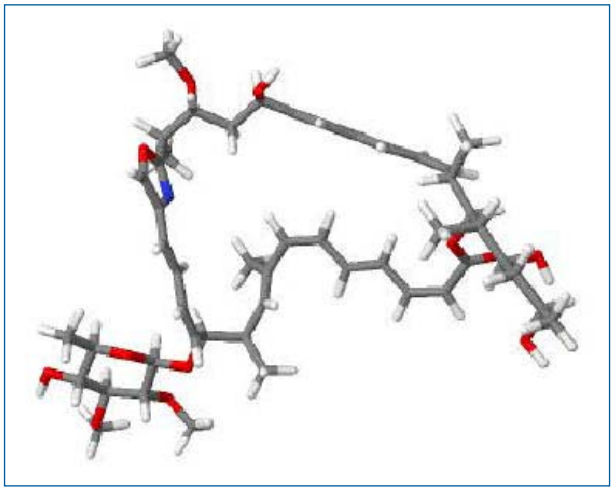
PROJECT LEADER | **Prof. Dr. Markus Kalesse | Department of Medicinal Chemistry | mka05@helmholtz-hzi.de**

PROJECT MEMBERS | Dr. Jutta Niggemann | Dr. Larissa Jundt

This project deals with the chemical synthesis of antibiotics in order to provide new and altered structures for anti-infectives research. The identification of pharmacophoric groups and the subsequent synthesis of tools for chemical genomics are two of the major tasks. Additionally, we will investigate the development of new synthetic strategies and methods that can be applied to modern syntheses platforms, such as automated SPOT synthesis or PASS-flow syntheses.

Synthesis of Corallopyronin and Ripostatin For the development of new and promising anti-infectives, it is necessary to fully understand the molecular architecture of the compounds involved. Their pharmacophoric groups need to be identified and their overall framework, which is often necessary for selectivity and specificity, needs to be unravelled. In some limited cases this can be done through modern spectroscopic methods. More reliable and general is to provide analogs through chemical synthesis. This analoging first requires the synthesis of the very natural product. This paves the way for the implementation of automated synthesis which, in turn, can provide a fine pattern of similar structures around the privileged compound.

The synthesis of natural products, therefore, is the starting point for medicinal chemistry. One of the projects along these lines involves the development of ripostatin and corallopyronin as promising inhibitors of bacterial RNA-polymerases. Unfortunately, the *in vivo* activity is significantly reduced. We hope to provide analogs with advanced biological profiles through chemical synthesis. Along these lines, we are about to establish methods for the parallel synthesis of improved analogs. This can be done through immobilized intermediates. We have so far established a protocol that allows the synthesis of acyl enamines on solid support through rearrangement reactions. Additionally, the first total syntheses of corallopyronin and ripostatin are close to being finalized.



The 3D-solution structure of chivosazol, a potent macrolide antibiotic Sorangium cellulosum.

Chivosazol and Thuggacin Another project revolves around the configurational assignment and synthesis of the macrolide antibiotic chivosazol and the novel anti-tuberculosis lead, thuggacin. The antibiotic, chivosazol, contains 10 centers of chirality. In order to unravel the SAR, these unknown stereocentres have to be established. We were able to assign 5 out of the 10 stereocentres through chemical degradation and chemical synthesis. The remaining stereocentres were assigned with the aid of NMR-analysis and analysis of the keto reductase gene sequence. Moving forward, the first synthetic steps have been accomplished. The same strategy has been applied to thuggacin, for which the first stereocenters have been assigned as well.

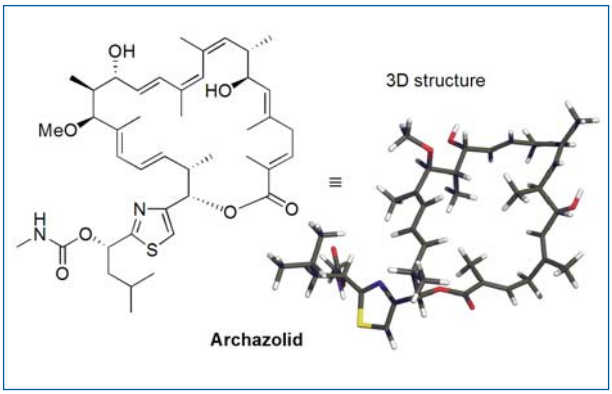
04.3 Development of Novel Antibiotics from Natural Sources

PROJECT LEADER | **Dr. Dirk Menche | Research Group Structure and Function of Antibiotics | mch@helmholtz-hzi.de**

PROJECT MEMBERS | Fatih Arikan | Dr. Jorma Hassfeld | 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 anti-infective research. Natural products continue to command attention, whether these are used to probe biological mechanisms, or to provide the basis for pharmaceutical drug discoveries. Research in this project is focused on various aspects of natural product chemistry and anti-infective research, ranging from natural product isolation and the development of new synthetic methods, to novel strategies in natural product synthesis and analogue design.

Focus: chiral amines A central focus of the programmes directed towards novel methodologies is chiral amines. They are key structural elements in a multitude of antibiotics, biologically active natural products and pharmaceuticals, rendering their synthesis an objective of high priority from the perspective of drug discovery. The aim of our research project is the development of novel methods (that is, ones that are more direct and effective than known procedures) to synthesize chiral amines in only one chemical *(‚stereoselective')* transformation from readily available building blocks, *‚ketones'* (‚direct asymmetric reductive amination'). Based on a novel biomimetic approach, we have developed a completely novel method for the direct reductive amination of both ketones and aldehydes which relies on the highly selective formation of hydrogen bonds. We have demonstrated the efficiency of this method for the synthesis of bioactive amines. The mild and nonacidic conditions, together with the high chemoselectivity of this protocol, should also enable applications for complex and acid-sensitive structures. Furthermore, the underlying catalytic cycle and the modular structure of the organocatalyst should allow the development of asymmetric variants and the adaptation of our approach to other direct procedures with imines as reaction intermediates. It is then planned to use this method for a combinatorial synthesis of antiinfectives.



The 3D-solution structure of archazolid, a potent macrolide antibiotic from the myxobacterium Archangium gephyra.

Archazolid and Etnangien – two potent natural antibiotics Besides conventional target-oriented natural product synthesis, we are interested in the development of simplified, equipotent analogs. Molecular modelling and modern NMR-techniques developed for determination of solution structures hold promise for the rational design of such substances. Current targets include the structurally complex myxobacterial natural products, archazolid and etnangien, present in highly potent macrolide antibiotics. While archazolid is a highly potent inhibitor of vacuolar type ATPases (V-ATPases), etnangien inhibits RNA-polymerase. 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 their relative stereochemistry and ground state conformation in 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, we have also conducted an analysis of *residual* dipolar couplings, as well as gene-based methods. This understanding of the 3D structure now enables a directed total synthesis and the design of analogs and opens the way for a rational design of simplified analogs and an understanding of SAR-data.

** nuclear Overhauser experiment: NMR measurement that allows to determine the distance of protons through space*

04.4 Chemical Biology of Infectious Diseases

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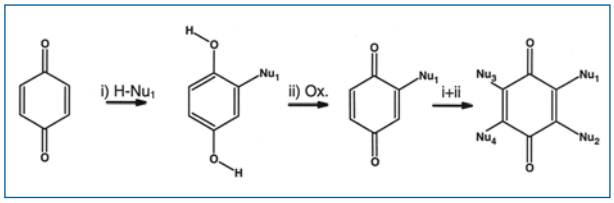
The central objective of this project is the elucidation of molecular mechanisms of infectious processes, using low molecular weightchemicals as tools. With the development of dedicated assays and screening technologies, bioactive compounds are selected from large chemical libraries and analyzed. Results from these analyses will lead to the discovery of new antibiotics, chemotherapeutics and immune modulators. Knowledge about their mechanisms of action will provide novel targets for therapeutic intervention.

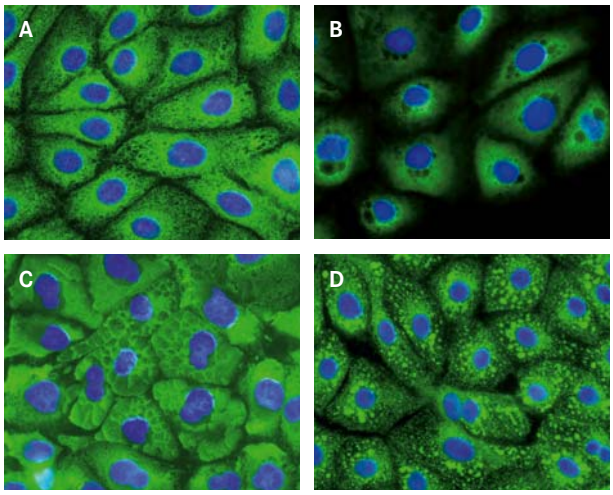
Combinatorial chemistry The major part of our compound repertoires for screening comes from combinatorial synthesis of analogues around chemical core structures, so-called scaffolds. The selection of new scaffolds for the generation of chemical libraries is a crucial step taking into account several synthetic requirements such as compatibility with synthesis technologies and biological relevance. The latter presumes the scaffold to be prone to interact with biological targets – privileged structure. Scaffolds taken from natural products are considered to be privileged because of their natural origin. For example, we have taken the p-Benzoquinone scaffold to design a process for a step-by-step assembly of analogues. This involves Michael addition of a nucleophile yielding a hydroquinone derivative, followed by oxidation, to regenerate the quinone scaffold. Theoretically, this can be repeated four times to reach a tetra-substituted benzoquinone. Intermediate Diels-Alder reactions of a bi-substituted quinone intermediate with appropriate diens, as well as the derivatisation of the hydroquinone hydroxyls, allow further routes for diversification. A series of such analogues were synthesized in solution and tested for biological activity. A solid-phase version of this process is under development.



Concept of the sequential Michael addition process to generate a combinatorial library of benzoquinone derivatives.

High content analysis The probability of identifying a selective interaction between a compound and a biological target increases with the number of compound-target pairs assayed. This is the basic concept of empirical high throughput screening (HTS). In the case of a single compound, the number of biological targets available for screening determines the success. A cell is a complex biological system containing a huge number of target molecules that are all functionally connected: a high content bio-assay system. The cell reacts to the action of the compound by developing a distinct phenotype. Thus, cell-based phenotypic screening is particularly effective in the search for bioactive compounds, although the target is not immediately apparent and needs to be identified subsequently. Often, the resulting phenotype is already known and can suggest potential target molecules.

We, therefore, routinely investigate novel compounds for their phenotypic effects on a series of selected cell types. In the case of our quinone derivatives, one of these perturb the ER of Ptk2 cells in very similar way to a natural product isolated from the marine sponge, *Aka coralliphaga.* Coralliddictyal also carries a benzoquinone scaffold that is most probably the responsible pharmacophore.



Phenotypic effects of benzoquinone derivatives on the endoplasmatic reticulum of PtK2 cells. The cells were stained for ER (green) and nuclei (blue). The control cells (A) show a normal ER network. Incubation of the cells with different benzoquinones induced different alteration in the ER structure. We observe vacuoles (B), cushion like structures (C) and vesicles (D) Photos: HZI, Sasse

04.5 Identification of Molecular Targets of Anti-Infectives

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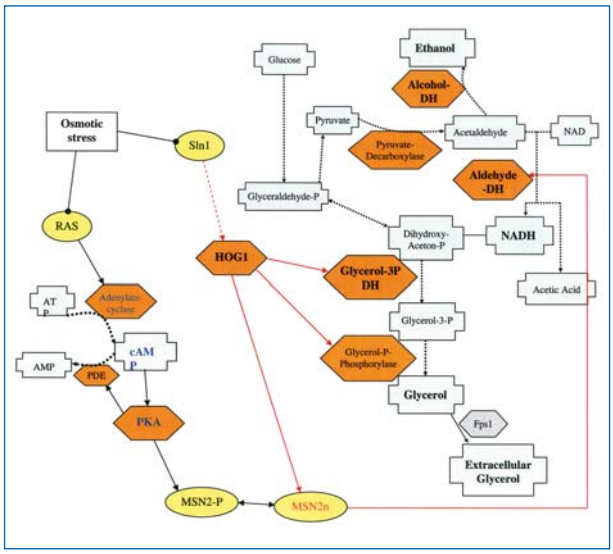
PROJECT MEMBERS | Dr. Florenz Sasse | Bianca Lüderitz | Janine Wesolowski | Nina Klippel

Primary screening for chemical compounds with anti-infective activities is usually based on whole cell assays indicating the growth inhibitory effect of the sample on target organisms, such as fungi, or bacteria. This approach requires subsequent investigations to elucidate the molecular target of the active compound. Due to the increasing relevance of fungal infections, in particular for immunocompromised patients, we decided to focus on compounds, which interfere either with the growth of fungi, or with functions of the innate immune system.

High Osmolarity Glycerol pathway We selected ambruticins, jerangolides and phenylpyrroles as anti-mycotic natural products, which lead to glycerol accumulation in reactive strains. However, the molecular target and the molecular mechanisms are not yet known. Due to its high relevance as a pathogen, we chose *Candida albicans* for the molecular analysis. Glycerol is produced from dihydroxyacetonphosphate, *i.e.* it is a byproduct of glycolysis. The expression of the corresponding enzymes – glycerol-3-phosphate dehydrogenase (GPD) and glycerol-3-phosphate phosphorylase (GPP) – is regulated by anaerobic and by osmotic stress conditions, because glycerol plays a role in the redox balance during anaerobic conditions and in the compensation of hyperosmotic pressure.

