Dina Hilmi
Dr. sc. hum

The impact of S. *aureus* intra-strain and intra-species variability on immune recognition and functional properties

Promotionsfach : Infektiologie
Doktorvater: Prof. Dr. med. Klaus Heeg

S. aureus is a Gram-positive bacterium, member of the normal mucosal flora but also acts as a human pathogen, which causes severe infections such as pneumonia, endocarditis and sepsis.Over the last decade, multiple studies have described and explained that genetic variation accounts for intra-species variability in *Staphylococcus aureus* isolates and differences in the host- pathogen interaction and other functional characteristics responsible for invasiveness of *S. aureus* strains.

The aim of this thesis was to study and to compare clinical isolates of *S. aureus* strains colonizing the respiratory tract of cystic fibrosis patients and other S. *aureus* strains recovered from patients with invasive staphylococcal disease and ultimately, to determine the functional consequences of genetic differences among these S. *aureus* isolates, which determine the intra-strain or intra-species variation, influence the host-pathogen interaction and affect functional properties.

In our collection of clinical *S. aureus* strains, 12 of the CF (cystic fibrosis) isolates were small colony variants (SCVs), 10 of them isolated from the same CF patient over a period of two years. Our results obtained in Pulsed field gel electrophoresis (PFGE), *spa* typing and Matrix assisted laser desorption-ionization (MALDI) show that these isolates are genetically related and derived from the same ancestral strain, although one SCV isolate had a different *spa* type and a truncated *spa* fragment size as revealed by sequence and electrophoresis of the PCR product. In addition, differences in *spa, agrA and rnaIII* mRNA expression levels correlated with differences in protein A expression levels in Western blot analysis and with differences in TLR2-activity and biofilm formation among these SCV isolates. Thus, intra-strain variability also affects their functional level.

In the second part of this project we compared different *S. aureus* clinical isolates in regards to their TLR2-activity by measuring IL-8 induction in TLR2-transfected HEK293 cells. IL-8 induction was used in this study as a parameter for the assessment of the immune stimulatory activity. The main results obtained in these assays revealed marked differences among *S. aureus* isolates. Mature lipoproteins were confirmed to represent the specific ligands for TLR2 since the mutant, which lacks acylated lipoproteins (Δ*lgt*) was unable to trigger IL-8 through TLR2 stimulation. Thus, loss of TLR2 stimulatory capacity in some isolates could be explained either by reduced lipoprotein sorting to the cell wall because interference with cell wall integrity through antibiotics or heat-treatment of the cells enhanced TLR2 activity, or could be due to down-regulation of TLR2-active lipoproteins such as SitC. Additional experiments show that the SCV phenotype or expression of capsular polysaccharides can be associated with reduced TLR2 activity. Our results further provided evidence that the presence of DNA of different origins enhances IL-8 induction in HEK293 cells transfected with pTLR2. Although, the mechanism responsible for this effect is not well understood, our results clearly indicate that the expression and stimulation of TLR2 is essential for IL-8 induction. Moreover, IL-8 secretion was significantly decreased in the presence of the G-rich inhibitory ODN PZ3, which has been postulated to act as an inhibitor of TLR9. Furthermore, the synergistic effect between DNA and TLR2 was independent of caspase activation. Thus, we hypothesize that DNA recognition in HEK293 cells could be at least partially mediated through cytoplasmic nucleic acid sensing receptors. Taken together these data provide the notion that TLR2 enhances DNA sensing, which is required for IL-8 induction in HEK293 cells. Further relevancy of these findings was found in experiments performed using human monocytes where TLR2 stimulation represented a prerequisite for DNA recognition and induction of cytokines such as IL-1 beta, TNF-alpha and IL-6.Altogether, the data presented in this study demonstrate major differences between the clinical *S. aureus* SCV and normal isolates obtained in our diagnostic unit and assess the functional impact of intra- strain and intra -species variation on the host-pathogen interaction.