Role of the chaperone gp96 for intestinal barrier function and the induction of tolerance

Original title / Originaltitel
Bedeutung des Chaperons gp96 für die intestinale Barriere und die Induktion von Toleranz

Summary / Zusammenfassung
Recent evidence suggests an important role of the chaperone gp96 for intestinal barrier function and the induction of tolerance at the gut mucosa. Gp96 protein is normally located in the endoplasmatic reticulum (ER). It is able to bind peptides (antigens) and can be secreted. Following re-internalization of the gp96-peptide complex by antigen presenting cells (APCs) the antigens can be transferred to MHC molecules mediating immune reactions or tolerance depending on the local concentration of gp96. Recently, gp96 has been shown to be the major chaperone for Toll-like receptors (TLRs). A lack of correct TLR protein folding is associated with disturbed recognition of bacterial products and impaired innate mucosal immunity.

Intestinal macrophages (IMACs) represent one of the largest macrophage populations of the human body. They constitute a tolerogenic cell type without expression of “typical” macrophage activation receptors. IMACs are of crucial importance for pathogen recognition at the mucosal surface and an impairment of their innate immune functions has been associated with the pathogenesis of chronic inflammatory bowel diseases such as Crohn’s disease (CD). Studying this cell population since 1995 in several projects funded by the German Science Foundation (DFG) we were able to isolate and purify IMACs and to subsequently clone genes specifically induced during the differentiation of IMACs. Among those genes was gp96 which turned out to be absent or decreased in IMACs in CD. In a mouse model of colitis we demonstrated amelioration of intestinal inflammation by treatment with gp96.

Based on these findings we hypothesize that 1) Gp96 is essential for the maintenance of tolerance against food antigens and commensal bacteria mediated by IMACs (“function in normal mucosa”) and 2) that the loss of gp96 protein in IMACs during CD contributes to the loss of tolerance and subsequent IMACs activation followed by chronic inflammation. (“contribution to CD pathogenesis”)

Specific aims and experimental design
A. Function of gp96 in IMACs in normal mucosa. Basal gp 96 expression, cellular gp96 protein content and gp96 secretion will be compared in macrophages, dendritic cells and isolated human CD33+ IMACs. Regulation of gp96 expression, gp96 effects on TLR folding and cellular recognition of bacterial wall products will be studied. IMAC innate immune functions will be investigated after lentiviral gp96 knock down on.

B. Pathways of gp96 binding, internalization and antigen processing by IMACS. Expression and function of gp96 receptors such as CD91, MRS-1 and SCARF-1 will be investigated by staining techniques and neutralizing antibodies.

C. T-cell response upon peptide presentation via gp96 and IMACs. Generation of regulatory T-cells. Studies will be performed in mouse models and isolated human cells with mixed lymphocyte cultures studying T-cell phenotype, activation and function.

D. Role of gp96 in IMACs in vivo. As a simple knock out of gp96 is lethal we already generated a mouse model for a conditional knock out which will allow cell type specific deletion of gp96 in the intestinal mucosa.

The long term goal will be a better understanding of mucosal tolerance and the development of new treatment options for chronic mucosal inflammatory diseases such as CD.
Keywords / Suchbegriffe
gp96, intestinal macrophages, TLRs, Crohn’s disease, innate immunity, PRR

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