In the yeast *Saccharomyces cerevisiae,* the osmotic stress response pathway is well described as a High Osmolarity Glycerol (HOG) pathway. Most proteins of this pathway are also present in *C. albicans.* Thus, we will use a new mathematical *S. cerevisiae* model as a basis for the analysis of the dynamic response of *C. albicans* to compound treatment. Accordingly, we investigated the effects of the selected compounds on growth, not only of various *C. albicans* wild-type strains, but also on the *S. cerevisae* mutant library. Additionally, we set up the quantification of extracellular ethanol and glycerol and of diverse intracellular parameters. The first experiments showed a strong influence of the genetic background of the target organism on the effects of the compounds. Only 2 out of 5 wild-type strains of *C. albicans* showed reduced growth, due to compound treatment. *S. cerevisiae* proved to be resistant, as did most of the single-gene mutants.



Some metabolites and proteins of the osmotic stress response network of S. cerevisiae: Hyperosmotic stress leads to the activation of the MAPKinase HOG1 via the osmotic stress sensor protein Sln1 and to the inhibition of the cAMP-dependent pathways via Ras proteins. Activation of the HOG pathway induces the expression of glycerol-3 phosphate dehydrogenase (glycerol-3P DH), which is the rate limiting enzyme of glycerol biosynthesis. A low activity of the cAMP dependent protein kinase (PKA) favors the unphosphorylated form of the general stress response transcription factor MSN2, which is localised in the nucleus (MSN2n). Parameters, of which the dynamic response will be determined, are indicated in bold.

Macrophages as representative of the immune system Because the innate immune system is the primary line of defence of the host, we selected the macrophage cell line RAW 264.7 to represent the immune system of the host. The interaction of pathogens, such as bacteria or fungi, with macrophages leads to phagocytosis, *i.e.* internalisation of the pathogen, followed by killing of the pathogen using "chemical weapons", such as the low pH and degrading enzymes in the phagolysosome, and reactive oxygen, *e.g.* H_2O_2, and nitrogen species, *e.g.* NO. Additionally, macrophages stimulate other cells of the immune system by messenger compounds, such as interleukins or TNFα. These reactions are the result of signal transduction cascades, which have their origin in various membrane-bound receptors.

Chemicals can disturb these pathways by the modification of protein activities, *e.g.* the polymerisation of the cytoskeleton of cells. Those compounds inhibit phagocytosis of bacteria and yeasts. But we also observed stimulation or inhibition in the production of messenger compounds in response to different chemicals. Here, detailed investigations of the involved proteins have started.

04.6 Antigen Delivery Systems and Vaccines

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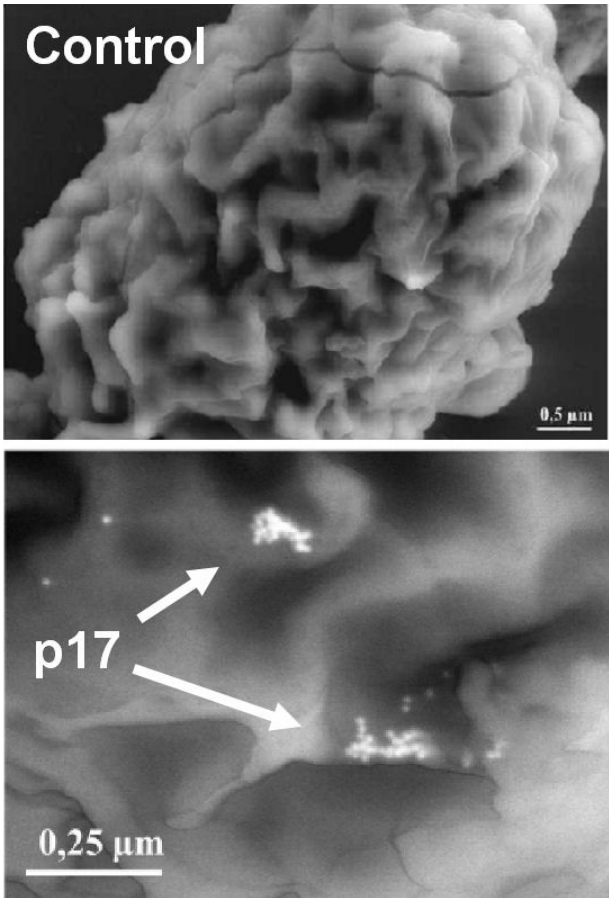
Vaccination is the most cost-effective strategy to prevent microbial infections. The aim of this project is the development of tools and strategies to optimize the delivery of vaccine antigens, particularly by the mucosal route.

Mucosal delivery of the HIV-1 matrix protein p17 The p17 is a structural protein essential to the viral life cycle, which supports HIV replication and spreading. The presence of p17-specific neutralizing antibodies and CTL also correlates with a slower progression to AIDS. Intranasal vaccination with p17 and the TLR2/6 agonist MALP-2 triggered strong humoral and cellular immune responses at systemic and mucosal levels. The antibodies blocked binding of p17 to its receptor, thereby neutralizing its virokine activity. These results suggest that formulations based on MALP-2 and p17, alone or in combination with other structural or regulatory proteins, are attractive candidates for vaccination against HIV/AIDS.

B-cell activation is critical for MALP-2 activity Functional studies performed using cells from animals deficient in TLR2, T cells and B cells demonstrated that MALP-2 promotes activation and maturation of follicular, B-1a and marginal zone B cells via TLR2, and increases the frequency of IgM and IgG secreting cells. Immunization of mice lacking B cells also demonstrated that B cells are critical for MALP-2-dependent improvement of T-cell responses. B-cell stimulation by pattern-recognition receptors appears to be a basic mechanism, which can be exploited to improve vaccine efficacy.

Identification of a T-cell subpopulation with immune modulatory properties A new B220lowCD3lowCD4−CD8−CD19−c-kit+ subpopulation with a T-cell precursor phenotype was identified in murine nasal associated lymphoid tissues (NALT). Fas-independent apoptosis was their main mechanism of death and they were able to down-regulate the activation of mature T cells. Interestingly, they showed high expression of TLR2 and were activated after NALT stimulation with MALP-2. Thus, these cells seem to modulate immune responses by maintaining a local tolerogenic milieu through their pro-apoptotic status and suppressive activity, which can be reverted through stimulation of TLR-signalling cascades.

SopB-mediated immune escape mechanism in *Salmonella* spp. The effector protein SopB, which is translocated through a type III secretion system, promotes immune escape of *Salmonella enterica*. Wild type bacteria and *sseC* or *aroA* attenuated mutants exerted stronger cytotoxic effects on dendritic cells (DC) than their SopB-deficient derivatives. DC infected with *sseC sopB, phoP sopB* and *aroA sopB* mutants also exhibited higher expression of activation markers and stronger antigen processing and presentation capacities. The incorporation of a *sopB* mutation into *sseC, phoP* or *aroA* attenuated strains resulted in improved humoral and cellular immune responses following oral vaccination. Thus, a new immune-escape strategy of this important pathogen was defined, which can be subverted to optimize the performance of *Salmonella*-based live attenuated vaccines.



Binding of the HIV matrix protein p17 to its receptor on the surface of B lymphocytes (indicated by arrows). Photos: HZI

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. Andrea Kröger | Dr. Ute Pägelow | Dr. Lars Macke | Bin Ma | Wilhelm Meyring

One important task of the immune system is to recognize and to eliminate tumours and persistently infected cells. However, tumour cells and persistent pathogens have evolved mechanisms to escape normal immune attack. Therefore, the basic problem for immunotherapy of these diseases is the fact that tolerogenic, anergy-inducing responses and other immune escape mechanisms prevent therapeutic effects, despite an existing immune response.

For the development of effective therapeutic cellular vaccines it is essential to better understand the critical cellular interactions. The functional significance of these interactions has to be confirmed in relevant animal models. Translational research is necessary to develop optimised immunotherapies.

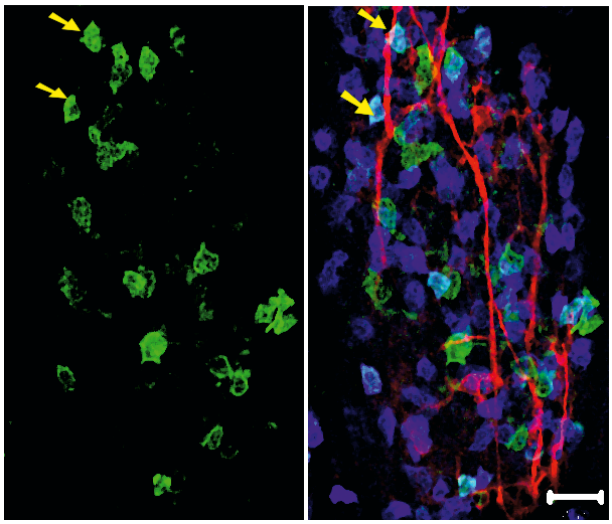
Visualisation of cellular interactions Specific imaging techniques have been established to investigate the highly complex and dynamic interactions of cells in lymph node, spleen and intestine. Special staining protocols and software tools were developed for simultaneous, three-dimensional high resolution imaging of up to six different cell types. These studies allowed visualizing the interactions between cells of the immune system and cells of other tissue, *e.g.* blood and lymph vessels, neural tissue. Confocal microscopy was used to investigate infection, cell migration and dynamic interactions in vitro, in organ culture and even in the living mouse. Detailed structural information was gained by electron microscopy in cooperation with Dr. M. Rohde, Department of Microbial Pathogenicity.

Modulation and functional analysis of the immune response in murine model systems Modulation of cellular interactions was achieved through genetic modification of immune cells, or tumour cells, using vectors which encode antigens and costimulatory molecules.

A transgenic murine model system from the Department of Mucosal Immunity, which uses influenza hemagglutinin as autoantigen and as a surrogate tumour antigen, is used for functional analysis. In this partially tolerant, or anergic setting, animals were immunized with adenovirally modified HA-antigen-presenting dendritic cells to investigate the factors which determine induction of anti-tumour immunity or autoimmunity.

In a second tumour model, activation of the interferon regulatory factor-1 (IRF-1) led to the inhibition of tumour growth and to the regression of tumours. IRF-1 causes transcriptional activation, upregulation of MHC class I, and, secretion of IFN-β suggesting that IRF-1 is counteracting oncogenic growth also by induction of innate and adaptive immune mechanisms.

Tools for production of therapeutic human cells For the translation of basic research into products for cell therapies, we developed cGMP-compliant production schemes for adenoviral vectors and modified cells. The biosafety level 2-GMP facility established for such a process was approved for GMP production by the local authorities. For the safe and reproducible production of genetically modified dendritic cells, a consortium of clinical and industrial partners developed an integrated bag system, which is closed from the isolation of patient cells by leukapheresis until the final formulation. This closed system has important advantages for the generation and handling of dendritic cells and will be extended to the cultivation of other cell types.



Multicolour imaging of intestine nerve fibers surrounded by B and T cells. B cells, T cells, and nerves in the villus of jejunum were stained with anti-B220 (green), anti-CD3 (blue) and anti-PGP9.5 (red), respectively. The yellow arrows mark cells which are positive for anti-B220 and CD3 in close contact with nerve fibers (red). Scale bar = 20 μm. Photo: HZI, Rohde

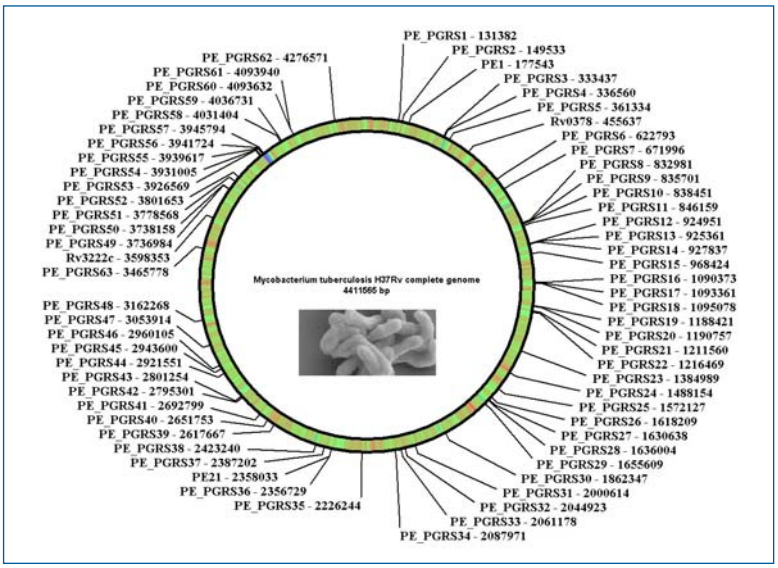
GENOME AND HEALTH RESEARCH

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

Pathogenesis often results from the complex interplay between genotypic and phenotypic traits. Besides inherited genetic defects or dispositions, factors such as age, lifestyle, host-pathogen interactions and environmental stress predominantly contribute to disease processes. The comparative analysis of genome information is an essential element in studying genotype-phenotype relationships for 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. This research programme, therefore, combines the experimental functional characterization of genomes with comprehensive genome-based bioinformatics.

Furthermore, the specific interactions between gene products, *i.e.* proteins, and their ligands are investigated using synthetic chemistry. The design and generation of synthetic mimetics containing discontinuous binding sites leads to novel inhibitors of protein-ligand interactions with high biomedical relevance.



