ELSEVIER

Journal of Reproductive Immunology 73 (2007) 74–84

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

www.elsevier.com/locate/jreprimm

Reduced expression of IL-6 and IL-1α mRNAs in secretory phase endometrium of women with recurrent miscarriage

Melinda J. Jasper*, Kelton P. Tremellen, Sarah A. Robertson

Research Centre for Reproductive Health, School of Paediatrics and Reproductive Health, The University of Adelaide, Adelaide, SA 5005, Australia

Received 22 March 2006; received in revised form 20 June 2006; accepted 20 June 2006

Abstract

A diverse array of cytokines is implicated in regulating the immune adaptation and endometrial tissue remodelling events that facilitate successful embryo implantation and early placental development. The aim of this study was to evaluate expression of mRNAs encoding a panel of immunoregulatory cytokines in the endometrium of fertile women and women experiencing recurrent miscarriage using highly sensitive, quantitative RT-PCR assays. Endometrial biopsies were collected during the mid-secretory phase of the menstrual cycle from women classified as proven fertile (control; $n = 12$) and women experiencing unexplained recurrent miscarriage (RM; $n = 9$). Reduced IL-6 mRNA and reduced IL-1α mRNA were independently associated with recurrent miscarriage. Altered expression was evident after accounting for variation in the composition of endometrial biopsies by normalization of data to epithelial and mesenchymal cell-specific transcripts, cytokeratin-18 mRNA and vimentin mRNA, respectively. The relative abundance of mRNAs encoding LIF, GM-CSF, IFNγ, IL-1β, IL-4, IL-5, IL-10, IL-12p40, TNFα, TGFβ1, TGFβ2 and TGFβ3 were not altered in recurrent miscarriage tissue. Associations between expression of IL-10, LIF, GM-CSF and TGFβ2 suggest that regulatory circuits link the transcription of these cytokine genes. Inadequate expression of IL-6 and IL-1α mRNAs in endometrial tissue may predispose to recurrent miscarriage through a perturbed maternal immune response, effects on decidual tissue remodeling and angiogenesis, or dysregulated trophoblast differentiation and invasion. Quantitative RT-PCR assays for these cytokines in endometrial biopsies may be a realistic strategy for development of novel diagnostics for predisposition to recurrent miscarriage.

Keywords: Endometrium; Cytokine; T-lymphocyte; Interleukin-6; Interleukin-1; Recurrent miscarriage

1. Introduction

Recurrent miscarriage, defined as three consecutive first trimester pregnancy losses, affects 0.5–2% of women (Stirrat, 1990). No apparent cause is identifiable in approximately 50% of cases, and these unexplained miscarriages are attributed to unknown endometrial disorders leading to placental insufficiency (Li et al., 2002). An aberrant maternal immune response, whereby the requisite adaptations to support conceptus development fail, is one likely mechanism contributing to unexplained recurrent miscarriage (Laird et al., 2003). The maternal immune response is principally regulated by an array of cytokines with characteristic expression patterns over the course of the menstrual cycle linked with generation of endometrial receptivity for embryo implantation during the mid-secretory phase. Endometrial cytokines act in complex networks to orchestrate the changes in leukocyte populations required to protect the conceptus from fetal immune rejection (Thellin et al., 2000; Trowsdale and Betz, 2006) and to facilitate the tissue remodeling processes necessary for decidualisation and adequate

* Corresponding author. Tel.: +61 8 8303 5100; fax: +61 8 8303 4099.
E-mail address: melinda.jasper@adelaide.edu.au (M.J. Jasper).

0165-0378/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jri.2006.06.003

placental development (Croy et al., 2002; Dimitriadis et al., 2005).

Prevention of conceptus assault by the maternal immune response is largely achieved by preventing uncontrolled cell-mediated (type 1) immune activation (Thellin et al., 2000; Wegmann et al., 1993). This occurs through local and systemic skewing towards type 2 immunity (Lin et al., 1993; Saito, 2000), as well as by tolerogenic mechanisms including T regulatory (Treg) cells (Saito et al., 2005; Trowsdale and Betz, 2006). Women experiencing recurrent miscarriage show evidence of excessive type 1 immune parameters and reduced type 2 and Treg immune parameters, both systemically and in endometrial tissues (Hill et al., 1995; Michimata et al., 2003; Piccinni et al., 1998; Saito et al., 2005; Sasaki et al., 2004; Yamada et al., 1994). Immunohistochemical and flow cytometric analyses suggest that, even in the non-pregnant state, predisposition to miscarriage is characterized by changes in the balance between endometrial lymphocyte subpopulations (Laird et al., 2003).

