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Toll-like receptor 2 and 4 expression in the pregnant and non-pregnant human uterine cervix

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ABSTRACT

Pelvic infections and sexually transmitted diseases place a burden on health resources and may be associated with premature birth. The mechanisms by which the female reproductive tract (FRT) combats these infections remain ill understood, but are likely to involve the pattern recognition Toll-like receptors (TLR). We sought to compare the expression of TLR-2 and -4 by human pregnant and non-pregnant ectocervical epithelium as a prelude to the investigation of the function of these receptors in this tissue during pregnancy. Using the techniques of reverse-transcriptase polymer chain reaction (RT-PCR) and immunohistochemistry, the gene and protein expression of TLR-2 and -4 were studied in the biopsies of ectocervix obtained from non-pregnant premenopausal women ($n = 21$) undergoing hysterectomy, women in the first trimester of pregnancy undergoing non-medically indicated suction pregnancy termination ($n = 6$), and women at term undergoing elective caesarean section ($n = 11$). The expression of TLR2 and TLR4 genes and proteins were upregulated in early and late pregnant ectocervical epithelium, compared with non-pregnant tissue. These findings suggest that the upregulation of TLR2 and TLR4 in the lower FRT may play a key role in the modulation of the innate immune and inflammatory mechanisms of the ectocervix during pregnancy, interacting with other neuroendocrine factors.

1. Introduction

Sexually transmitted infections (STIs) are a major worldwide health problem, compromising reproductive fecundity, causing morbidity, and reducing life expectancy (WHO, 2012; Adams et al., 2000). Maternal urogenital infections may lead to miscarriage, chorioamnionitis and congenital foetal infection and appear to be implicated in premature births (Lyon et al., 2010), the principal cause of perinatal mortality and handicap in surviving children (Christiaens et al., 2008).

The vagina and ectocervix are heavily colonised by microbial organisms whilst the endocervix, endometrium and fallopian tubes are sterile (Mardh, 1991). The modulation of this microbial commensal distribution, and the immunological mechanisms that prevent ascending

Abbreviations: FRT, female reproductive tract; LCM, laser capture micro-dissection; PRR, pattern recognition receptors; STIs, sexually transmitted infections; TLR, toll-like receptor; TBO, toluidine blue O; OCT, optimal cutting temperature; RT, reverse transcriptase; TAE, tris-acetate and EDTA; HECECs, human ectocervical epithelial cells.

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infections are poorly understood. The mucosal surface of the genital tract is the first barrier against the entry of most STIs (Starnbach and Roan, 2008). Recent studies suggest that Toll-like receptors (TLR), a family of pattern recognition receptors (PRR) that mediate innate immunity, expressed by cells that are the first line of host defence (Kumar et al., 2011; Playfair and Chain, 2013), might play a role. The expression profiles and activity of the Toll family of receptors in different tissues and organs have been detailed (Backhed and Hornef, 2003; Basu and Fenton, 2004), but relatively little is still known about their role in the human female reproductive tract (FRT) during pregnancy.

During pregnancy the immune system must adapt to support a feto-placental unit that is immunologically distinct (Wira et al., 2005). FRT must deal with sexually transmitted bacterial and viral pathogens, allogeneic spermatozoa, and the immunologically distinct foetus. Since two-thirds of pregnancy-related infections ascend the cervix into the uterus (Romero and Mazor, 1988; Racicot et al., 2013), a breech in this immunological barrier is implicated in the pathogenesis of most infections associated with pregnancy. There is also emerging evidence that dysregulated Toll-mediated signalling might be implicated in premature birth (Elovitz et al., 2003; Riley and Nelson, 2010). For instance, a variant in the human TLR4 gene has been associated with an increased risk of premature birth (Lorenz et al., 2002). This may be mediated by pro-inflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF (Lyon et al., 2010) through NF-κB activation by signalling mechanisms that appear crucial for prelabour cervical remodelling and myometrial activation (Sennstrom et al., 2000).

