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**A role for type I interferon and plasmacytoid dendritic cells in immune defense against  
*Staphylococcus aureus***

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The objective of the thesis presented was set out to investigate the mechanisms involved in stimulation of human pDC by extracellularly located bacteria. We chose *S. aureus* since it has repeatedly been shown to induce type I IFN in human peripheral blood leukocyte.

Our data indicate that *S. aureus* is a potent IFN- $\alpha$  inducer and that IFN-induction is independent of  $\alpha$ -toxin. Importantly, bacterial viability was crucial for pDC activation and crude cell wall extracts or bacterial culture supernatants were not able to activate pDC on IFN- $\alpha$  production.

When compared to *S. aureus* Coagulase-negative staphylococci displayed only low to absent IFN- $\alpha$  induction with high donor variability. Subsequently it was understood that this was due to variations in species-specific IgG concentrations in the donor serum. PDC stimulated with *S. aureus* in IgG-depleted serum or in chicken serum were not able to respond with IFN- $\alpha$  secretion; IgG restored the IFN response. Titration experiments with human IgG in chicken serum revealed that *S. aureus*-induced IFN- $\alpha$  levels depended on the concentration of specific IgG.

PDC express CD32A receptor which binds IgG immune complexes. Our data show that pDC can take up IgG opsonized bacteria which will be recognized by intracellular PRR what will stimulate IFN- $\alpha$  production. By blocking CD32A receptor on pDC by neutralizing antibody we were able to diminish IFN- $\alpha$  secretion caused by *S. aureus*.

Interestingly, in individual donors certain *S. aureus* strains were able to induce IFN- $\alpha$  in the absence of IgG, e.g. in an CD32A-independent manner. Although differences in the IFN- $\alpha$  induction potential of *S. aureus* strains with high or low expression of protein A were not observed in the presence of autologous serum, protein A expression was found to correlate with IFN- $\alpha$  secretion in CD32-independent pDC activation.

To date the only well described stimuli for human pDC activation are microbial nucleic acids that engage TLR7 and TLR9. Our data showed that isolated DNA and RNA from coagulase –positive and –negative strains were capable to induce IFN- $\alpha$  in human pDC. DNA fraction showed better results than RNA in IFN- $\alpha$  production. Of note, no differences in that context between coagulase –negative and –positive strains were observed.

The experiments further revealed that CD32-dependent and -independent stimulation of pDC with *S. aureus* can be inhibited or significantly reduced by: cytochalasin D (indicating an endocytosis-mediated process), chloroquine and cathepsin B inhibitor CL074-Me (indicating endosomal and/or lysosomal involvement). Moreover, stimulation with *S. aureus* was inhibited by pretreatment of pDC with the G-rich oligodeoxynucleotide PZ3, which was previously described as a TLR9 inhibitor.

Based on the data we conclude that *S. aureus*-mediated CD32-dependent and -independent pDC activation is most likely mediated via TLR9. However, we cannot exclude a role of other cytosolic receptors involved in sensing of the pathogens.

We provide evidence that - in contrast to other innate immune cells - the recognition of staphylococci by human pDC is independent of TLR2 and specific for coagulase-positive staphylococci. This specificity is mediated by preformed anti-staphylococcal IgG and CD32A engagement. Based on our findings we propose that pDC activation is a hallmark of an antigen-specific memory response rather than an early event in a primary immune response.