Tackling tuberculosis: M. tuberculosis *genome map showing the family of elements potentially involved in host pathogen interactions.* *Collage: HZI*

01 Inhibitors of Protein-Ligand Interactions

PROJECT LEADER | **Priv.-Doz. Dr. Jutta Eichler | Research Group Conformational Protein-Ligand Interactions | jei@helmholtz-hzi.de**

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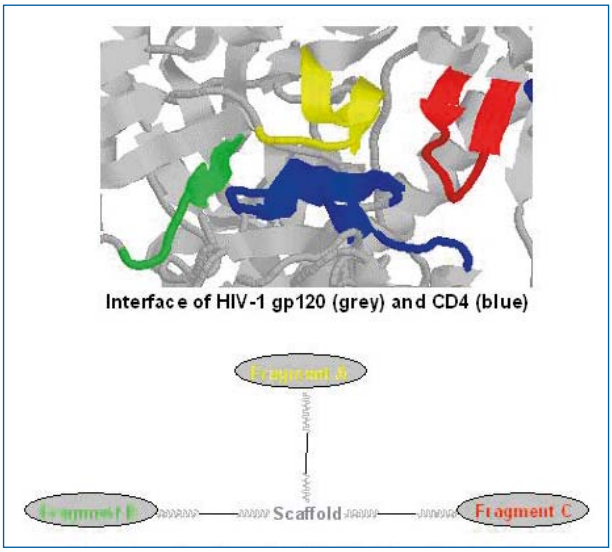
Specific interactions of proteins with their ligand are the molecular basis of essentially all biological processes. The exploration of these interactions at the molecular and atomic level is an important step towards the modulation of protein function through controlled interference with underlying binding events. The design and generation of molecules, which, due to their specific molecular architecture, 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 localized in short, 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. We have recently developed a range of synthesis methods for the generation of structurally diverse assembled and scaffolded peptides. The goal of our current projects is to use these methods to mimic the sequentially discontinuous binding sites of a range of biomedically relevant proteins, including interaction domains (hYAP WW and Mena EVH1 domains), the cytokine receptor gp130, as well as viral proteins (HIV-1 gp120 and SARS-CoV S1), and to use these mimetic molecules als competitive inhibitors of the respective interactions.



Interface of HIV-1 gp 120 (grey) and CD4 (blue) (upper imagine); synthetic mimetic of the CD4 binding site of gp120 (lower imagine). *The image has been taken from the publication: Raimo Franke, Tatjana Hirsch, Heike Overwin, Jutta Eichler (2007) Synthetische Mimetika der CD4-Bindungsstelle von HIV-1 gp120 für das Immunogen-Design. Angewandte Chemie 119(8): 1275-1277. Published Online: 9 Jan 2007.DOI: 10.1002/ange.200603274. The permission of Wiley-VCH publishers is gratefully acknowledged.*

02 Generation and Exploitation of DNA Sequence Data

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

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We focus on the genome-wide study and in-depth analysis of genomic information. This involves high-throughput sequencing of DNA, as well as annotation, up to the level of metabolic and regulatory pathways. More than 50 percent of the department's activities are devoted to bioinformatics work.

Sequence analysis projects DNA sequencing is one of the key technologies in modern biological research and application. 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 working on the complete sequencing and functional analysis of chimpanzee chr X (international consortium). We analysed selected regions from the horse, pig and cattle genomes, most of which are suspected to be disease-related. We have begun to analyse bacterial communities in the gut of mice to study the influence of the nutrition on the composition of gut flora (metagenomics). After the sequencing phase of the genomes of *Sorangium cellulosum, Bordetella petrii* and *E. coli* Nissle 1917, the deep annotation of these genomes is approaching completion.

Going analog! A novel bioinformatics technology was successfully applied where conventional methods fail to establish new principles and new functional properties Photo: HZI

MycoGenomes Supported by an EU project "NEWTB-DRUGS", we identified several drug targets for persistent and multiple drug-resistant tuberculosis. NrdI and ALADH, which are targets involved in DNA or cell-wall biosynthesis respectively, were purified and characterised. NrdI is absent in the host and is upregulated in *M. tuberculosis* under intracellular stress in macrophages. It exists as a monomer in the reduced and as a dimer in the oxidized form, and contains three conserved cysteine residues, suspected to be involved in dimerization.

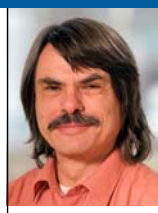
We crystallised the 240kDa large hexameric ALADH. The crystals gave excellent diffraction pattern and the elucidation of the crystal structure is in progress.

In addition, we have established amplification conditions for more than 40 GC-rich genetic elements (PE-PGRS) from *M. tuberculosis.* These elements show highly similar DNA sequences and are currently the intensive subject of tuberculosis research with respect to pathogenesis as well as vaccine and drug development.

For drug screening, a novel assay for RNA polymerase was developed which does not require any labelling and uses natural nucleotides as substrates.

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. In the DNA field, our approach is fully established as the tool which will replace similar programmes when it comes to functional analysis of DNA stretches. We were able to show that the system works as proposed in theory and that the hardware implementation (DSP card) allows for the searching of large eukaryotic genomes in an acceptable timeframe– all this was done with artificial and natural sequences. Systematic selectivity and sensitivity investigations have been carried out with 38 different encoding schemes. The results of these experiments confirm that our system is able to spot property-dependent similarities where letter code-based systems fail.

Our software implementation "FeatureScan" is publicly available through a web site or as a real web service, following the "HOBIT" standards of the Helmholtz Association (http://genome.helmholtz-hzi/featurescan).



GENES, ENVIRONMENT AND HEALTH

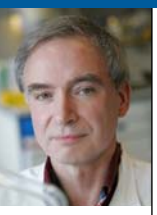
PROGRAMME SPEAKER | **Dr. Wolf-Rainer Abraham | Research Group Chemical Microbiology | wab@helmholtz-hzi.de**

Microorganisms are ubiquitous and because they can tolerate environmental conditions far too extreme for higher organisms, their habitats define the biosphere. Microbial activities profoundly influence global processes - like the carbon cycle and global warming – and local ones, such as animal and plant disease. They also provide essential nutrients for plants and animals. Microbes have a critical impact on human beings and their activities, in a multitude of both positive and negative ways: some are responsible for the greater portion of human disease and mortality, whereas others provide us with antibiotics to treat disease, and still others play a critical role in cleansing our environment of organic waste. Much of biotechnology is based on microbes and their products. Our ability to influence microbial activities – to obtain greater benefit from the positive aspects and diminish the effects of the negative repercussions – requires an understanding of how microbes live and function in their habitats, and how their activities are regulated.

Classical microbiology focuses on the study of pure cultures growing under laboratory conditions. However, microbes in nature grow as complex, diverse and dynamic communities, the members of which interact and share available resources in complex ways. It is these interactions, and interactions with other biotic and abiotic components of their environment, that determine community activities. At present, we have no general understanding of such interactions.

The goals of the Environmental Microbiology research programme are to understand microbial communities as functional units, to elucidate the critical interactions that regulate community activities, to develop and validate interventions that result in optimizations of microbial activities, promoting beneficial and minimizing detrimental ones, and to discover new microbial products and metabolic activities by exploring microbial diversity. The research programme is characterised by a multi-scale approach – gene, organism, community; test tube, chemostat, natural habitat – and a multi-disciplinary one – microbial ecology, physiology, phylogeny, biochemistry, analytical chemistry, genetics/genomics, bioinformatics, and modelling. Although the results obtained are generally applicable to most types of microbial communities, our research focuses on microbial communities that either cause disease in humans or live in extreme environments. An important goal of the programme is to make key contributions to the sustainable development of our society.

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



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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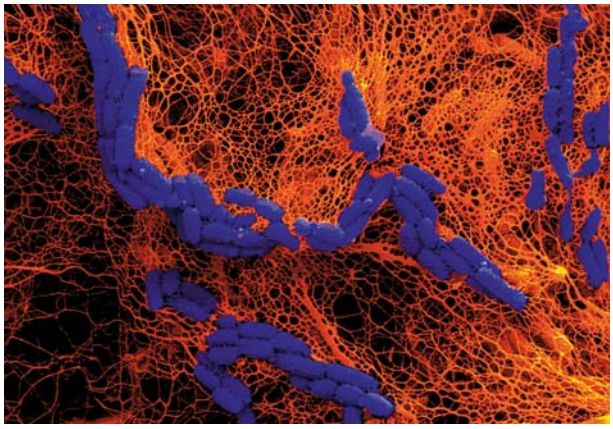
Alcanivorax borkumensis is one of a number of cosmopolitan marine "hydrocarbonoclastic" bacteria that utilize exclusively oil hydrocarbons as sources of carbon and energy. Such bacteria are present at low concentrations in unpolluted environments but bloom after an oil spill. *A. borkumensis* is unusual in that it dominates the microbial communities of most oil-polluted waters or oil-spiked seawater-based experimental microcosms. It has become the paradigm of hydrocarbonoclastic bacteria and, for this reason, was selected for a genome sequencing/functional genomics analysis.

This analysis has revealed multiple determinants of functions for the efficient utilization of a wide range of aliphatic hydrocarbons. *In silico* deductions are supported by proteomics data showing hydrocarbon induction of membrane-localized monooxygenases, which act in concert with rubredoxins/rubredoxin reductases in the terminal oxidation of hydrocarbons. *A. borkumensis* also has an extensive array of determinants of functions for the scavenging of nutrients and oligoelements in the nutrient-poor open sea, and specifies systems for biofilm formation at the oil-water interface, biosurfactant production and niche-specific stress responses.

Collectively, these features provide *Alcanivorax* with a significant advantage over its competitors in oil-polluted environments and explain its dominating role in hydrocarbon degradation in most marine settings. The decoding and annotation of the *Alcanivorax borkumensis* genome marks a milestone towards new strategies for the biomitigation of oil contamination of marine systems.

Biotechnological potential of *Alcanivorax* The functional genomic analysis of *Alcanivorax* has revealed further biotechnological potential. Firstly, one transposon mutant generated, that is defective in biofilm formation, has a unique and entirely unexpected phenotype, namely the hyperproduction and extracellular deposition of polyhydroxyalkanoate (PHA) food storage material, a starting substance for environmentally-friendly bioplastics. Since the main problem with the exploitation of PHA for industrial polymer production, the cost and environmental burden of solvent extraction of intracellular PHA granules from producing cells, is circumvented in this mutant, it represents a potential "petrochemical biofactory", a cell factory for the production of bioplastics from petroleum oil.



Alcanivorax borkumensis *SK2 mutated by tesB-like hydroxyacyl-coenzyme A-specific thioesterase gene overproduces and excretes polyhydroxyalkanoates.* *Photo: HZI, Lünsdorf*

Moreover, a genomic expression library of *Alcanivorax* has yielded a number of new carboxylesterases with high specific activities (1-2 orders of magnitude higher activities than those of typical esterases) and excellent enantioselectivity (E>100) in the kinetic resolution of a variety of chiral synthons, including (R)-geranyl acetate and L-tryptofan methyl ester, (R)-menthyl acetate, methyl(R)-3-bromo-2-methylpropionate and N-benzyl-D-proline ethyl ester, which make them potentially useful for the synthesis of certain fine chemicals and drug precursors.

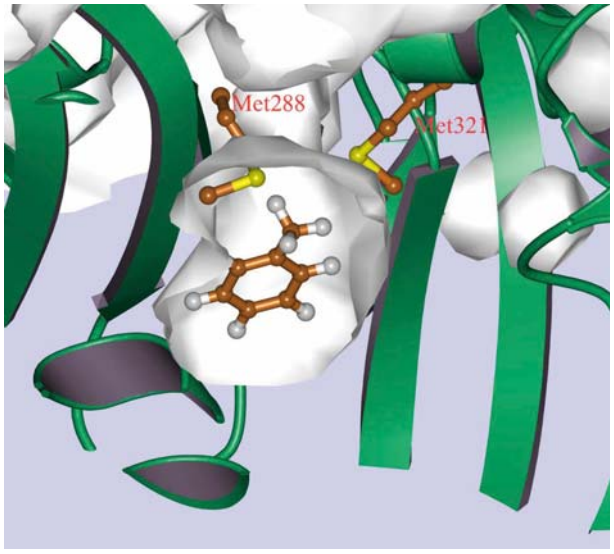
02 Metabolic Diversity

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

PROJECT MEMBERS | Hamdy Aly | Dr. Beatriz Camara | Dr. Howard Junca | Macarena Marin | Dr. Hannes Nahrstedt | Britta Scheithauer | Robert Witzig | Dr. Melissa Wos

The goal of the project is to quantify, predict and influence metabolic processes under *in situ* conditions. To gain an understanding of the activities and adaptation abilities of bacterial communities, we performed a detailed analysis in pure cultures and model communities and developed methods allowing analysis in complex systems. The project concentrated on the network for the metabolism of aromatic pollutants and is now focused on understanding intestinal ecosystems because microbial gut microflora has a profound influence on human health. Due to its high metabolic diversity, the microflora have an immense impact on the fate of nutritive and non-nutritive food components.

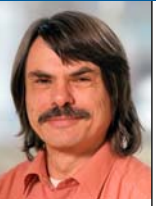
New biocatalysts Despite the difficulties and artificiality of isolating and propagating microbes in pure culture, this is an appropriate way to obtain detailed information on their metabolic routes and networks. We obtained new knowledge on how microorganisms and their key oxygenase enzymes can transform highly toxic dioxin-like pollutants, such as chlorinated dibenzofurans. We evaluated the metabolic capacities of a new *Pseudomonas* species for the degradation of salicylates as crucial metabolites in the degradation of dibenzofurans, and found evidence for the presence of unusual catabolic routes and the involvement of enzymes not or only distantly related to previously described ones.



Model of the active site of an isopropylbenzene dioxygenase indicating the influence of two bulky methionine residues on the substrate binding pocket. The substrate toluene is shown in the active site pocket. Graphic: HZI

Analysis of community functions A detailed picture of the catabolic gene structure in environmental samples will increase our knowledge of the functional potential and evolution of microbial communities. Genetic fingerprinting techniques were used to assess the diversity and distribution of genes encoding oxygenases as key activities for the degradation of various environmental pollutants. Functional genotype fingerprinting of the central cores revealed a substantial diversity in soil samples differently contaminated with mainly benzene. In contrast to the expected benzene dioxygenases, isopropylbenzene dioxygenases were found to be predominant. Analysis of abundant sequence types showed differences in amino acids, which are localized at the substrate binding pocket. While isolates containing a protein with isoleucine and leucine at these positions were capable of degrading benzene and toluene, isolates containing two methionine substitutions were capable of degrading benzene only, indicating that the more bulky methionine residues significantly narrowed the available space within the substrate-binding pocket. These findings exemplify how extreme contamination conditions could select for gene polymorphisms, encoding genes with altered active site structure to act on specific substrates.