Using immunohistochemistry, we determined that early pregnancy is associated with increased expression of TLR2 and TLR4 receptors in cervical tissue. We then sought to determine whether such upregulation during pregnancy persisted into the third trimester by performing semi-quantitative RT-PCR. The present studies were aimed to provide a prelude to mechanistic studies exploring the role of TLR2 and TLR4 in the immunological changes of pregnancy at this mucosal interface.

2. Materials and methods

2.1. Study design

The South Sheffield Research Ethics Committee approved the study. Written informed consent from participating patients was obtained before collection of the samples.

2.2. Receptor protein expression studies: immunohistochemistry

2.2.1. Subjects and immunostaining of cervical tissue samples

Fresh, full-thickness biopsies of the ectocervix were obtained from premenopausal women (age 35–45 years) undergoing hysterectomy for dysfunctional uterine bleeding ($n = 6$). The biopsies were acquired using an 8-mm Stiefel punch biopsy forceps immediately after the cervix and uterus had been excised. All subjects had had a negative urinary pregnancy test, a normal cervical smear within the previous 3 years, negative swabs for genital infection, and were not taking hormonal contraceptives at least six weeks before surgery. Biopsies were also obtained using a Tischler punch biopsy forceps from women in the first trimester of pregnancy (age 28–40 years) undergoing non-medically indicated suction pregnancy termination ($n = 6$). All cervical tissue specimens were collected under sterile conditions, transported in Hank's Balanced Salt Solution (HBSS; Invitrogen, Paisley, UK) on ice, and immediately processed. Small sections (5 mm × 5 mm) were quickly fixed in 10% (w/v) formalin. Tissue sections (3 mm) for immunohistochemical study were prepared from these samples.

Formalin-fixed sections were dewaxed in xylene twice for 5 min, followed by rehydration in a graded series of ethanol washes. Endogenous peroxidase activity was quenched in the paraffin sections by incubating in 3% (v/v) hydrogen peroxide in methanol for 20 min. Antigen retrieval on these sections was performed by microwave irradiation for 12 min in 10 mmol/l sodium citrate (pH 6.0). Sections were allowed to cool for 15 min and then washed in PBS. Formalin-fixed slides were then stained using a Vectastain Elite ABC peroxidase kit (Vector Laboratories, Peterborough, UK). This kit contains normal serum, biotinylated universal secondary antibody and preformed Elite ABC reagent. In addition, to avoid non-specific binding, an avidin/biotin blocking kit (Vector) was used. Briefly, slides were blocked for 1 h at room temperature in PBS containing 0.2% (v/v) horse serum and 25% (v/v) avidin supplied in the blocking kit. The block was removed and slides were incubated overnight at 4 °C in primary antibody at an appropriate dilution and 250 ml of biotin per ml of diluted antibody. Binding was visualised by incubation with peroxidase substrate AEC (3-amino-9-ethylcarbazole; Vector) for 10 min, washed in distilled water for 3 min and counterstained in 10% haematoxylin for 10 min. Slides were washed in tap water for 2 min and mounted with Aquamount (VWR, Radnor, PA, USA).

Optimal staining was achieved by incubating tissue sections with different concentrations of TLR antibodies. Control sections were obtained by excluding the primary antibody. Specific TLR antibody staining was blocked by co-incubation of diluted TLR antibody with 20-fold concentration of the corresponding specific peptide overnight at 4 °C. The blocked primary antibody was then used in immunoperoxidase staining as described above.

2.2.2. Reagents and antibodies

Antibodies and peptides were obtained from Santa Cruz Biotechnology (Dallas, TX, USA): goat polyclonal antibodies specific for the N-terminal domains of TLR2 (catalogue no. sc8689) and goat polyclonal antibody specific for C-terminal domains of TLR4 (catalogue no. sc8694). Blocking peptides specific for the respective antibodies (sc8689P and sc8694P) were used at a 20-fold excess following the manufacturer's guidelines.