Working group Prof. Dr. med. Barbara C. Kahl

Cystic fibrosis is one of the most prevalent hereditary diseases in the Caucasian population. Cystic fibrosis (CF) patients suffer from recurrent and chronic bacterial airway infections, which eventually lead to lung insufficiency and preterm death. *Staphylococcus aureus* is one of the first pathogens, which can be isolated from the airways of CF patients and in most patients, the same clone persists for many years. The main focus of our research group is the persistent *S. aureus* colonization/infection in the airways of CF patients. We are studying the mechanisms of adaptation and microevolution during long-term persistence of *S. aureus* in the airways of CF patients. Therefore, we characterize virulence regulators and genes in clinical *S. aureus* isolates including small colony variants (SCVs). Furthermore, we investigate the epidemiology and colonization dynamics of *S. aureus* in CF patients, the adaptive processes of *S. aureus* during persistence, which include characterization of virulence patterns in *in vitro* cell culture models.

Staff

Group leader	Prof. Dr. med. Barbara C. Kahl
Postdocs	Dr. rer. nat. Cathrin Baum
	Dr. rer. nat. Nina Hirschhausen
	Dr. rer. nat. Claudia Neumann
PhD student	Desirée Block (B.Sc., M.Sc.)
Cand. B.Sc.	Barbara Müther
Cand. med.	Johannes Birtel
	Charlotte Hasenkamp
	Katharina Higelin
	Theda Jansen
	Daniel Köhler
	Johanna Thoring
	Susanne Weber
	Nadya Braun
	Katharina Dueck
	Kristina Dyck
	Kathrin Heidenreich
Technicians	Susanne Deiwick
	Damayanti Kaiser
	Katrin Wardecki
Students	Lena Raidt (SHK)
	Felix Stader (SHK)

Projects

1. Infection genomics: Host-pathogen interactions: Effects of secreted proteins of *Staphylococcus aureus* on cells and components of the immune system.
(B. Kahl, M. Hussain, G. Peters, funded by BMBF)

Duration: 0 1.09.2010 - 30.08.2013

Staphylococcus aureus pathogenicity is determined by the expression of a large set of virulence factors. Extracellular proteins constitute a reservoir of virulence factors. The function of at least 70% of the extracellular proteins encoded by *S. aureus* is not yet clear and a possible role in virulence has to be elucidated. The present project focuses on the characterization of these particular proteins. Mutants of the corresponding genes, recombinant proteins and corresponding polyclonal antibodies will be generated and used for functional

characterization. The functional analysis includes binding to cells of the immune system and non-professional phagocytes (epithelial and endothelial cells), the response of these cells to the proteins, the humoral and cellular immune response, interaction with components of the innate immune system and virulence in animal models. With this, we want to gain new insights into immune evasion mechanisms of *S. aureus*. Moreover, we will define potential candidates for the development of new vaccines aiming at mitigating or preventing *S. aureus* infections. Finally, a protein array will be developed which can be used for detection of early *S. aureus* infections.

2. Pathophysiology of staphylococci in the post-genomic era: *Staphylococcus aureus* in the airways of cystic fibrosis patients, a human model for long-term adaptive interaction
(A. Mellmann, B. Kahl, funded by Transregional Collaborative Research Centre 34)

Duration: 01.07.2010 – 30.06.2014

The lungs of cystic fibrosis (CF) patients are often infected by a predominant *Staphylocccus aureus* clone for many years. During persistent infection, *S. aureus* needs to adapt to the hostile environment of the lungs. In this project, we study adaptive mechanisms of *S. aureus* isolates recovered from the airways of individual CF patients several years apart using different approaches: comparison of proteome and transcriptome, assessment of the role of small non-coding RNAs, and investigation of the virulence potential of isolates. On the host site, the immune proteome of CF patients in response to chronic *S. aureus* infection will be analyzed.

3. *In vivo* evolution of *Staphylococcus aureus* during chronic airway infection of cystic fibrosis (CF) patients.
(B. Kahl, funded by the Interdisciplinary Clinical Research Center (IZKF), Münster)

Duration 01.01.2009 – 31.12.2011

One of the first pathogens that colonizes and infects the respiratory tract of CF patients is *Staphylococcus aureus*. The certified CF center in the Pediatric Department of the Universtity Clinics in Münster cares for more than 80 CF patients. Within the last years we were able to show that *S. aureus* colonization and infection in CF patients is chronic, persists for many years and in most patients is caused by a predominant *S. aureus* clone. We are interested whether long-time persistence of *S. aureus* in the CF lung accompanies genomic adaptations of strains to this environment. For that, we access a unique collection of *S. aureus* airway isolates from CF patients who have been persistently infected/colonized. Early and late *S. aureus* isolates of 3 patients recovered 12 years apart, were chosen for whole genome sequencing. Clonal identity of the respective strain pairs was ensured by MLST, PFGE, *agr*-, and *spa*-typing prior to WGS. The genome sequences of early and late isolates were compared to detect SNPs (*short nucleotide polymorphism*), insertions, and deletions. Sanger resequencing will be performed to confirm genomic alterations and a potential impact of detected mutations on protein composition and functionality. In parallel, virulence of the strain pairs will be compared focusing on biofilm formation, adhesive and invasive properties, the capacity to induce apoptosis and host inflammatory response, as well as the virulence potential in an *in vivo* murine chronic pneumonia model.

4. Dissection of *Staphylococcus aureus* infection from colonization in cystic fibrosis (CF) patients, a non-interventional, prospective, longitudinal multicenter study
(B. Kahl, H. Wittkowski, funded by Mukoviszidose e.V.; ClinicalTrials.gov Identifier: NCT00669760)

Duration: 01.01.2008 – 31.12.2011

S. aureus is not only one of the first pathogens infecting the airways of CF patients, but also a highly prevalent microorganism (>60% of all CF patients; European and American CF registries), which often persists for several years in the respiratory tract of CF patients. The purpose of this study is to dissect infection by *S. aureus* from colonization. Therefore, the following non-interventional, prospective, longitudinal multicenter study will be conducted to verify the following hypothesis: CF patients with high bacterial loads are more likely to be infected by *S. aureus* than patients with low bacterial loads.

The primary endpoint is the bacterial load of sputum and throat cultures. Secondary endpoints are: Nasal carriage, molecular analysis of *S. aureus* (monoclonal/polyclonal); Serum: *S. aureus*-specific antibodies, S100A12, IL-8, TNF-α; Sputum: S100A12, IL-8, myeloperoxidase; *S. aureus* therapy regimens; Lung function analysis: FEV1, ΔFVC, ΔMEF25; BMI development

Inclusion criteria are *S. aureus* cultures for more than 6 months within the last year, children (>6 years) and patients, who are able to perform lung function tests. Exclusion criteria are *P. aeruginosa* and/or *B. cepacia* cultures for more than 6 months within the last year before recruitment or during the study period.

In addition to microbiological investigations and clinical laboratory tests, the actual clinical situation will be evaluated and reported during the study period. The results of this observational study will be used to carefully plan a clinical interventional study. Furthermore, it might be possible to characterize a subpopulation of patients, which is at particular risk for *S. aureus* infections.