Communities in biliary stents Biliary stents are catheters placed to overcome obstructions of the biliary duct. This artificial surface introduced into the human body is prone to microbial colonisation. Ultimately the stents become occluded by biofilms and have to be replaced. Because knowledge on the microbes colonising the stent might lead to strategies for prevention of biofilm formation, stents from medical clinics have been analysed for their microbial community composition. Members of the gastrointestinal microflora were observed to be predominant in these biofilms. Overall, the establishment of biliary stent biofilm communities could be shown to be a strongly host-dependent process with a high variance between different patients.



03 Biofilm Communities in Environment and Health

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

PROJECT MEMBERS | Jörgen Haneke | Dr. Heinrich Lünsdorf | Dr. Alexandre Macedo | Sonja Pawelczyk | Esther Surges

This project aims to understand microbial communities as functional units and to discover novel metabolic activities by exploring microbial diversity. We focus our research efforts on microbial communities living in biofilm communities in order to generate the knowledge base for controlling them.

Dynamic biofilm communities on PCB The ability of microbial communities from polluted habitats to degrade xenobiotic compounds was explored. Soil samples were taken from a polluted site and served as inocula to grow biofilm communities on polychlorinated biphenyls (PCBs). We observed in the microscope that, contrary to our expectations, the bacteria did not grow at the beginning directly on the PCB-oil droplet but around it. After 7 days the microbial diversity in the biofilm community increased and the bacteria started to colonize the PCB oil. At the same time, the biofilm, to this point rather homogenous, started to form aggregates, and the diversity of the biofilm community continued to increase. Although degradations of less chlorinated compounds were observed right from the beginning, this degradation came almost to an end during the process of colonization of the PCB-oil. However, after 21 days and the complete colonization of the PCB-droplet the diversity of the biofilm community decreased and the degradation started again comprising now for the first time higher chlorinated compounds. We assume that anaerobic pockets formed in the biofilm where these compounds were reductively dechlorinated and the resulting metabolites diffused to the surface of the aggregates where they were degraded aerobically. To our knowledge this is the first characterization of such a coupling of anaerobic and aerobic degradation in biofilms.

The girder has been exposed during 5 months to the deep waters (> 25 m) of the opencast mining lake at Merseburg Ost. Now it is been harvested with the surface-grown biofilms in a joint action with scientists from the UFZ Halle-Leipzig. The deep waters of this lake are cold and contain high salt concentrations, and we discovered here many bacteria, which we have known until now only from the Antarctica.
Photo: HZI, Abraham

Biofilms from highly saline mining lakes A former open cast mining site of lignite near Merseburg was initially flooded by highly saline groundwater which was then covered by freshwater during the controlled flooding. Currently, the lake has a sharp transition between aerobic freshwater and anaerobic salt water. The salt water has a temperature of 6-8°C over the whole year. We were interested in the microbial diversity of this extreme habitat because microbial communities of such habitats have only been described from Antarctica. The next relatives of isolates from the salt water layer came as expected from marine and arctic areas. Furthermore, we isolated bacteria which produce natural products active against multiresistant clinical isolates. These bacteria are currently under further investigation. To learn more about the functional diversity of the microbial communities in the anaerobic salt water we exposed inert substrata in the salt water in order to obtain biofilms. Their analyses revealed many sulphate reducers and the formation of minerals obviously connected with the biofilms. Our results demonstrate that, even in Germany, a habitat can be found where arctic bacteria live and which also comprise novel producers of biologically active compounds.

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.

Part of the groups of the technological platforms are working in the "Gründerzentrum"-building Photo: Radde

01 Central Animal Facility

HEAD | **Dr. David Monner** | **Central Animal Facility** | **dmo@helmholtz-hzi.de**

The purpose of the Central Animal Facility is to care for and provide research animals – exclusively mice – for the scientists at the HZI and to monitor compliance with the guidelines of the federal Animal Welfare Act. All animals are kept under state of the art conditions in individually ventilated cages (IVC).

The facility consists of a building dedicated to breeding mice under specific pathogen-free (SPF) conditions, a separate quarantine and a bio-safety level 2 unit (BSL-2) for performing infection experiments under SPF conditions. Over 70% of the mouse colonies are now kept under SPF conditions with regular health monitoring.

In addition to caring for breeding colonies, both under specific pathogen-free conditions and in quarantine, duties of the facility include performing back crosses and experimental breedings to create new mouse lines, routine health monitoring, the rederivation of strains by embryo transfer, archiving of strains by embryo and sperm cryopreservation, maintenance of nuclear breeding colonies, and the breeding and provision of donor animals and pseudo-pregnant females for the generation of new genetically-modified mouse lines by ES-cell blastocyst injection.

Dr. Monner moving new mouse cages to their place.
Photo: HZI, Bierstedt

Services During 2006, cage occupancy in the facility remained relatively constant at about 3,600. Currently, over 150 different mouse lines are housed in the facility. Besides providing standard animal care, the animal technicians carry out all experimental breeding with attendant data bank administration and perform a number of other services, including biopsies, blood sampling, immunisations and other manipulations.

In 2006, rederivations were performed on six lines, while five lines were archived by embryo cryopreservation. *In vitro* fertilization techniques were further refined during the year.

The training program for laboratory animal technicians had a successful year. Two apprentices finished their training and received their certification. Presently, one apprentice is in the third and final year of training, and four are in their second and first years, respectively.

Operation of the infection platform The dedicated animal care unit for performing infection experiments at BSL-2, located in an annex in a separate building (D) and with a capacity of 1,728 cages, has now been in operation for three years. Only SPF-certified animals, whether purchased or from our own production colonies, are allowed into the unit and all activities, experimental breedings as well as infection experiments, are performed under correspondingly controlled conditions.

Planning for the new animal facility on the HZI campus
The planning for a new facility with a capacity of approximately 10,000 cages began in January, 2004. This year the tenders and submissions of bids for most of the construction were completed. Commissioning of the building is planned for summer, 2008.

The animal facility in the Twincore Building in Hannover
The Twincore Research Centre in Hannover was founded in the fall, after the HZI and the MHH purchased the building that had housed the MPI for Experimental Endocrinology. The planning for Twincore calls for the establishment of an animal facility with the same hygienic standard as the HZI facilities, namely SPF using a gnotobiotic background.

02 Analytical Instruments

HEAD | Dr. Victor Wray | Research Group Biophysical Analysis | vwr@helmholtz-hzi.de

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

This platform is a facility for determining the three dimensional structure of all types of natural products and is equipped to carry out mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), X-ray crystallography, protein sequencing, electron microscopy and confocal laser microscopy. For the majority of low-molecular natural products, their structure can be 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. Mass spectrometry has the important advantage of providing information about very small amounts of compound.
The secondary and tertiary structure of peptides and proteins can be elucidated in solution using multidimensional NMR spectroscopy when appropriately labelled material (^{15}N and ^{13}C) is available. Automated MS micro-techniques are used for the identification and characterization of proteins from 2D gels for “Proteomics” and from “gel-less” techniques, through the determination of the molecular weight of their proteolytic fragments using MALDI/TOF-MS/MS and HPLC-ESI-MS/MS.

X-ray crystallography The main emphasis in X-ray crystallography is the structural analysis of proteins at the atomic level. A pipette-robot and a 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.

Edman degradation N-terminal protein sequencing is performed by automated Edman degradation. Applications include the elucidation of new protein sequences, the identification of proteins in data bases, as well as checking the identity and purity of recombinant proteins. Samples, either in solution, or bound to PVDF-membranes, may be analysed in the low picomolar range.

FESEM-techniques Electron microscopy is used to visualize the adherence to, and invasion of, host cells by a wide range of pathogens. Preparation protocols have been customized to undertake studies using high resolution field emission scanning electron microscopy (FESEM) revealing distinct pathways for invading the same host cell. In addition, a methodology has been developed to immuno-localize-pathogenicity factors using FESEM, not only on the bacterial cell surface or the interface between bacterial and host cell membrane, but also inside the host cell using antibodies and colloidal gold-particles.

Preparing a probe for an analysis at the NMR.
Photo: HZI, Gramann

03 Gene Expression Analysis

HEAD | **Dr. Robert Geffers** | **Research Group Mucosal Immunity** | **rog@helmholtz-hzi.de**

SCIENTIFIC COLLABORATOR | Andreas Jeron

The Array Facility is a central service unit at the HZI, performing micro array based expression analysis. Following the principle of high throughput analysis, particular emphasis is placed on the automation of sample preparation and development of customer adapted micro arrays. This allows multiple screenings for gene sets of interest. The customized expression analyses are complemented by highly standardized GeneChip expression arrays manufactured by Affymetrix. Alongside Affymetrix, tiling arrays and SNP genotyping arrays for mouse and human are available for transcript mapping, promoter studies and genotyping on genomic level.

Services The Array Facility offers expression, genotyping and promoter analysis services for HZI researchers and external collaborators. In 2005, a total of 550 expression arrays were performed, with 450 using the Affymetrix GeneChip technology. Out of these 450 expression arrays, 250 were requested by HZI researchers and 200 by external research groups und collaborators. Some 100 experiments were performed on self-printed custom chips, so called theme arrays.

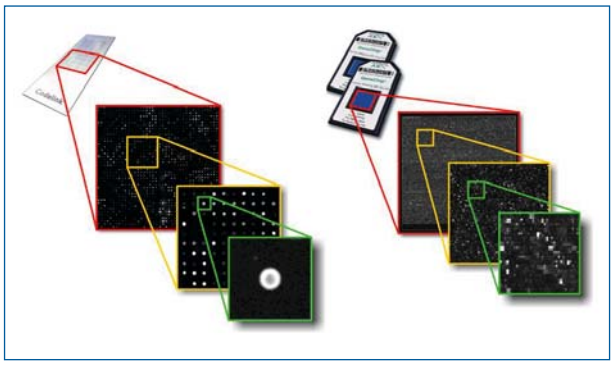
For the development and manufacturing of theme arrays, the array facility advises users on the optimum choice of materials and shipping conditions. The manufacture of the arrays is guaranteed by the Array Facility, and standardized quality controls are applied to each batch of arrays. Furthermore, optimized protocols for their application are published for our customers.

In addition, to array manufacturing and sample preparation, data storage is organized on the basis of international standards allowing for a micro array data exchange with other micro-array platforms.

Research... In the course of numerous internal and external collaborations, analyses were performed by the facility relating to tumor growth and type, host-pathogen interactions – Streptoccoci, Pseudomonads and Mycobacteria – and immune responses.

Two examples: We have developed, together with the RWTH Aachen and HZI researchers, a unique whole genome *Streptococcus mutans* expression micro array that we have applied to the uncovering of molecular mechanism in quorum sensing. The aim of the project is to develop new therapies to keep these antibiotica resistant bacteria under control. Together with researchers at the MHH, we have used a combination of expression analysis and genotyping to identify a new factor that is critical to the functioning of neutrophils, B cells, cytotoxic T cells and melanocytes and plays an important role in a novel human primary immunodeficiency disorder.

...and Development In the course of custom array development and improvement, several new theme arrays were generated by researchers at the HZI and MHH. Further individual chip designs are intended for 2007.



Chips for the Array Facilities. *Photo: HZI*

04 Peptide Systhesis

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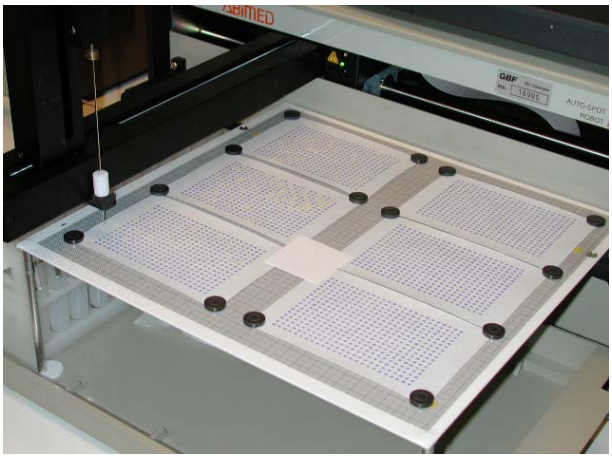
SCIENTIFIC COLLABORATOR | Dr. Ronald Frank

Since its inauguration as a service unit in 1990, the platform generates synthetic peptides, both in soluble form and immobilized in the form of arrays for many different scientific HZI projects. State-of-the-art equipment is employed for the synthesis, characterization and purification. By pursuing our own research projects, our methodological repertoires are continuously updated and extended.

Soluble peptides To date, over 2,500 soluble peptides have been generated in the platform and handed over to the researchers. Soluble peptides are characterized using HPLC and MALDI mass spectrometry. If necessary, further characterization is carried out by amino acid analysis, protein sequencing, special mass spectrometry techniques and NMR in the HZI Department 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 phosphorylation, biotinylation, lipid conjugation, branched peptides and cyclizations.

SPOT-arrays In the platform, immobilized 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 by close cooperation and collaboration with the users. The SPOT-arrays are generated semi-automatically and fully automatically on paper sheets or other polymeric supports. Each year, approximately 15,000 peptides and peptide mixtures are generated in an array format and utilized for the investi gation of *e.g.* protein-protein interaction and enzyme-substrate recognition.



