Pushing the barriers of knowledge

Members of the Breaking Barriers research team, Germany, Principal Investigators Professor Cornelius Faber, Dr Bettina Löffler, and Professor Georg Peters describe their research, the importance of understanding pathogen-host interactions on a cellular scale and their ambition to use this knowledge to create new clinical interventions

To begin, can you summarise the different areas of research that are investigated within the Collaborative Research Centre (SFB) 1009 Breaking Barriers. What is the main goal of this consortium?

GP: Infectious diseases and autoimmune or rheumatic disorders represent enormous medical and economic burdens to society. Common features of such inflammatory processes include their complexity and the frequent involvement of multiple organ systems. New strategies for therapy are urgently needed. Antibiotic therapy for infection is becoming increasingly restricted, mainly due to the development of pathogen resistance. Therapy against autoimmune and rheumatic diseases is still limited to mainly arbitrary suppression of inflammatory cytokines. Central to both infectious and inflammatory diseases are cellular barriers which, in physiological conditions, act to restrict pathogen entry and regulate immune cell surveillance of tissues. The main goal of our SFB 1009 is the targeting of components of cellular barriers for future diagnostic, therapeutic or preventive strategies.

What are the main mechanisms governing the passage of foreign bodies whilst facilitating the transport of necessary signalling molecules and other host substances into and out of cells?

GP: Cellular and associated extracellular matrix barriers fulfil important functions not only by establishing a physical barrier between the exterior and interior but also by coordinating physiological processes at their surfaces and regulating the transport of nutrients, metabolites and even cells between two principally different compartments (outside-inside, blood or lymphatic vessel and surrounding tissue). The entire process of barrier penetration is orchestrated by multiple interactions between the immigrating and barrier forming cells and is crucially modulated by soluble factors that activate or inactivate specific responses of the two cell populations.

Individual research projects are organised into 'the integrity and cellular penetration of barriers' and 'cellular barriers as targets for infection and immune processes'. What is the reason for this classification?

GP: Projects in part A of our SFB 1009 aim to decipher the basic biological principles that underlie the establishment, dynamics and functional complexity of endothelial barriers and the regulation of leukocyte activation in the course of transendothelial cell migration. In part B, the scientific programme projects address questions of pathogen interactions with cellular barriers and their consequences for the development of inflammatory reactions. Altogether, the research programmes of all projects synergistically work on new interventional strategies against inflammatory diseases with substantial innovation and added value potential.

One special feature of your centre is the use of non-invasive imaging technologies, in particular MRI. Can you provide some examples of the research options made possible with this technique?

CF & BL: Using the techniques of bacterial iron-labelling and MRI we developed a model of endocarditis in mice. Using these methods we could monitor the development of endocarditis non-invasively. An endocarditis model in mice is very difficult to perform, and obtaining high resolution MRI of the extremely fast moving structures in the beating heart is a challenge. Furthermore, MRI provides a tool to assess other barriers properties in the organism. By parametric imaging one can assess integrity of the blood-brain barrier, micro vessel density or mean vessel size in the brain, or angiogenesis in solid tumours.

The bite-sized approach to cellular barriers

The Collaborative Research Centre 1009 in Münster, Germany, is attempting to elucidate the processes and mechanisms involved in infectious disease and autoimmune disorders. Led by Professor Georg Peters, it is developing new techniques to gain insight into pathogenic interaction with cellular barriers and host inflammation response

Have you faced any particular challenges in seeking an understanding of the molecular and cellular mechanisms of cell barrier regulation, in particular during bacterial infections? What strategies have you used to overcome these?

BL: Some toxins of *Staphylococcus aureus* are very cell- and species-specific, such as Panton-Valentine Leukocidin (PVL), which only acts on neutrophils, monocytes and macrophages isolated from humans or from rabbits. As PVL has no effect on murine cells, mice models cannot be used to study the role of PVL in disease development. To overcome this limitation we developed a murine model combined with human cells of necrotising pneumonia. In this model we could demonstrate that PVL is an effective toxin to lyse human neutrophils, which causes massive tissue damage in the lung.

Are you collaborating with other projects/ laboratories in the course of your investigations?

CF & BL: Besides all the member laboratories within the Collaborative Research Centre, we are collaborating with the other local Collaborative Research Centres in Münster and with many national and international institutes working on staphylococcal pathogenesis and on immune reactions of the host. A main focus of our research is the host-pathogen interaction at different stages of the infection we always require combined expertise regarding the virulence strategies of the pathogens and the defence mechanisms of the host. In order to improve imaging technology we are in constant exchange with other leading groups in Europe via national, European and international societies.