The abundance and phenotype of lymphocytes in peripheral tissues is generally understood to be regulated by local immunoregulatory cytokines, particularly those influencing antigen-presenting cell (APC) function (Moser and Murphy, 2000) and phenotype acquisition and maintenance in T helper cells (Reiner, 2001). This raises the prospect that elevated type 1 immune responses in the implantation site are secondary to altered expression of local immunoregulatory cytokines (Saito, 2000). Both leukocytes and non-leukocytic cells contribute to endometrial cytokine synthesis. T helper type 1 (Th1) cells differentiate in response to IL-12-secreting APCs, resulting in their expression of the hallmark Th1 cytokine IFNγ, while T helper type 2 (Th2) cells express the Th2 signature cytokines IL-4, IL-5 and IL-10. Development of regulatory T cells (Treg cells) is favored in the presence of TGFβ and Treg cells characteristically secrete abundant TGFβ and IL-10 (von Boehmer, 2005). Uterine luminal and glandular epithelial cells, as well as decidual fibroblasts and endothelial cells, are potent sources of many additional cytokines including LIF, GM-CSF, IL-1α, IL-1β, IL-6, IL-12, TNFα and TGFβ (Dimitriadis et al., 2005; Robertson et al., 1994). These cytokines regulate immune response outcomes through their effects on the phenotype and function of dendritic cells and macrophages, and also target endothelial cells, epithelial cells and stromal cells to mediate the vascular and tissue remodeling changes essential for implantation success.

The possibility that unexplained recurrent miscarriage is the consequence of altered expression of regulatory cytokines in the endometrium has begun to be examined. Using qualitative RT-PCR, mRNAs encoding several type 1 cytokines were detected more frequently, while the type 2 cytokine IL-6 was detected less often, in endometrial tissue from miscarrying women than in normal fertile women (Lim et al., 2000). Similar skewing was seen using RNAse protection assays to quantify the most abundant endometrial cytokines, with a reduction in mean expression levels of both IL-6 and IL-1β mRNA in a cohort of women experiencing recurrent miscarriage compared with fertile controls (von Wolff et al., 2000). However, the mRNA quantification techniques used in these studies lack the combination of sensitivity and precision required to quantify accurately mRNA transcripts expressed in minor populations of endometrial lymphocytes, and also fail to take into account the possibility that differences in biopsy cell lineage composition contribute to detected changes in transcript abundance.

The objective of this study was to investigate the physiological significance in fertility status of expression of several cytokines implicated directly and indirectly in regulation of the endometrial Th1/Th2/Treg immune balance using real-time quantitative RT-PCR assays. Endometrial tissue was collected during the mid-luteal phase of the menstrual cycle from a cohort of women experiencing unexplained recurrent miscarriage, and from a group of proven fertile controls. Biopsies were analyzed by quantitative, real-time RT-PCR assays in an experimental strategy that included normalization to cell lineage-specific transcripts for cytokeratin-18 and vimentin to account for potential variation in biopsy content of epithelial cells and stromal tissue. This highly sensitive technique allowed precise evaluation of the relative abundance of cytokines and their association with fertility status, even those cytokines undetectable by other approaches.

2. Materials and methods

2.1. Subjects and endometrial biopsy collection

This study was approved by the North Western Adelaide Health Service (118/2001) and the Adelaide Women's and Children's Hospital (REC1216) and was conducted using tissue collected at Repromed Pty Ltd., the University of Adelaide's Reproductive Medicine Unit, at either the Queen Elizabeth Hospital (Woodville, Australia) or the Wakefield Hospital (Adelaide, Australia). All participants were 18 years or older and provided informed consent prior to sample collection.

Endometrial biopsies were performed in the mid-luteal phase of the menstrual cycle (day 5–9 post-ovulation), timed according to the last menstrual period