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

05 Histo-Pathology Platform

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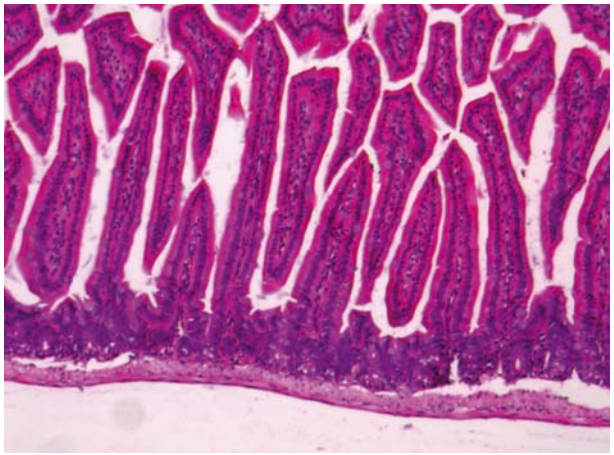
SCIENTIFIC COLLABORATOR | Priv.-Doz. Dr. Reinhard von Wasielewski

A new histo-pathology platform was established in 2006 as a central service unit at the HZI. The unit 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 defense in genetically diverse mouse strains and mutant lines. Thus, the need for histological and pathological analysis of *in vivo* experiments has greatly increased. The histo-pathology unit now offers a central customized 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 these tasks themselves.



Intestine of a mouse (10X), not infected, HE-coloured. Photo: HZI

At present, paraffin sections are offered on a routine basis. Cryostats are available and a limited set of immune-histochemical analyses is available which will be continuously expanded. More specialized services can be performed in collaboration with the Department of Pathology at the Hannover Medical School (MHH). Pathological expertise and support for the planning of experiments and interpretation of results is being provided by a professional pathologist who is regularly present at the HZI, or can be contacted if needed.

06 Protein Expression

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Protein production at an 100L scale. *Photo: HZI, Bierstedt*

The recombinant protein expression group (RPEX) of the structural biology division is coping with the task of producing both bacterial as well as mammalian proteins for structural analysis. Nowadays, it is possible to clone almost all genes, but the success of expression depends on the availability of an efficient expression system. Therefore, four major expression systems have been established in the RPEX facility: *E. coli, P. pastoris*, insect cell and mammalian cell cultures. This allows the production of "simple" proteins as well as proteins with complicated modifications, often designated as "difficult proteins".

In June 2006, the RPEX group moved as an integral part of the Division of Structural Biology into a new building on the HZI campus. The new facility houses state-of-the-art infrastructure and services for structural biologists at the HZI, as well as other Helmholtz research centers and academic institutions, to produce proteins for structure analysis using X-ray crystallography, NMR spectroscopy and electron microscopy.

Services The RPEX group is comprised of scientists with a profound knowledge of cloning, gene expression, production, purification and refolding of proteins. Currently, about 50% of the capacity is used for the production of proteins for in-house structural analysis. The second half is dedicated to the newly established Helmholtz Protein Sample Production Facility (PSPF), which is located at the HZI and the Max-Delbrück-Center (MDC) Berlin-Buch. The aim of the HZI activities is the production of sufficient amounts of ultra-pure proteins for structural characterisation using animal cell culture techniques. The PSPF is supported by additional funding from the Helmholtz Association of German Research Centres.

Research The RPEX group is investigating new strategies to improve the expression of multiprotein complexes and to establish novel methods for protein production using the latest technological developments, mainly in the field of mammalian and insect cell culture expression systems. The main challenges for the facility.are to pursue a scale-down by increasing the productivity of the expression systems, reducing the development time scale from clone to stable expression and automation.

07 Biotech Facilities

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In 2005/2006, the Biochemical Engineering Division – Biotech Facilities – of the Helmholtz Centre for Infection Research (HZI) continued to act as a national technology service provider for clients both inside and outside the Helmholtz Association. These services comprised the development and scale-up of cultivation processes for microbial and animal cells, and purification processes for the isolation of biomolecules, such as proteins, nucleic acids and antibodies from cell mass and supernatant.

At the HZI, various biotechnological pilot plants are available for this purpose, housing numerous bioreactors, centrifuges, chromatographic and filtration systems. The facilities have been licensed since 1997 for the production of GMP-material, in accordance with the German Drug Act (AMG), thus enabling novel active pharmaceutical ingredients to be produced for clinical research. In compliance with the regulations, a highly compartmentalized clean room pilot plant (GMP I) was installed in 1999. In order to satisfy increasing demand for capacity and quality, an additional plant was installed in 2001 (GMP II). The commissioning and qualification of GMP II is currently ongoing. Since 2004, the HZI has been the first and until now only German institution with a general and non-product-specific GMP manufacturing license.

During 2005, more than 300 microbial and mammalian cell cultivations were performed, of which 40% were for external clients from academic institutes and industry.

Three projects with biopharmaceutical active proteins as their target have been carried out for German pharmaceutical and biotech companies and are targeted for GMP-manufacturing of pharmaceutical grade drug substance /API. A long term cooperation focused on the development of animal cell culture processes was initialized in early 2006 with the Swiss-based pharmaceutical company F. Hoffmann La-Roche Ltd..

Dr. Neophytos Papamichael controlling the water supply system of the GMP-unit II at the HZI. *Photo: HZI, Bierstedt*

People at the HZI

Photo: HZI

Photo: Koch

Photo: HZI

Photo: HZI

Photo: HZI

Photo: Koch

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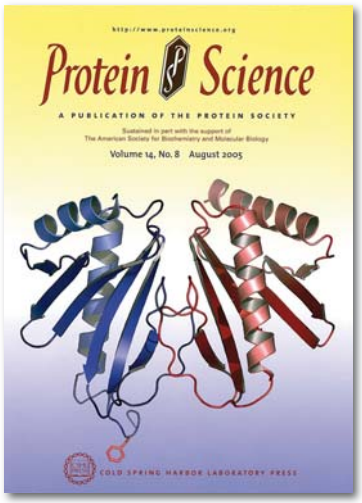

Cover picture of the journal Protein Science, Vol. 14 (8), 2005, on the occasion of the publication of the article by Büttner, C. R.; Cornelis, G. R.; Heinz, D. W., and Niemann, H. H.. Crystal structure of Yersina enterolitica *type III secretion chaperone SycT.* ***Protein Science.*** *2005.* ***14 (8)****: 1993 – 2002. The permission of Cold Spring Harbor Laboratory Press is gratefully acknowledged.*

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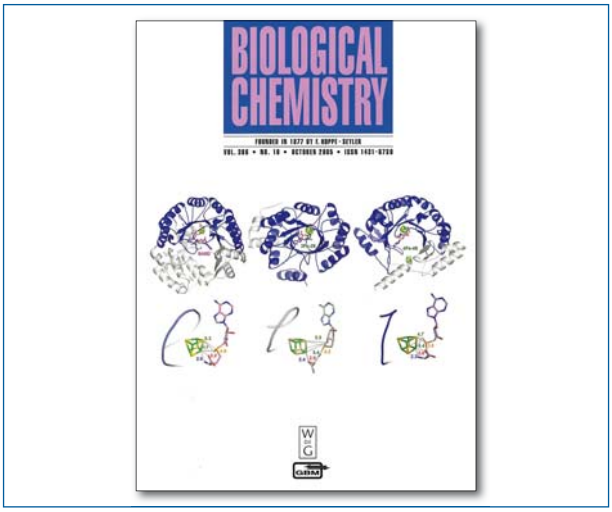

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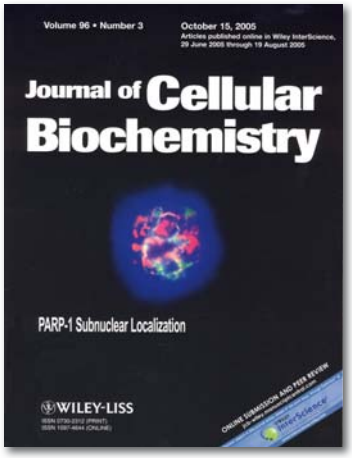

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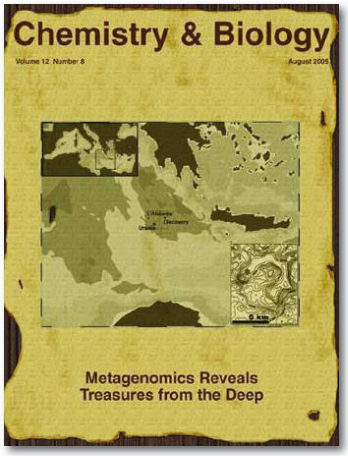

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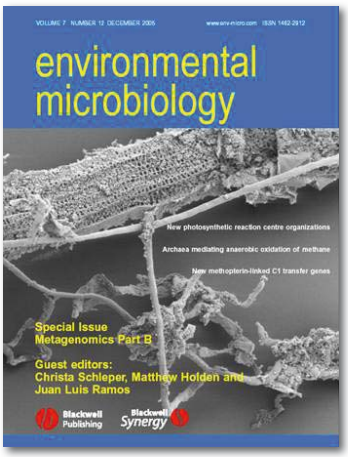

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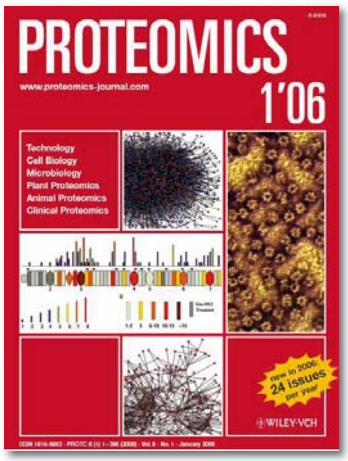

Cover picture of the journal Proteomics, Vol. 6 (1), 2006, on the occasion of the publication of the article by Strocchi, M.; Ferrer, M.; Timmis, K. N., and Golyshin, P. N.. Low temperature-induced systems failure in Escherichia coli: *Insights from rescue by cold-adapted chaperones. **Proteomics**. 2006. **6 (1)**: 193 – 206. The permission of Wiley-VCH is gratefully acknowledged.*

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*Cover picture of the Journal of Bacteriology, Vol. 188 (24), 2006, on the occasion of the publication of the article by Sabirova, J. S.; Ferrer, M.; Lünsdorf, H.; Wray, V.; Kalscheuer, R.; Steinbüchel, A.; Timmis, K. N., and Golyshin, P. N.. Mutation in a "*tesB*-like" hydroxyacyl-co-enzyme A-specific thioesterase gene causes hyperproduction of extra-cellular polyhydroxyalcanoates by "*Alcanivorax borkumensis*" SK2.* ***Journal of Bacteriology.*** *2006;* ***188 (24):*** *8452-8459. The permission of the American Society for Microbiology is gratefully acknowledged.*

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- Yakimov,M.M., Giuliano,L., Cappello,D., Denaro,R., & Golyshin,P. N. (2007) Microbial community of a hydrothermal mud vent underneath the deep-sea anoxic brine lake urania (eastern mediterranean). In: Origins of Life and Evolution of the Biospheres.

Platforms – 2007

- Gutzeit,D., Wray,V., Winterhalter,P., & Jerz,G. (2007) Preparative isolation and purification of flavonoids and protocatechuic acid from sea buckthorn juice concentrate (*Hippophaë rhamnoides* L. *ssp. rhamnoides*) by high-speed counter-current chromatography. *Chromatographia* **65(1-2)**, 1-7.
- Wei,M., Fujiki,K., Ando,E., Zhang,S., Ozaki,T., Ishiguro,H., Kondo,T., Nokihara,K., Wray,V., & Naruse,S. (2007) Identification of key residues that cause differential gallbladder response to PACAP and VIP in the guinea pig. *American Journal of Physiology -Gastrointestinal and Liver Physiology* **292(1)**, G76-G83.

Further Publications – 2007

- Fürch,T., Hollmann,R., Wittmann,C., Wang,W., & Deckwer,W.-D. (2007) Comparative study on central metabolic fluxes of *Bacillus megaterium* strains in continuous culture using 13C labelled substrates. *Bioprocess and Biosystems Engineering* **30**, 47-59.

