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Lipopolysaccharide-binding protein is produced in the epididymis and associated with spermatozoa and prostasomes

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Abstract

Lipopolysaccharide-binding protein (LBP) is an acute phase protein known to play a central role in the defense against Gram-negative bacteria. It binds lipopolysaccharides of Gram-negative bacteria and, after binding to CD14, the complex signals through Toll-like receptor (TLR)-4, eliciting host-defense responses, such as cytokine production, in inflammatory cells. The present study demonstrates constitutive expression of the gene encoding lipopolysaccharide-binding protein in the epithelium of the human epididymis by in situ hybridization. Using immunohistochemistry lipopolysaccharide-binding protein was shown to be present in the same cells and also attached to the heads and tails of spermatozoa. Cell-free seminal plasma, lysed spermatozoa and lysed prostasomes were subjected to Western blot; all showed immunoreactive bands corresponding to the size of lipopolysaccharide-binding protein. Gel filtration demonstrated that lipopolysaccharide-binding protein colocalizes with prostasomes. The concentration of lipopolysacharide-binding protein in seminal

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plasma was 127 ± 42 ng/mL (mean ± S.D.; range 73–215 ng/mL). Taken together, our results suggest roles for lipopolysaccharide-binding protein during human reproduction.

Keywords: Lipopolysaccharide-binding protein; Reproduction; Prostasomes; Spermatozoa

1. Introduction

Both human semen and prostatic fluid can exhibit antimicrobial activity. However, reports dealing with the in vitro inhibitory effect on the growth of various bacterial species are contradictory. Some authors claim that only Gram-positive bacteria are inhibited by factors in semen and prostatic fluid, others suggest that both Gram-positive and Gram-negative species are effected (Taylor and Morgan, 1952; Gupta et al., 1967). Recently, it has been shown that the antibacterial activity of semen, at least partly, is derived from prostasomes (Carlsson et al., 2000). These organells are membrane-surrounded vesicles produced by prostate epithelial cells. Prostasomes are expelled with the prostate secretion at ejaculation (Ronquist et al., 1978a,b) and appear in the seminal plasma. They have a diameter between 40 and 500 nm, regardless of whether they are found intra- or extra-cellularly. The *mean* size is 150 nm (Ronquist et al., 1990). Prostasomes are claimed to have several functions. For example, after fusion with spermatozoa (Arienti et al., 1997), they enhance sperm motility (Stegmayr and Ronquist, 1982). In addition, prostasomes possess immunosuppressive functions (Kelly et al., 1991; Skibinski et al., 1992). Lipopolysaccharide-binding protein (LBP) is an acute-phase protein that is constitutively synthesized by hepatocytes, and, during inflammation its concentration increases up to 30-fold in the blood circulation (Schumann et al., 1990). LBP (60 kDa) has lipopolysaccharide (LPS)-binding properties. The lipopolysaccharide-binding protein plays an important role in the host defense against Gram-negative bacteria. It functions as an opsonin and after binding to LPS the LBP/LPS-complex can activate monocytes/macrophages (Wright et al., 1990). Human LBP binds to the LPS of Gram-negative bacteria and this complex binds to either soluble or membrane bound CD14. This complex can activate Toll-like receptor (TLR)-4 present on the surface of inflammatory cells, eliciting functional responses (Yang et al., 1998). In addition to its expression in the liver, LBP is expressed in cell-lines derived from gastrointestinal and airway epithelium after stimulation by proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β (Vreugdenhil et al., 1999; Dentener et al., 2000). In the present study, we investigated the presence of LBP in the male reproductive system. The LBP gene expression was detected by in situ hybridisation, and the protein was detected by immunohistochemistry in the epithelium of epididymis. Furthermore, immunocytochemistry showed presence of LBP on the surface of the heads and tails of spermatozoa. Immunoreactive LBP was identified by Western blot in seminal plasma, and in homogenates of spermatozoa and prostasomes, respectively. The association of LBP with prostasomes was further investigated by gel filtration and colocalization with the prostasome marker dipeptidyl peptidase IV (CD26). Our findings suggest roles for LBP in the host defense of the male reproductive system and during reproduction.