Regulation of innate and adaptive immune responses by Gram-positive and Gram-negative bacteria

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Bacteria are classified as Gram-positive or Gram-negative, depending on their cell wall structure. The role of the bacterial cell wall in immune regulation is the focus of the current work. Most Gram-positive bacteria stimulate monocytes to produce large amounts of IL-12. IL-12 induces production of IFN-γ in T cells and NK cells, which, in turn, activates the bactericidal capacity of the phagocyte in synergy/concert with TNF, produced by macrophages. We studied the bacterial structures and signalling pathways involved in IL-12 production in response to intact Gram-positive bacteria. This production depended on phagocytosis and activation of the JNK, NF-κB and PI3K pathways. Gram-positive bacterial fragments inhibited IL-12 production, which may serve as a negative feedback to turn off phagocyte activation the bacteria have been destroyed.

S. pneumoniae is a Gram-positive pathogen with a peculiar habit to disintegrate in stationary culture, due to activation of autolytic enzymes that degrade the cell wall. We demonstrated that pneumococci undergoing autolysis generate bacterial fragments that shut off monocyte production of TNF, IFN-γ and IL-12, thereby counteracting phagocyte activation. Further, the cytoplasmic pneumococcal toxin pneumolysin that was released upon autolysis dramatically augmented radical oxygen production in human neutrophils. Notably, ROS were foremost produced into intracellular compartments, probably affecting neutrophil function.

We also studied differences in how Gram-positive and Gram-negative bacteria modulated presentation of a model antigen to naïve T cells. Different subsets of mouse antigen-presenting cells were fed soluble ovalbumin (OVA), or OVA produced inside transgenic Gram-positive (lactobacilli/lactococci) or Gram-negative (*E. coli*) bacteria. Proliferation and cytokine production by OVA-specific transgenic T cells (DO11.10) was used as read-out system. “Bacterial” OVA much more efficiently activated OVA-specific $CD4^+$ T cells, than did soluble OVA. Further, *E. coli*-OVA induced a greater T cell proliferation than did OVA expressed by Gram-positive bacteria. Splenic APCs pulsed with soluble OVA induced IL-13 production in the T cell culture, while *E. coli*-OVA induced both IFN-γ and IL-13 and lactobacilli-OVA induced a weak IFN-γ response. We also noted that peritoneal DCs induced a different T cell polarisation pattern compared to splenic DCs, supporting production of more IL-17 and IL-10, but less IL-13. Furthermore, the presence of peritoneal macrophages inhibited $CD4^+$ T cell activation to bacterial, but not to soluble, antigens.

Key words: Gram-positive, Gram-negative bacteria, Streptococcus pneumoniae, IL-12, autolysin, pneumolysin, monocytes/macrophages, dendritic cells, $CD4^+$ T cells

ISBN 987-91-628-7819-1

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Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs Universitet, kommer att offentligen försvaras i föreläsningssalen (plan 3), Guldhedsgatan 10A, Göteborg

Torsdagen den 11 juni 2009, kl. 13.00

av

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Kings College, London, UK

Avhandlingen baseras på följande arbeten:

I. Cecilia Barkman, Anna Martner, Christina Hessle and Agnes E. Wold. 2008. **Soluble bacterial constituents down-regulate secretion of IL-12 in response to intact Gram-positive bacteria.** *Microbes and infection* 10:1484-93.

II. Anna Martner, Susann Skovbjerg, James C. Paton, Agnes E. Wold. **Autolysis of *Streptococcus pneumoniae* prevents phagocytosis and production of phagocyte activating cytokines.** *Submitted.*

III. Anna Martner, Claes Dahlgren, James C. Paton and Agnes E. Wold. 2008. **Pneumolysin released during *Streptococcus pneumoniae* autolysis is a potent activator of intracellular oxygen radical production in neutrophils.** *Infection and immunity* 76:4079-4087.

IV. Anna Martner, Sofia Östman, Samuel Lundin, Lars Axelsson and Agnes E. Wold. **Gram-negative bacteria are superior $CD4^+$ T cell activators compared to Gram-positive bacteria.** *In manuscript.*