Lectures 2005-2007

Lectures held at the HZI by external scientists

Lectures 2005

- Ackermann, Karin, Dr.: *Bone metastasis: The crosstalk of protate tumor cells with osteoblasts;* DKFZ. Heidelberg, Germany
- Anderluh, Gregor, Dr.: *Membrane binding processes of pore-forming toxins studied by surface plasmon resonance;* University of Ljubljana. Ljubljana, Slovenia
- Anderson, Kurt I., Dr.: *The leading edge is a lipid diffusion barrier;* Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
- Baumann, Jörg, Dr. *The interaction of virus and host: Cellular factors influencing retroviral infection and transmission;* HIV Drug Resistence Program, National Cancer Institute at Frederick. USA;
- Bennett, Simon T: *Ultra high throughput DNA sequencing for comprehensive whole genome analyses;* Firma Solexa. Essex, UK
- Berger, Imre, Dr.: *New Baculovirus expression tools for multiprotein applications;* ETH Zürich. Switzerland
- Brakhage, Axel, Prof. Dr.: *Molecular mechanisms of virulence of the fuman pathogenic fungus Aspergillus fumigatus;* Dept. Molecular and Applied Microbiology, Leibnitz Institute for Natural Products Research and Infection Biology. Jena, Germany
- Brueck, Thomas, Dr.: *Biotechnological routes to identify and exploit bioactive secondary metabolite producers associated with marine soft coral;* Center of Excellence in Biomedical and Marine Biotechnology , Boca Raton, Florida, USA
- Craig, Benham, Prof. Dr.: *Structural transitions in stressed DNA and their roles in regulation;* University of California, Davis. Davis, USA
- Dröge, Marcus, Dr.: *The Genome Sequencer 20 System from Roche Applied Science – A Revolution in Genome Analysis;* Global Marketing Director, Roche Applied Science. Penzberg, Germany
- Edenhofer, Frank, Dr.: *Cell penetrating proteins in stem cell biology;* University of Bonn. Bonn, Germany
- Essen, Lars-Oliver, Prof. Dr.: *DNA photolyases: A structural base for light-mediated DNA repair;* University of Marburg. Marburg, Germany
- Gomelsky, Mark, Prof. Dr.: *Novel protein domains in bacterial sensory transduction: from sequence to biology;* University of Wyoming, Dept. of Molecular Biology. Wyoming, USA
- Grez, Manuel , Dr.: *Correction of Chronic Granulomatous Disease by Gene Therapy;* Georg-Speyer-Haus. Frankfurt, Germany
- Grosse-Hovest, Ludger, Dr.: *T lymphocytes to melanoma cells;* Institute for Cell Biology, Dept. of Immunology, Eberhard Karls University. Tübingen, Germany
- Hauck, Christof, Dr.: *Eyploitation of cell adhesion molecules by bacterial pathogens;* Centre for Infection Research at the University of Würzburg. Würzburg, Germany
- Heidtke, Karsten R., Dr. *Data analysis;* Bioinformatics – German Resource Center for Genome Research. Berlin, Germany
- Häupl, Thomas, Dr.: *Lost and Found: Secrets of Inflammation in Floods of Expression Data;* Charité Universitätsklinikum. Berlin, Germany
- Hövelmeyer, Nadine: *Targeted mutation of the NFkB deubiquitinating enzyme CYLD leads to major immunological deficiencies;* University of Köln. Köln, Germany
- Just, Lothar, Dr.: *The intestinal stem cell compartment: Analysis of neural and epithelial progenitor in the gut;* University of Tübingen. Tübingen, Germany
- Kempermann, Gerd, Dr.: *Adult neurogenesis: neuronal development under the conditions of the adult brain;* Forschungsgruppe "Neurogene Permissivität" der Volkswagenstiftung, Abt. Experimentelle Neurologie, Charité Universitätsmedizin Berlin und Arbeitsgruppe "Neuronale Stammzellen" des MDC für Molekulare Medizin. Berlin, Germany
- Kiefer, Friedemann, Dr.: *Endothelioma cells: A model for embryonic and proliferating endothelium;* Max-Planck-Institut für Zellbiologie. Münster, Germany
- Kloer, Daniel: *Retinal biosynthesis in eubacteria: the crystal structure of apocarotenoid-15,15'-oxygenase from Synechocystis;* University of Freiburg. Freiburg, Germany
- Klugewitz, Katja, Dr.: *LSEC (liver sinusoidal endothelial cells) promote transmigration of CD4+ T-cells by efficient surface presentation of chemokines;* Charité Berlin. Germany
- Klump, Hannes, Dr.: *Converting Embryonic Stem Cells into Blood - HOXB4 mediate Stem Cell Tuning;* Dept. of Hematology and Oncology, Hannover Medical School. Hannover, Germany
- Kracht, Michael, Prof. Dr.: *Effector mechanisms that translate inflammatory signalling into gene regulatory responses;* Hannover Medical School, Hannover, Germany
- Kretschmer, Karsten, Dr.: *Inducing and expanding CD25+Foxp3+ regulatory T cells by foreign antigen*; Boston, USA.
- Kriete, Andreas, Prof. Dr.: *Systems Biology and Animal Models;* Drexel University. Philadelphia, USA
- Lu, Mengji, PD, Dr.: *Kontrolle der Hepatitis B Virusinfektion durch Induktion spezifischer Immunantworten: Studien im Woodchuck-Modell;* Institut für Virologie, Universitätsklinikum, Essen. Germany
- Maltsev, Valeri: *Scanning Flow Cytometry: the next generation in flow cytometry instruments;* Forschungszentrum Vector in Novosibirsk. Novosibirsk, Russia
- Martynyuk, Raisa A., Dr. and Karpenko, Larisa I., Dr.: *Advances in HIV-1 vaccine development at VECTOR;* Russian State Research Center of Virology and Biotechnology (Vector). Koltsovo/Novosibirsk
- Meyer-Hermann, Michael, Dr.: *Predictions from theory about selection mechanisms in the adaptive immune response;* Frankfurt Institute for Advanced Studies (FIAS), Johann Wolfgang Goethe University. Frankfurt, Germany
- Mielenz, Dirk, Dr.: *Analysis of B cell lipid rafts by 2D gel electrophoresis and mass spectrometry;* Nikolaus-Fiebiger-Zentrum. Erlangen, Germany
- Multhaup, Gerd, Prof. Dr.: *Copper in Alzheimer's disease, what do we really know?;* Institut für Chemie – Biochemie, Freie Universität Berlin. Berlin, Germany

- Nagel, Stefan, Dr.: *The BCL11B regulatory oasis and leukemia: Ectopic activation of homeobox genes by a conserved non-coding region;* DSMZ, Department of Human and Animal Cell Cultures. Braunschweig, Germany
- Nitschke, Lars: *CD22: a negative regulator of B cell activation;* Institute of Genetics, University of Erlangen. Nürnberg, Germany
- Panet, Amos, Dr.: *Virus tropism in solid tissues and application to the delivery of therapeutic proteins;* The Hebrew University. Jerusalem, Israel
- Pawelec, Graham, Prof. Dr.: *Is immunosenescence infectious?;* University of Tübingen. Tübingen, Germany
- Riedl, Petra, Dr.: *Oligonucleotide-cationic peptide complexes: a novel adjuvant system for CD8+ T cell priming;* Universitäty of Ulm, Molekulare Infektionsimmunologie. Ulm, Germany
- Rudolph, Lenhard, PD Dr.: *Senescence and DNA damage responses during aging, regeneration and carcinogenesis;* Hannover Medical School, Abt. Gastroenterologie. Hannover, Germany
- Schubert, Mario, Dr.: *Studies of RNases and glycosidases using NMR spectroscopy and X-ray crystallography as complementary techniques;* ETH Zürich. Zürich, Switzerland
- Schubert, Ulrich, Prof. Dr.: *The role of the ubiquitin-proteasome-system in HIV replication: potential targets for anti-retroviral therapy;* Institute of Clinical and Molecular Virology, University of Erlangen. Germany
- Schwanbeck, Ralf, Dr.: *Investigation of Chromatin Proteins by Hydroxyl Radical Footprinting;* NCI, National Institutes of Health. Bethesda, Maryland/USA
- Steiniger, Birte, Prof. Dr.: *Microanatomy and function of the spleen;* Institute of Anatomy and Cell Biology, University of Marburg. Germany
- Sühnel, Jürgen: *Neue Einsichten in die Struktur von Proteinen durch Methoden der Strukturbioinformatik;* Biocomputing Group, Institut für Molekulare Biotechnologie, Zentrum für Bioinformatik. Jena, Germany
- Süssmuth, Roderich, Prof. Dr.: *Investigations on structure, biosynthesis and mode of action of antibiotic natural products;* Technical University Berlin, Institute of Chemistry. Berlin, Germany
- Tan, Tianwei, Prof. Dr.: *Production of chemicals from bioconversion;* Key Lab of Bioprocess of Beijing and College of Life Science and Technology, Beijing University of Chemical Technology. Beijing, China
- Verhoeyen, Els, Dr.: *Lentiviral vectors displaying "early acting cytokines" for selective gene transfer of hematopoietic stem cells: evaluation in a rhesus macaque pre-clinical AIDS model;* Ecole Normal Supérieure de Lyon (ENS). Lyon, France
- Vingron, Martin, Prof. Dr.: *Computational Methods in Gene Expression and Gene Regulation;* MPI for Molecular Genetics, Computational Molecular Biology. Berlin, Germany
- Walker, Mark, Prof. Dr.: *The biological consequences of plasminogen – streptococcal interaction;* University of Wollongong, Australia
- Wood, Keith, Dr.: *Engineering novel light-emitting probes for bio-chemical and cellular analysis;* Promega Corporation. Madison, USA
- Zawatzky, Rainer, Dr.: *The growing family of interferon-induced genes: isolation and initial characterization of new members;* DKFZ. Heidelberg, Germany
- Zhou, Weichang, Ph.D.: I*ntegrating Process Development Activities with Product Quality Assessment for Large-scale Manufacturing of Monoclonal Antibodies;* Process Sciences and Engineering, Protein Design Labs, Inc. Fremont, California/USA

Lectures 2006

- Becker, Regina, Dr.: *Neuigkeiten zum 7. Forschungsrahmenprogramm der EU;* Helmholtz-Büro. Brüssel, Belgium
- Beigier-Beompadre, Macarena, Dr.: *Toll like receptors in the human immune response against Mycobacterium tuberculosis;* Institut für Klinische Mikrobiologie, Immunologie und Hygiene Friedrich-Alexander Universitäty of Erlangen, Nürnberg, Germany
- Benuit, Martin, Dr.: *How strong is a single cell adhesion molecule and how sticky are cells? Forces measured by Atomic Force Microscopy;* Institut für Physik, Ludwig-Maximilians-Universität. München, Germany
- Blaut, Michael, Prof. Dr.: *Influence of intestinal bacteria on bio-availability of dietary lignans;* Deutsches Institut für Ernährungs-forschung, Abt. Gastrointestinale Mikrobiologie. Potsdam – Rehbrücke, Germany
- Bogdan, Christian, Prof. Dr.: *The innate immune response to/ Leishmania/parasites: The role of type I interferons and toll-like receptors;* Universitätsklinikum Freiburg. Freiburg, Germany
- Braus, Gerhard, Prof. Dr.: F*ungal Models for Infection and Disease;* Georg-August-University, Dept. of Molecular Microbiology and Genetics. Göttingen, Germany
- Bulyk, Martha L., Prof. Dr.: *Genomic analysis of transcription factors and cis regulatory elements;* Division of Genetics, Dept. of Medicine, Dept. of Pathology, Harvard-MIT Division of Health Sciences and Technology, Brigham & Women's Hospital and Harvard Medical School. Boston, USA
- Cleary, Patrick, Prof. Dr.: *Disruption of the tonsil reservoir: Will vaccines eliminate serious complications associated with group A streptococcal infections?;* University of Minnesota. USA
- Collins, Mary, Prof. Dr.: *Lentiviral vectors for gene therapy;* University College London. London, UK
- Dehio, Christoph, Prof. Dr.: *Role of type IV secretion in bacterial pathogenesis: the Bartonella paradigm;* Biocenter Basel. Switzerland
- Dersch, Petra, Prof. Dr.: *Enteropathogenic Yersinia: Internalization into humn cells and beyond;* Institute of Microbiology, Technical University of Braunschweig. Germany
- Dittmar, Ulf , Dr.: *Regulatory T cells in chronic retroviral infection;* Universitätskrankenhaus. Essen, Germany
- Fackler, Oliver, Dr.: *Modulation of actin dynamics by the HIV-1 pathogenicity factor Nef;* Hygiene Institut des Universitätsklinikum Heidelberg, Abteilung Virologie. Heidelberg, Germany
- Haas, Hubertus: *The role of siderophores in iron metabolism and virulence of Aspergillus;* Dept. of Molecular Microbiology at Innsbruck Medical School. Innsbruck, Austria
- Hamacher, Kay, Dr.: *Reduced Molecular Models - Increased System Knowledge;* Center for Theoretical Biological Physics, University of California at San Diego. La Jolla, USA
- Heil, Matthias, Dr.: *Cellular and Molecular Pathways of Arteriogenesis;* Max-Planck-Institut für physiologische und klinische Forschung. Bad Nauheim, Germany
- Hufton, Andrew, Dr.: *A genomic view of Xenopus organizer function;* Stanford University, Dept. of Genetics. Stanford, UK
- Jacobs, Thomas, Dr.: *New strategies to induce protective CD8+ T cells in a murine model of malaria;* Bernhard-Nocht-Institut für Tropenmedizin. Hamburg, Germany
- Jores, Jörg, Dr.: *MLST_Analysis of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) E. coli;* International Livestock Research Institute (ILRI). Nairobi, Kenia

