

Inflammatory mediator response to Gram-positive and Gram-negative bacteria *in vitro* and in middle ear infections

Akademisk avhandling

Som för avläggande av doktorexamen vid Sahlgrenska akademien, Göteborgs Universitet kommer att offentlig försvaras i föreläsningssalen (plan 3), Bakteriologiska Laboratoriet, SU/Sahlgrenska, Guldhedsgatan 10A, Göteborg

Torsdagen den 4 mars 2010, kl. 9.00

av

Susann Skovbjerg

Fakultetsopponent:

Professor Kristian Riesbeck

Institutionen för Laboratoriemedicin, Medicinsk mikrobiologi,
Skånes universitetssjukhus, Malmö

Avhandlingen baseras på följande arbeten:

- I. Skovbjerg S, Martner A, Hynsjö L, Hessele C, Olsen I, Dewhirst FE, Tham W, Wold AE. **Gram-positive and Gram-negative bacteria induced different cytokines from human mononuclear cells irrespective of taxonomic relatedness.** J Interferon Cytokine Res. 2010 Jan;30(1):23-32.
- II. Martner A, Skovbjerg S, Paton JC, Wold AE. ***Streptococcus pneumoniae* autolysis prevents phagocytosis and production of phagocyte-activating cytokines.** Infect Immun. 2009 Sep;77(9):3826-37.
- III. Skovbjerg S, Roos K, Nowrouzian F, Lindh M, Holm SE, Adlerberth I, Olofsson S, Wold AE. **High cytokine levels in perforated acute otitis media exsudates containing live bacteria.** Clin Microbiol Infect. 2009 Oct 14. [Epub ahead of print]
- IV. Skovbjerg S, Roos K, Holm SE, Grahn Håkansson E, Nowrouzian F, Ivarsson M, Adlerberth I, Wold AE. **Spray bacteriotherapy decreases middle ear fluid in children with secretory otitis media.** Arch Dis Child. 2009 Feb;94(2):92-8.



UNIVERSITY OF GOTHENBURG

Inflammatory mediator response to Gram-positive and Gram-negative bacteria *in vitro* and in middle ear infections

Susann Skovbjerg, Department of Infectious Medicine, University of Gothenburg, Guldhedsgatan 10A, SE 413 46, Gothenburg, Sweden, susann.skovbjerg@vregion.se

Abstract

Based on the structure of the cell wall, bacteria are divided into Gram-positive and Gram-negative. While the cell wall of Gram-positive bacteria is thick, the cell wall of Gram-negatives is very thin and surrounded by an outer membrane with LPS. Previous studies have shown that Gram-positive bacteria induce much more IL-12, TNF and IFN- γ from human peripheral mononuclear cells (PBMC) than do Gram-negatives, which instead induce more IL-6, IL-8 and IL-10 than do Gram-positive bacteria. In this thesis we confirm this cytokine pattern and show that the capacity of Gram-positive and Gram-negative bacteria to induce these distinct cytokine profiles is independent of their taxonomic relatedness. One exception from this general pattern was *Listeria monocytogenes*, which was a poor inducer of IL-12, TNF and IFN- γ despite being Gram-positive. Since an intricate interplay between IL-12, TNF and IFN- γ results in enhanced killing capacity of macrophages, these cytokines are recognized as phagocyte-activating cytokines. Another striking exception was *Streptococcus pneumoniae*, which induced no IL-12 despite its close relation to the viridans streptococci, which induced high levels. Aging pneumococci decompose due to the action of autolysin. We show that autolyzed pneumococci induce very little IL-12, TNF and IFN- γ and also inhibit the production of phagocyte-activating cytokines in response to intact bacteria. Further, fragments partly blocked phagocytosis of intact pneumococci. Thus, fragments generated by autolysin may paralyse phagocyte defenses and contribute to virulence.

To investigate the response to Gram-positive and Gram-negative bacterial infection, middle ear fluid was collected from children with acute otitis media (AOM) and from children with long-standing secretory otitis media (SOM). In SOM, Gram-negative bacteria were more prevalent than in AOM. Further, fluids with no cultureable bacteria were often positive by PCR in SOM, but not in AOM. This suggests that bacterial DNA is eliminated soon after killing of bacteria in AOM but not in SOM, or that bacteria remain dormant in the middle ear cavity of the SOM patients. The levels of inflammatory mediators in the fluids did not relate to the etiological agent. Instead, most cytokines, especially IL-1 β , were highly elevated in middle ear fluids containing live, cultureable bacteria compared to negative fluids, even if microbial DNA could be detected by PCR. In contrast, high levels of IL-6 and PGE₂ were measured also in AOM fluids with no detectable bacteria, and might be important in the resolution phase of the infection.

Nasal spray treatment with viridans streptococci and lactobacilli were tested as a method to speed up resolution of SOM in a placebo controlled double blind pilot study. One third of children sprayed with viridans streptococci showed significant clinical improvement, while treatment with lactobacilli was less effective. Clinical recovery was not associated with changes in the nasopharyngeal flora, or the expression of inflammatory mediators in nasopharynx or in the middle ear. Spray treatment with viridans streptococci could be an alternative to surgery due to SOM, but the mechanism of the beneficial effects remains to be elucidated.

Key words: Gram-positive, Gram-negative, bacteria, cytokines, monocytes/macrophages, phagocytosis, *Streptococcus pneumoniae*, autolysin, acute otitis media, secretory otitis media, children, probiotics