INFECTIOUS AND AUTOIMMUNE disorders are two of the biggest burdens on quality of life. These disorders – whether mediated by a foreign pathogen such as bacteria, or caused by a damaging autoimmune response – have several similarities. In the case of infectious disease, the pathogen in question invades and infects the body by passing through physical barriers such as epithelial and endothelial cell layers. The action of each pathogen and its host interactions will depend on the specific biochemical and physiological adaptations of that pathogen, but almost all foreign bodies share the need to cross the same cellular barriers. Once inside the host, most detected pathogens will trigger an immune response which includes inflammation.

The inflammation process is similar in both pathogenic infections and autoimmune disorders. The cellular barriers which attempt to block invading pathogens are also involved in regulating inflammatory processes in both disease types, making cellular barriers a common component in a huge swathe of the global disease burden.

Today, the importance of these cellular barriers in biomedical research and clinical intervention is clear. As such, a large multidisciplinary team from Medical and Biological Faculties of the University of Münster, Germany, is working to elucidate the components and mechanisms of cellular barriers, with the aim of identifying and targeting specific regions of those barriers for diagnostic, therapeutic and preventive strategies.

BARRIERS

Cellular barriers are integral to almost all processes in complex organisms. "In humans, polarised endothelial and epithelial cell layers and their underlying basement membranes are fundamental to the maintenance of normal organ function and tissue homeostasis," explains the Speaker of the Collaborative Research Centre (SFB) 1009, Professor Georg Peters. While cellular barriers are clearly an essential defence against pathogens, some viruses and bacteria have evolved ways of exploiting the structure and behaviour of barriers to gain access to cells and surrounding tissue. In this context, it is clear that understanding the mechanisms of barrier processes is essential.

Cellular barriers themselves are one part of a wider homeostatic mechanism. The behaviour of cellular barriers and their interaction with the extracellular matrix and surrounding cells is controlled at least in part by important signalling molecules: "Such factors include cytokines and chemokines but also other molecular complexes associated with tissue damage or pathogen recognition systems," highlights Peters. In combination, cellular barrier behaviours and the molecular regulation of the setting creates a strictly controlled system of immigration and emigration through epithelial and endothelial membranes. Critically, if this complex balance is disrupted, the door is left ajar for pathogenic invasion.

THE CASE OF STAPHYLOCOCCUS AUREUS

Within its overarching aims, the SFB 1009 is running multiple sub-projects. One of these is looking at the behaviour of a common family of bacteria in infected host models.

Staphylococcus aureus is one of the most clinically relevant families of bacteria known. It is a common passenger in our bodies, often asymptomatically in areas such as the nasal cavity. *Staph. aureus* can present more serious complications if it enters the body, causing infection, tissue damage and even death. As is the case with all bacteria, *Staph. aureus* infection if identified can be treated with antibiotics. However, some *Staph. aureus* strains are well-known examples of antibiotic resistant bacteria (for example methicillin-resistant *Staph. Aureus* - MRSA). As such they often present a serious issue in clinical settings.

The German team is attempting to elucidate the mechanisms which these bacteria employ to overcome host defences and invade tissue. If achieved, they hope to use this knowledge to design therapeutic intervention which overcomes the growing resistance of *Staph. aureus* to antibiotics. The bacteria are impressively diverse in their strategy to gain access to host tissue: "*Staph. aureus* disposes of

BREAKING BARRIERS

COLLABORATIVE RESEARCH CENTRE 1009 OF THE GERMAN RESEARCH FOUNDATION

OBJECTIVES

The Collaborative Research Centre 1009 consists of 17 collaboratively working projects with 48 scientists from 16 participating institutions. They address fundamental questions concerning cellular barrier functions and basic mechanisms of barrier penetration by immune cells or pathogens, as well as the interplay between them.

KEY COLLABORATORS

Professor Dr Dietmar Vestweber • Professor Dr Lydia Sorokin • Professor Dr Heinz Wiendl • Professor Dr Christian Klämbt • Professor Dr Alexander Zarbock • Professor Dr Volker Gerke • PD Dr Ursula Rescher • Professor Dr Carsten Müller-Tidow • Professor Dr Thomas Pap • Professor Dr Georg Peters • PD Dr Bettina Löffler • PD Dr Christina Ehrhardt • Professor Dr Stephan Ludwig • Professor Dr M Alexander Schmidt • Professor Dr Helge Karch • PD Dr Alexander Mellmann • Professor Dr Ulrich Dobrindt • Professor Dr Karin Loser • PD Dr Thomas Vogl • Professor Dr Dirk Föll • Professor Dr Johannes Roth • Dr Sabine Blass-Kampmann • Professor Dr Cornelius Faber