- Keil, Günther, Dr.: *Furin processed viral glycoproteins as transporters or carriers for biologically active proteins: a new protein expression approach;* Friedrich Löffler Institut, Bundesforschungsinstitut für Tiergesundheit. Greifswald, Germany
- Klausdeinken, Franz-Josef, Dr.: *Influence of Phytoestrogens on Animal Models with focus on the Immune System;* Harlan Winkelmann. Germany
- Koch, Ina, Prof. Dr.: *Bioinformatics approaches for qualitative modelling in systems biology;* Bioinformatics TFH Berlin. Germany
- Koop, Ronald , Dr.: *Discovery in the living organism – bioluminescence and fluorescence in vivo imaging;* Xenogen Corporation. Hopkinton, USA
- Kuttler, Christina, Dr.: *Modelling of Cell-Cell Communication in Gram-genative Bacteria;* Institute of Biomathematics and Biometry, GSF . Neuherberg, Germany
- Levi, Ben-Zion, Prof. Dr.: *IRF-8, a master regulator of macrophage activity: Pathogen elimination and tumor suppression;* Technion-Israel Institute of Technology. Haifa, Israel
- Lohoff, Michael, Prof. Dr.: *The role of IRF's for T helper cell differentiation;* Philipps-Universität Marburg. Marrburg, Germany
- Luers, Thorsten, Dr.: *Towards a Molecular Understanding of Amyloidosis: 3D Structures of Prions Proteins and Alzheimer Fibrils;* The Salk Institute, Structural Biology Lab. La Jolla, USA
- Lynch, Nick, Dr.: *More harm than good? The lectin pathway of complement activation;* Dept. of Infection, Immunity and Inflammation of the University of Leicester. Leicester, UK
- Malek, Nisar, Dr.: *Control of cell cycle progression and tissue homeostasis by p27kip1 and cyclin E;* Dept. of Gastroenterology, Hepatology and Endocrinology, Hannover Medial School, Hannover, Germany
- Manz, Rudi, Dr.: *Plasma cell differentiation and homeostasis;* Deutsches Rheumaforschungszentrum. Berlin, Germany
- Maruyama, Mitsuo, Dr.: *Zizimin2, a novel Cd42 guanine nucleotide exchange factor (GEF) in Lymphoid lineage;* National Center for Geriatrics and Gerontology. Tsukuba, Japan
- Meinzinger, Stefan, Dr.: *Amaxa Nucleofection technology;* Amaxa GmbH. Braunschweig, Germany
- Messerle, Martin, Prof. Dr.: *Genetic strategies for the characterization of cytomegalovirus immuno-modulatory functions;* Hannover Medical School, Molecular Virology. Hannover, Germany
- Miyazaki, Tadaaki, Dr.: *Apoptosis signal in T and B cells;* National Center for Geriatrics and Gerontology. Tsukuba, Japan
- Montagutelli, Xavier, Dr.: *Interspecific recombinant congenic strains as a tool to analyze traits of complex genetic determinism;* Pasteur Institute. Paris, France
- Mulaa, Francis: *Plasmodium falciparum 4-methyl-5-beta-hydroxy-ethylthiazole kinase as a drug target;* Department of Biochemistry, University of Nairobi. Nairobi, Kenia
- Neuhaus, Klaus, Dr.: *EHEC and environmental organisms – monitoring of relevant genes by lux fusions;* TU München. München, Germany
- Nielsen, Lars K., Prof. Dr.: *True and pseudo-stochastic behaviour in haematopoiesis – when does noise reflect a significant biological feature as opposed to reflecting a poor model or poor measurements?;* Dept. of Chemical Engineering, University of Queensland. Queensland, Australia
- Niessing, Dierk, Dr.: *Yeast ASH1 mRNA transport as model system for eukaryotic mRNP translocation;* GSF, München. Germany
- Pietschmann, Thomas, Dr.: *The level of CD81 cell surface expression is a key determinant for productive entry of Hepatitis C Virus into host cells;* Ruprecht-Karls-Universität, Abt. Molekulare Virologie. Heidelberg, Germany
- Prangishvili, David, Prof. Dr.: *A mysterous world of archaeal viruses;* Institute Pasteur. Paris, France
- Rademann, Jörg, Prof. Dr.: *A synthetic rhamnolipid library for the stimulation of the innate immune system;* Research Institute for Molecular Pharmacology, FU Berlin. Berlin, Germany
- Ritter, Christiane, Dr.: *Determination of the structural basis for infectivity and regulation of the [Het-s] prion;* Structural Biology Laboratory, The Salk Institute. La Jolla, USA
- Rottenberg, Martin, Prof. Dr.: *Interferon-gamma and regulation of immune defense against infections;* Karolinska Institute. Stockholm, Sweden
- Rudolph, Cornelia, Dr.: *Die Bedeutung der chromosomalen Instabilität in hämatologischen Neoplasien – Ergebnisse aus murinen in vitro- und in vivo-Modellen;* Hannover Medical School. Hannover, Germany
- Schaper, Fred, Prof. Dr.: *Mechanisms for regulating IL-6-type cytokine signalling;* Dept. of Biochemistry, Medical School, RWTH Aachen. Aachen, Germany
- Scheu, Stefanie, Dr.: *Activation of the integrated stress response during T helper cell differentiation and the type I interferon response/in vivo/: an IFNb reporter mouse model;* Institute of Medical Microbiology, Heinrich-Heine-University Düsseldorf. Düsseldorf, Germany
- Schröder, Martina, Dr.: *Vaccinia virus antagonists of toll-like receptor signalling;* School of Biochemistry and Immunology, Trinity College Dublin. Dublin, Ireland
- Schüler, Herwig, Dr.: *Actin based motility of the Malaria parasite;* Max-Delbrück-Centre. Berlin, Germany
- Schweizer, Herbert, Prof. Dr.: *Efflux-mediated drug resistance in bacteria: implications for therapy and drug discovery;* Colorado State University, Dept. of Microbiology, Immunology and Pathology. Fort Collins, USA
- Schwienhorst, Andreas, PD Dr.: *Selective targeting of proteins - from biochemical tools to drug candidates;* Institute of Microbiology and Genetics, University of Göttingen. Göttingen, Germany
- Sleeman, Jonathan, PD Dr.: *The relationship between tumors and the lymphatic system: consequences for metastasis;* Forschungszentrum Karlsruhe, Institut für Toxikologie und Genetik. Karlsruhe, Germany
- Sorg, Isabel, Dr.: *Needle length control in the Yersinia type III secretion system;* University of Basel. Basel, Switzerland
- Stülke, Jörg, Prof. Dr.: *Carbon metabolism in* Mycoplasma pneumoniae – *regulation and relation to pathogenicity;* Institute of Microbiology and Genetics, Universitäty of Göttingen. Göttingen, Germany
- Takeuchi, Yasuhiro, Dr.: *Porcine endogenous retroviruses as potential risk in xenotransplantation;* University College London. London, UK
- Tylzanowski, Przemko, Dr.: *When too little is too much – BMP signaling in developing limb;* Universitäty of Leiden. The Netherlands
- Uetz, Peter, Dr.: *Protein interaction maps in viruses and bacteria;* Institute of Genetics, FZ Karlsruhe. Karlsruhe, Germany
- Van Ham, Marco, Dr.: *Microtubule plus-end-tracking proteins and their role in cell migration;* Dept. of Cell Biology and Genetics at the Erasmus Medical Centre. Rotterdam, The Netherlands
- Wang., Beinan, Dr.: *TGFβ1 upregulates group A streptococcal invasion of mammalian cells;* University of Minnesota. USA

- Weber, Friedemann, PD Dr.: *Inhibition of interferon induction by SARS - Coronavirus and bunyaviruses;* Dept. of Virology, University of Freiburg. Freiburg, Germany
- Wenzel, Wolfgang, Dr.: *Biomolecular structure prediction with stochastic optimization methods;* Forschungszentrum Karlsruhe, Inst. für Nanotechnologie. Karlsruhe, Germany
- Wolff, Thorsten, PD Dr.: *Influenza virus: Virulence and host range factors of a devious pathogen;* Robert Koch Institute. Berlin, Germany

Lectures 2007

- Brown, Eric, Prof. Dr.: *Screening in academe: it's a small molecule world;* McMaster University. Ontario, Canada.
- Feldmeier, Hermann, Prof. Dr.: *Pyoderma, group A streptococci and parasitic skin diseases – a dangerous relationship;* Institute of Microbiology and Hygiene, Charité-University Medicine. Berlin, Germany.
- Frank, Johannes. *Mapping temporally varying quantitative trait loci in time – to failure experiments;* Penn State University, USA
- Graef, Ralph, Prof. Dr.: *Functional Analysis of Dynein-Associated Centrosomal Proteins in Dictyostelium;* University of Potsdam. Germany.
- Hildebrandt, Jan-Peter, Prof. Dr.: *Signaltransduktion in Epithelzellen – Steuerung von Sekretion und Zellproliferation;* Zoologisches Institut der Universität Greifswald. Greifswald, Germany.
- Penninger, Josef, Prof. Dr.: *Learning from SARS infections – ACE2 family proteins;* Institute of Molecular Biotechnology. Wien, Austria.
- Pruzzo, Carla , Prof. Dr.: *Vibrio cholerae survival in the environment and link with human infection;* University of Genova. Genua, Italy.
- Ruland, Jürgen, Dr.: *Signal Specific Activation of NF-kappaB in Immunity and Lymphomagenesis;* Technical University of München. München, Germany.
- Thery, Manuel: *Microfabrication techniques and theoretical physics bring insight into the relationship between cell adhesion to the internal organisation of actin and microtubule networks;* Laboratoire Bipuces at CEA Grenoble. Grenoble, France

Impressions of the HZI Campus

Photo: Radde

Photo: HZI, Bierstedt

Photo: HZI, Bierstedt

Photo: HZI, Jonas

Photo: Radde

Photo: HZI, Jonas

Photos: left: Participants of the 2005 InWEnt-GBF-Biotech Course and other guests during the official launching of the virtual ASEAN-South American-German Biotechnetwork at the BIOTECHNICA Fair 2005 in Hannover | centre: The flags of the HZI at the new entrance of the Centre | right: Aerial photo of the HZI Campus (2005) | Photos: HZI, Jonas (le) | HZI, Bierstedt (ce) | HZI, Hans (ri)

FACTS AND FIGURES

Facts and Figures

Prof. Dr. Rainer Jonas | Department of Scientific Information | rjo@helmholtz-hzi.de

In 1965 the GBF 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 Lower Saxony (Niedersachsen) took over the Centre, now called “German Research Centre for Biotechnology” (GBF). Since then the BMFT/BMBF as well as the State of Lower Saxony jointly finance the GBF. In 2002, the GBF took the decision to focus its research activities towards the understanding of basic mechanisms of infectious diseases. 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 2006 the total costs of the HZI amounted to 50.3 Mio. € with more than three quarters, 39.0 Mio. €, devoted to the programme “Infection and Immunity”.

External Funding More than 70% of the external funding came from national research programmes. About 12% and 14% were from EU programmes and industry, respectively.

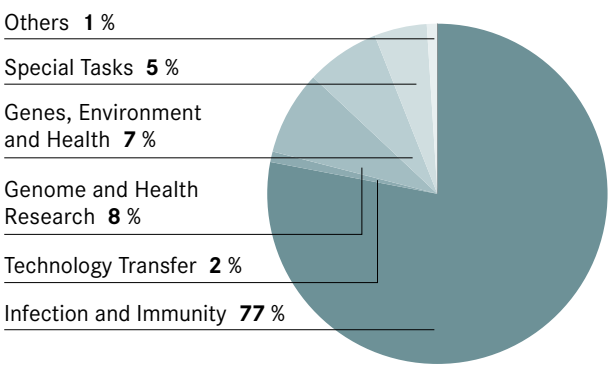
Costs per programme (in T€)

Research Area	Programme	Full Cost
Health	Infection and Immunity	39 024
	Genome and Health Research	4 025
Environment and Health	Genes, Environment and Health	3 326
Technology Transfer		998
Special Tasks		2 450
Others		481
Total Sum		**50 304**

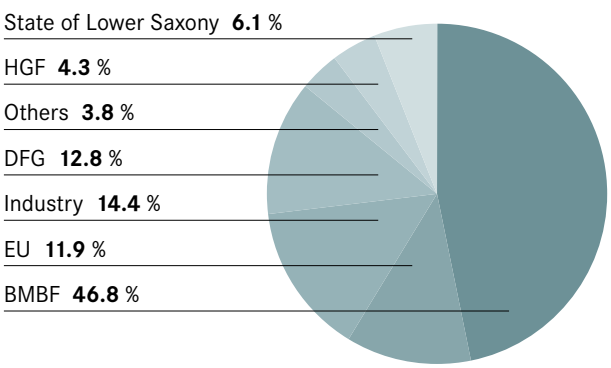
External financing (in T€)

Source	Full Cost
BMBF	6 579.56
DFG	1 797.97
EU	1 668.29
Industry	2 017.83
HGF	607.44
State of Lower Saxony	856.46
Others	530.18
Total Sum	**14 057.75**

Full Costs 2006



External funding 2006 – by source



Property Rights / Licences In 2006, six patents were applied for, all outside of Germany. Five of these patents were originated in the research area "Health", the other in the research area "Environment".

Patents and property rights, licences, year 2006

	Total number	Germany	
Priority based applications (2006)	6	0	6
Priority based applications, total number	80	46	34
Granted patents (2006)	8	2	6
Total number of held property rights	173	16	157
Licence agreement, total number	161	7	154
Licence proceeds* (in T€)	756	275	481

** 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 (for further details see under "Publications" in the section *Scientific Reports*).

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

Participation of HZI scientists in national and international research programmes (main activities)

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
SSP 1069	Mouse model of the Preeclampsia
SSP 1087	Selenoproteins
SSP 1089	New Vaccine Strategies
SSP 1150	Signal Pathways to the Cytoskeleton and Bacterial Pathogenecity
SSP 1160	Colonisation and Infection through Human-Pathogen Fungi
FOR 119	Hepatocellular Carcinoma
FOR 471	Cell Differentiation
FOR 629	Antibodies and Proteinanalysis

NGFN II (National Genome Research Network)	
Network	Infection and Inflammation
Network	Ecological Genomics
SMP	Mammalian Models
SMP	Protein
SMP	DNA
SMP	Cell
EP	Antibody Factory

Genomic (National Microorganism Network)	
Sorangium cellulosum	*Alcanivorax borkumensis*
Streptococcus pyogenes	*Listeria monocytogenes*
Bordatella	Metagenome of Biofilms

EU Frame Programmes	
Food	Healthy Water
GCE	BIOTOOL
GCE	Marine Genomics
INCO	ASSIST
LSH	Genostem
LSH	AVIP
LSH	EPI-VECTOR
LSH	FPLFLEX
LSH	EUROPATHOGENOMICS
LSH	MUGEN
LSH	New TB Drugs
LSH	CASIMIR
LSH	Fastest TB
LSH	EUMODIC
LSH	Clinigene
NEST	PROBACTYS
NEST	EMERGENCE
NEST	MAMOCELL
NMP	BIOMERCURY
Marie Curie HR	BIOSAP
Marie Curie EST	MIDITRAIN
Marie Curie RTN	IMDEMI
QLK	STEMGENOS
QLK	EUMORPHIA
QLK	TSE-SOIL-FATE
RICA	ProteomeBinders
SP	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 GRK 705	"Characterization of Patho-Physiological Animal Models"
DFG-Graduate School GRK1273	"Chronical Infections"

Quantitative Parameters	Category	2004	2005	2006
Publications	ISI-listed Papers Published by the Centre	184	225	247
	Peer-reviewed non-ISI publications in journals	3	1	7
	Books/other non-ISI-listed publications	21/14	30/5	18/3
	Total number	**222**	**262**	**275**
	Habilitations	1	0	0
	Dissertations	33	21	15
Full Professorship Offerings (W2/W3)	Calls for professorship	0	3	4
Special DFG-Programmes	DFG-SFBs, Transregios	4	6	6
	DFG-Research Focus	4	5	5
	Graduiertenkollegs	3	3	3
	Total number	**11**	**14**	**14**
Guest Scientists		93	109	132

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 the BioRegioN and the "Transferkolleg Biotechnologie e.V.". Furthermore, the HZI is an active partner in BioRegioN GmbH as well as in *"BioProfil Functional Genome Analysis"*.

The HZI-Biotech Campus In 2006 the HZI started with the construction of the new mouse house. This will be ready in 2008.

