Proteases in Staphylococcal Arthritis

Akademisk avhandling

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av

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I. Calander A-M, Jonsson I-M, Kanth A, Arvidsson S, Shaw L, Foster SJ, Tarkowski A, *Impact of staphylococcal protease expression on the outcome of infectious arthritis.* Microbes Infect, 2004. 6(2): p. 202-6.

II. Calander A-M, Dubin G, Potempa J, Tarkowski A, *Staphylococcus aureus infection triggers production of neutralizing, V8 protease-specific antibodies.* Submitted.

III. Calander A-M, Starckx S, Opdenakker G, Bergin P, Quiding-Järbrink M, Tarkowski A, *Matrix metalloproteinase-9 (gelatinase B) deficiency leads to increased severity of Staphylococcus aureus-triggered septic arthritis.* Microbes Infect, 2006. 8(6): p. 1434-9

Proteases in Staphylococcal Arthritis

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Staphylococcus aureus (*S. aureus*) is a ubiquitous bacterium. Due to an increasing prevalence of immunodeficiency states in the world population and the emergence of antibiotic resistant strains, the incidence of *S. aureus* septicaemia and its complications is increasing worldwide, as is the mortality thereof. *S. aureus* has an impressive capacity to adjust to the environment since it has developed a multitude of invasive and evasive mechanisms to cope with host defence. Many studies have been performed mapping *S. aureus* virulence determinants with the aim of improving our ability to combat *S. aureus* infections. So far, little is known concerning the impact of *S. aureus* extracellular proteases on virulence.

The aim of the first part of this thesis was to investigate the role of bacterial extracellular proteases as potential virulence factors in *S. aureus* induced septic arthritis. Inherent to this goal was to investigate whether there is a specific immune response to *S. aureus* extracellular proteases and if so, whether protease specific antibodies have any inhibitory function on the protease activity and thereby modulating the biological properties of the bacteria. The second part of this thesis aimed at shedding light on the impact of a host protease, matrix metalloproteinase 9 (MMP-9) on the development of septic arthritis.

S. aureus strain 8325-4 with a known high production of extracellular proteases and its mutants lacking extracellular proteases, Aur^-, Ssp^-, $SspB^-$, were compared concerning their capacity to induce arthritis and to prevail in kidneys and joints. Total serum levels of IgG and IgM were measured, as were antibodies specific for the deleted proteases. Silencing of the *ssp*, *aur*, or *sspB* genes did not affect the clinical nor the histological course of septic arthritis. Polyclonal B-cell activation was illustrated by auto-antibody production and a 10-fold increase in total IgG and a 50% increase in total IgM. Specific antibody response was demonstrated since only mice infected with bacteria expressing SspB and V8 responded with anti-protease specific antibody production. Functional capacity of the specific antibodies was illustrated by the fact that the V8 protease antibodies inhibited the activity of the enzyme in vitro.

To study the production of host protease MMP-9 over time, zymographic analyses were performed of spleen homogenates at different time points after bacterial inoculation. At day 9 post inoculation there was a four-fold increase of MMP-9 expression. To further investigate the role of MMP-9 in infectious arthritis, MMP-9 KO mice and their littermates C57Bl/6 wt mice, were inoculated iv with *S. aureus*. MMP-9 deficient mice showed more clinical arthritis and thrived less well. Importantly, the MMP-9 KO mice harboured significantly more bacteria in kidneys and joints than did their congenic controls, indicating MMP-9 as an indispensable molecule in the clearance of the infective agent.

Altogether this thesis shows that *S. aureus* proteases can evoke a specific immune response in the host, but that they are not essential in mediating bacterial arthritis. Furthermore, host MMP-9 contributes to innate immune responses during septic arthritis.

Keywords: *Staphylococcus aureus*, septic arthritis, proteases, matrix metalloproteinases

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