FUNDING

German Research Foundation

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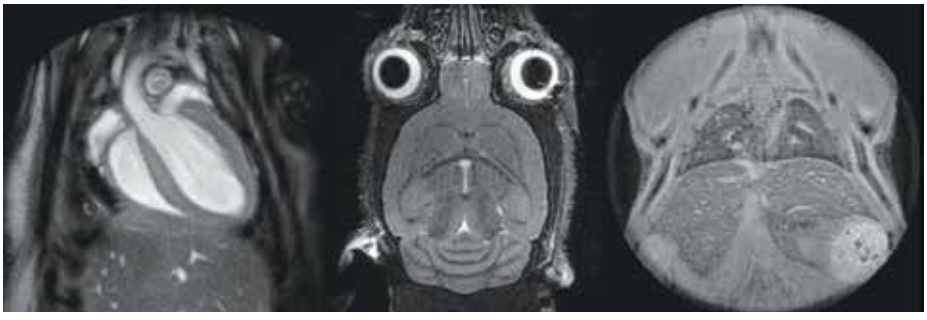
GEORG PETERS graduated in Medicine 1976 at the University of Cologne. In 1992 he became Full Professor and Director of the Institute of Medical Microbiology at the University of Muenster.

CORNELIUS FABER (physicist, PhD) developed the 17.6-tesla MRI laboratory at Würzburg University. Since 2008 he has been Professor of Experimental Magnetic Resonance at the University of Münster and focuses on development of MRI methods for neuroimaging and imaging of infections in animal models.

BETTINA LÖFFLER studied medicine at the University of Munich, LMU, Germany and graduated in 2000. Since 2002 she has been working in the Department of Microbiology, Münster and established a research group focusing on staphylococcal pathogenesis and host-pathogen interactions.

High resolution MRI provides non-invasive insight into the intact mouse model. *In vivo* images reveal anatomy and function of the mouse heart (left), brain (middle), or thorax (right). Based on such images tagged bacteria can be localised and followed in the body.



a multitude of virulence factors, such as adhesins, toxins and enzymes to invade host tissue and to escape from the host immune system," highlights project researcher Dr Bettina Löffler. The group has made notable progress in their attempts to combat this range of bacterial strategy: "Various anti-infective strategies are under development, such as vaccines against toxins or neutralisation of the toxin by the addition of polyvalent immunoglobulins containing antibodies against toxins," expands Löffler.

IMPROVING MR

Perhaps the most applicable and ergonomic breakthrough recently made by the SFB 1009 is the creation of a technique which allows the *in vivo* visualisation of real-time bacterial infection. The process, so far limited to murine models, involves tagging bacteria with iron oxide particles. These bacteria are then introduced to the host model. The team is then able to use magnetic resonance imaging (MRI) to identify and track the process of infection in the models without any form of surgical intervention. This represents an impressive step forward with regards to the tools and methodologies available to biomedical research teams across the world. The team has been able to use this technology to build an understanding of infection behaviour, inflammatory and immune responses and the locality of these processes. Due to the non-invasive nature of this technique the group hope that the technology may soon be transferred and further developed for the application in humans: "The methodology we have developed here can readily be applied to humans, as soon as suitable targeted MR contrast agents for bacteria are available," explains project researcher Professor Cornelius Faber.

COLLABORATION

Due to the size of the overarching research project and the complexity of the work in question, collaboration has been essential to the progress achieved by the group. Furthermore, it has been important in securing funding: "The collaborative nature of the research programme is a prerequisite for the funding of a Collaborative Research Centre by the German Research Foundation," points out Speaker Georg Peters. By engaging researchers of various expertises and bringing them together with one overarching goal, the group has been able to make notable progress, including the development of their new MRI technique – progress that has been dependent on interdisciplinary working.

In combination, the projects being conducted at this and other Collaborative Research Centres in Münster, Germany, represent one of the most holistic and far-reaching approaches to biomedical research in Europe. By tackling a huge scientific question with a multitude of collaborative projects, the SFB 1009 and its researchers are making notable inroads into the elucidation of pathogenic and autoimmune disorders. The future implications of such a large effort are hard to predict, but the provision of a novel MRI technique which might open new doors for scientists across the world, hints at the potential this project holds. In the future, the project leaders and Peters hope that new discoveries and breakthroughs can be translated into real life clinical intervention and that may have beneficial ramifications across a whole range of infectious and autoimmune diseases.

PIs inspect the preparation of an imaging experiment with the 9.4-tesla small animal MRI scanner.