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 For the 4th time OMNILAB organized, with the support from HZI, DSMZ, and BioRegioN, a small biotech-fair and symposium in the FORUM, which took place on 14 September 2006. About 60 enterprises presented their products and about 500 visitors from the region Braunschweig – Hannover – Magdeburg attended the fair. Eleven lecturers gave highly interesting seminars on new R&D items.

During the exhibition of the Biotech Fair organized by Omnilab at the HZI FORUM in September 2006 Photo: OMNILAB

Biotech Network Southeast Asian Region – Germany The GBF together with InWEnt, Paul Charlton Coaching, and the Ministry of Economics, Labour and Traffic of Niedersachsen inaugurated during the Biotechnica Fair 2005 the ASEAN-South American-German Biotechnetwork (asag-biotechnetwork), a virtual platform for networking in biotechnology. In September 2006 HZI together with InWEnt and other partners organized three workshops on "Technology Transfer" in Bangkok, Hanoi, and São Paulo. Except the workshop in São Paulo, they were supported by the Ministry of Economics, Labour and Traffic of Niedersachsen.

Former participants of the InWEnt/HZI-courses and Ms. Jeannie Scriven, HZI, during the workshop on Technology Transfer in Bangkok, October 2006. Photo: HZI, Jonas

List of the firms and special laboratories on the HZI campus, Status: 31.03.2007

Company	Contact person	Phone/Fax	E-Mail	Homepage
Ascenion	Dr. Sabina Heim Tina Damm	0531-6181-2090/-2091 Fax -2098	heim@ascenion.de damm@ascenion.de	www.ascenion.de
AIMS Scientific Products GmbH	Dr. Norbert Zander Fr. Dobberphul	0531-8760-4940 Fax -4949	cdo@aims-sci.de	www.aims-sci.com
Airport-Hopper	Martin Rolfes	0531-612121; Fax -610846		
AMODIA Bioservice GmbH	Drs. Sabine Peters U. Krause | F. Schwieger	0531-260-1764; Fax -1766	info@amodia.de	www.amodia.com
Cosmix molecular biologicals GmbH	Dr. Thomas Wagner Ute Heidrich (Secr.)	0531-12086-0; Fax -99	info@cosmix.de	www.cosmix.de
Forschungsgruppe Wundheilung der TU Braunschweig	Prof. Dr. Peter Mühlradt	0531-121795-4; Fax -8		www.malp-research.de
Glyco Thera GbR	Dr. Harald Conradt Dr. Eckart Grabenhorst	0531-12058-0; Fax -21	conradt.harald@glycothera.de grabenhorst@glycothera.de	www.glycothera.de
Hartmann Analytic GmbH	Dr. Ursula Hartmann	0531-26028-0; Fax -28	hartmann@hartmann-analytic.de	www.hartmann-analytic.de
IBA Biologics HmbH	Dr. J. Bertram Dr. Bernd Müller	0551-50672-118 0163-5067218	bertram@iba-go.com	
Lionex GmbH	Dr. Ralf Spallek Dr. Eva Gebhardt-Singh	0531-260-1266; Fax -1159	msi@lionex.de	www.lionex.de
RELIATech GmbH	Dr. Bernhard Barleon	0531-260-1831; Fax -1833	info@reliatech.de	www.reliatech.de
Schwarz, Peggy Ingenieursbüro	Peggy Schwarz Bärbel Fritz	0531-866-7003; Fax -8627	uwepschwarz@aol.com	www.reliatech.de
BIOS – Biotechnologisches Schülerlabor	Dr. Iris Eisenbeiser Arntraut Meyer	0531-6181-1900	bios.lab@helmholtz-hzi.de	www.reliatech.de

Crèche and Kindergarten for Children of HZI Employees The Helmholtz Centre for Infection Research (HZI) in co-operation with the Sterntaler Kindergarten in Braunschweig-Stöckheim offers childcare for those aged one year and older. Normally this is between the hours of 8 am to 5pm. Special arrangements can be made outside these hours. Since 2005, each year about three children of HZI employees were admitted to the kindergarten.

Special Programme for Female Scientists and Technicians after a Family Time Out In 2005 this programme was introduced and partially financed by HGF to help female scientists to return back to the laboratory after taking a break for family reasons. It will now continue at the HZI through institutional funding. Up to 2007 about five scientists have been employed within this initiative.

Since 2005 also two female technicians have been employed through an internally funded special programme.

HGF-Mentoring Programme 2007/8 On 22 May 2007 the 3rd HGF-mentoring programme 2007/8 will start. This serves for female scientists to get a better basis for promoting their career and to have better chances to get leading positions. Twenty-three women will take part in the programme, three of them from the HZI.

Personnel At the end of 2006 the HZI staff comprised 617 persons with full time and part time occupation. Additionally, 114 guests worked in various projects, receiving their payment from third parties. In total, 241 scientists were working at the HZI, including 76 postdocs, 72 PhD-students and 27 engineers.

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 Lower Saxony (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 33 elected scientists. 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. Ute Pägelow.

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, 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: 31.03.2007

Function	Name, Title	Organisation	Locality
Chairman SB	Lange, MinDirig 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	Bilitewski, Prof. Dr. Ursula	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 April 2007

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

Supervisory Board (AR)
MinDir Dr. P. Lange, BMBF, Chairman
MinDirig H. Gevers, Ministry for Science and Culture of Lower Saxony, 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) M. Sylla

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

Safety Officer (FaSi)
G.-W. Piehl

Equal Opportunity Officer (GB)
E. Rohn-Stenzel

Scientific Groups

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

- Dept. Experimental Immunology (EI) Prof. Dr. W. Müller
- Dept. Chemical Biology (CBIO) Dr. R. Frank
- Dept. Cell Biology (ZB) Prof. Dr. J. Wehland
- RG Mucosal Immunity (MI) Prof. Dr. J. Buer
- RG Cytoskeleton Dynamics (CYD) Dr. K. Rottner
- RG Mikrobial Communication (KOM) PD 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 Immunodynamics (ID) Dr. M. Gunzer
- RG Conformational Protein-Ligand Interactions (KPLI) PD Dr. J. Eichler
- JRG Chronic Pseudomonas Diseases (CPI) PD Dr. S. Häußler

Division of Microbiology (MIK)
Prof. Dr. G.S. Chhatwal*

- Dept. Microbial Pathogenicity a. Vaccine Research (MPAT) Prof. Dr. G. S. Chhatwal
  - RG Infection Immunology (INI) Dr. E. Medina
  - RG Biodegradation (DEG) PD Dr. D. Pieper
- Dept. Vaccinology (VAC) Prof. Dr. C.A. Guzmán
  - RG Chemical Microbiology (CMIK) Dr. W.-R. Abraham
- RG Environmental Microbiology (UMW) Prof. Dr. K. N. Timmis
- RG Directed Evolution (DirEv) Prof. Dr. J. Collins

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

- Dept. Genome Analysis (GNA) Dr. H. Blöcker
  - RG Systems Biology (SBIO) Prof. Dr. A.-P. Zeng
- Dept. Gene Regulation and Differentiation (RDIF) Dr. H. Hauser
  - RG Epigenetic Regulation Mechanisms (EPI) Prof. Dr. J. Bode
  - RG Molecular Immunology (MOLI) Dr. S. Weiß
  - RG Predictive Models for Infection a. Immunity (MSYS) Dr. D. Wirth

Division of Biochemical Engineering (BVT)
Dr. H. Ziehr*

- RG Quality and Project Management (QPM) Dr. H. Ziehr
- RG Upstream Processing (KVT) Dr. A. Roß
- RG Downstream Processing (ABT) K.-H. Kroner
- RG Quality Control(QC) Dr. J. Berlin

Division of Structural Biology (SB)
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

- Dept. of Medicinal Chemistry (MCH) Prof. Dr. M. Kalesse
  - JRG Structure and Function of Antibiotics (SFA) Dr. D. Menche
  - RG Microbial Drugs (MWIS) Prof. Dr. R. Müller
- Dept. of Experimental Mouse Genetics (EMG) Prof. Dr. K. Schughart (Prokurist)
  - RG Central Animal Facility (TEE) Dr. D. Monner
  - JRG Infection Genetics (ING) Dr. A. Lengeling

Abbreviations:
Dept Department
RG Research Group
JRG Junior Research Group
PWC Scientific Controlling
BCO Financial Controlling
DMC External Funding Controlling

* *temporarily acting*

Infrastructure

Scientific and Technical Services (WTD)
N.N.

- 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) U. Richter (Prokurist)
- Financial Affairs (FA) D.-M. Reinhardt*
- Purchasing and Material Management (EM) H. Ohrdorf
- Legal Affairs and Licences (JUR) Dr. C. Kügler-Walkemeyer
- Patents (PS) D. Meseke
- Technical Services (TB) O. Rabe

Staff Units

- Public Relations (ÖA) H. Schlender
- Controlling (CO) Prof. Dr. K. Schughart* (PWC) (Prokurist) S. Hempe* (BCO) Dr. M. Strätz (DMC)
- Organisation/SAP (ORG) R. Lomberg*
- Internal Auditing (IR) R. Lomberg (data security officer)

Research Report 2006/2007

Published by
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Member of Helmholtz Association of
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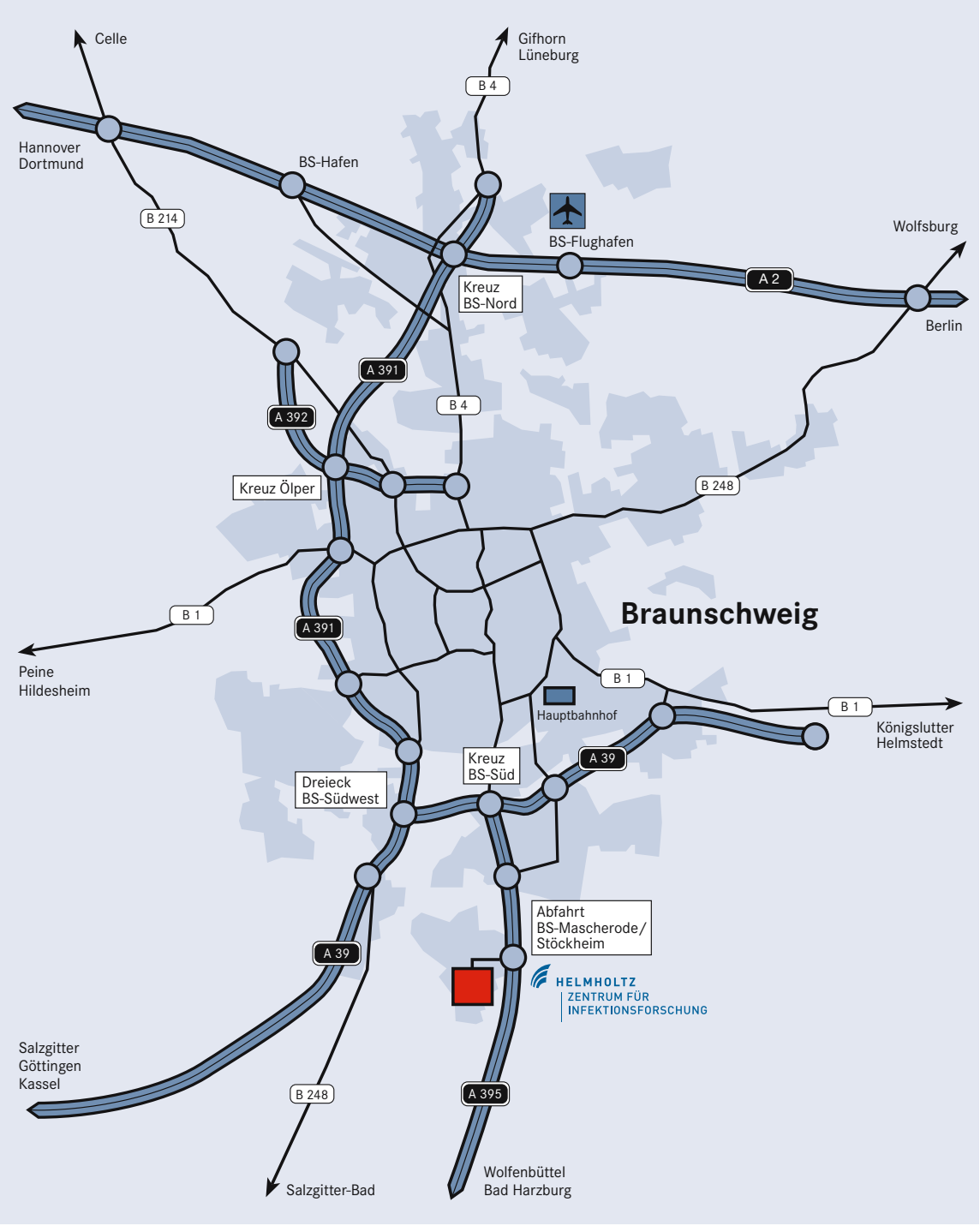
Photographs
The portraits were taken by
Thomas Ammerpohl – pages: 70,78, 84
Frank Bierstedt – pages: 3 (all), 21, 42 (Kalesse, Menche, Bilitewski), 46, 62, 66, 67, 75, 79, 82, 83, 87, 89, 92, 93, 95, 96, 97, 98, 99, 100, 101, 103, 104, 106, 108, 109, 111, 144
Annick Eimer – pages: 107
Heinz Gramann – pages: 5, 64, 86, 81
Dirk Hans – pages: 69, 74, 80
Susanne Hübner – pages: 86, 112
HZI archive – pages: 8, 16, 32, 42 (Frank, Gerth), 54, 59, 72, 73, 76, 77, 85, 88, 91, 94, 110
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:
Döring Druck, Druckerei und Verlag GmbH, Braunschweig
Printed on acid-free and chlorine-free paper
ISSN 0935-0497



Site plan of the Helmholtz Centre for Infection Research

ISSN 0935-0497

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