

Vaginal commensal bacteria

Interactions with cervix epithelial and monocytic cells and influence on cytokines and secretory leukocyte protease inhibitor, SLPI

Akademisk avhandling

som för avläggande av medicine doktorexamen vid Sahlgrenska akademien vid Göteborgs universitet kommer att offentlig försvaras i föreläsningssalen (plan 3)
Avdelningen för Klinisk Bakteriologi, Guldhedsgatan 10 A, Göteborg

Torsdagen den 4:e december 2008, kl. 9.00

av

Louise Strömbeck

Fakultetsopponent:

Docent Åsa Ljungh

Sektionen för Medicinsk Mikrobiologi
Lunds Universitet

Avhandlingen baserar sig på följande arbeten:

- I. Louise Strömbeck, Jens Sandros, Elisabeth Holst, Phoebus Madianos, Ulf Nannmark, Panos Papanou and Inger Mattsby-Baltzer
***Prevotella bivia* can invade human cervix epithelial cells, HeLa**
APMIS 115: 241–251, 2007
- II. Louise Strömbeck, and Inger Mattsby-Baltzer
Commensal vaginal lactobacilli and their regulatory effects on secretory leukocyte protease inhibitor (SLPI) and cytokine secretion in human monocytic cells
Submitted, 2008
- III. Louise Strömbeck and Inger Mattsby-Baltzer
Effects of commensal vaginal lactobacilli on the regulation of secretory leukocyte protease inhibitor (SLPI) and cytokine secretion in human cervix epithelial cells (HeLa)
Submitted, 2008

Göteborg 2008



UNIVERSITY OF GOTHENBURG

Vaginal commensal bacteria

Interactions with cervix epithelial and monocytic cells and influence on cytokines and secretory leukocyte protease inhibitor, SLPI

Louise Strömbeck

Department of Infectious Medicine, Gothenburg University

Abstract

Lactobacillus is the predominating species of the vaginal microbiota in women of childbearing age. Lactobacilli are thought to contribute to the local immune defence by producing a variety of antimicrobial substances and, thereby, attenuate growth of other opportunistic bacteria. A disturbed vaginal microbiota, with loss of lactobacilli and an overgrowth of other anaerobic bacteria such as occurs in cases of bacterial vaginosis (BV), increases susceptibility to sexually transmitted infections, risk of ascending infections to the upper genital tract and postoperative infections, which can pose a threat to pregnancy and cause preterm birth (PTB).

Antimicrobial polypeptides (AMPs) are important molecules of the mucosal innate immune defence. Secretory leukocyte protease inhibitor (SLPI) is a multifunctional AMP that is present at high concentrations in the healthy female genital tract. Thus, the presence of a healthy microbiota, such as lactobacilli, and the surveillance by the mucosal innate immune system are suggested to be important factors for the homeostasis of the lower genital tract.

Our aim was to analyse the occurrence of virulence features in the opportunistic Gram-negative bacterium *Prevotella bivia*, commonly found in BV, associated with upper genital tract infection and PTB, and to investigate whether vaginal lactobacilli interact with the host innate immune defence, by affecting the regulation of SLPI and pro-inflammatory cytokine responses in host cells.

Studies of the anaerobic bacterium *P.bivia* showed that one out of five strains tested had a high capacity to invade HeLa cells. The lack of adhesion structures on *P.bivia*, as well as a similar capacity of different strains to adhere to HeLa cells, suggested that *P.bivia* are endowed with some other factor important for the intracellular invasion. Only the most invasive strain of *P.bivia* gave rise to a weak IL-6 and IL-8 response. Thus, a high invasion capacity together with a low pro-inflammatory cytokine response in certain strains of *P.bivia* are suggested to be virulence factors in establishing a subclinical upper genital tract infection.

Analysis of isolates of the four most frequent vaginal *Lactobacillus* spp. (*L.crispatus*, *L.iners*, *L.gasperi* and *L.jensenii*), regarding the capacity to influence the secretion and expression of SLPI in monocytic- (THP-1) and cervix epithelial- (HeLa) cells, showed that *L.iners* could up-regulate the constitutive secretion of SLPI. However, high concentrations of *L.iners* down-regulated the SLPI secretion in both cell types. The largest difference between the four lactobacilli species in their regulation of SLPI was obtained between *L.iners* and *L.crispatus*. At the concentrations tested, *L.crispatus* gave rise to a strong reduction of SLPI in both cell types. A negative correlation was found between SLPI protein and mRNA expression levels in HeLa cells, but not in THP-1 cells. In both cell types, synergy effects in the pro-inflammatory cytokine response were obtained by co-incubation of lactobacilli with *E.coli*. Positive synergy effects were obtained for the IL-8 and TNF- α production in THP-1 cells and for IL-6 and IL-8 in HeLa cells. Negative synergy effects were obtained for IL-6 and IL-18 in THP-1 cells. Moreover, negative correlations were obtained between the cytokines and SLPI levels. Analysing the effects of the addition of recombinant SLPI to HeLa cells prior to the exposure to *E.coli* showed a significant reduction of the IL-6 and IL-8 responses in the cells.

The results indicated that vaginal lactobacilli can contribute to the regulation of SLPI and pro-inflammatory cytokine responses in host cells. However, our data also suggested that a dominating *Lactobacillus* spp., such as *L.iners* or *L.crispatus*, may influence the mucosal innate immune defense in different ways. Moreover, the regulatory effect on the SLPI secretion was inversely associated with the capacity of the bacteria to evoke pro-inflammatory cytokines in the host cells.

Key words: *Prevotella bivia*, bacterial vaginosis (BV), invasion, *Lactobacillus*, secretory leukocyte protease inhibitor (SLPI), cytokines, THP-1 cells, HeLa cells.