Immunoendocrine aspects of endometrial function and implantation

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Abstract

Effective ovarian and uterine function relies on a complex interplay between the endocrine and immune systems. It is generally accepted that in reproductive tissues, oestradiol and progesterone have pro- and anti-inflammatory activities respectively and, in this regard, the paracrine effects of the sex steroids on the ovary are similar to the endocrine effects on the uterus. Ovarian leukocyte recruitment and cytokine release are central to follicle development, ovulation and corpus luteum function. At the uterine level, the cyclical changes in sex steroids regulate the number and distribution of endometrial and decidual immune cells as well as other immune signalling and surveillance factors. The uterine mucosa is unique, in that it must tolerate sperm and the allogeneic blastocyst in a way that does not compromise uterine immune surveillance against bacteria, yeast and viruses. Crosstalk between the sex steroids and immune mediators (systemic and local) are central to these functions, and this article will review these mechanisms and their importance for successful reproductive function and pregnancy success.

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Introduction

Implantation and subsequent placental growth are complex processes biologically regulated by interplay between sex steroids and local cell signalling molecules, many of which have an immune function. There is increasing evidence that this interplay is essential for effective ovarian and uterine function, and thus there is now considerable blurring between the respective roles of endocrinology and immunology in reproductive biology. In terms of endocrinology, the priming of the endometrium by the sex steroids and the associated cyclical morphological and functional changes that occur in preparation for pregnancy are well documented. At the local tissue level, however, it is clear that central to these changes are alterations in both inter- and intracellular signalling molecules many of which have the capacity to alter immune processes. Moreover, the establishment of pregnancy requires further interaction between endocrine and immune mechanisms, which facilitate maternal–foetal crosstalk, regulate implantation, promote placental growth and prevent immunorejection of the semi-allogeneic foetus. The main focus of this review is the role of immunoendocrine crosstalk in endometrial function and implantation.

Immunoendocrine crosstalk in reproductive biology: the big picture

In the non-pregnant female reproductive system, immunoendocrine crosstalk is essential for normal ovarian function, and this, in turn, regulates the endometrial changes required for possible implantation. Under the influence of follicle-derived oestrogen and pituitary-derived gonadotrophins, follicle development, oocyte maturation and ovulation occur and all of these processes involve inflammatory-like mechanisms (Espey 1994). For example, the inflammatory features of ovulation include extracellular matrix degradation, vascular changes, the expression of chemokines, cell adhesion molecules and integrins and the ultimate recruitment of leukocytes from the circulation (Brannstrom & Enskog 2002).

In the absence of pregnancy, immune cells have been implicated in the process of luteal regression. Neutrophils, macrophages and T-lymphocytes predominate in the corpus luteum (CL) at around the time of luteolysis and may be directly involved in the destruction of the luteal cells and subsequent loss of progesterone secretion (Brannstrom *et al.* 1994, Pate & Landis Keyes 2001). Alternatively, it has been suggested that these immune cells are simply responding to the dead and

dying luteal cells and may have a clearing up function, thus preventing a deleterious immune response to the surrounding ovarian tissue (Pate & Landis Keyes 2001).

Following ovulation, anti-inflammatory mechanisms counteract the original ovulation-associated inflammatory processes. This is reported to be mediated in part by glucocorticoids of adrenal origin that act through the receptor (NR3C1: nuclear receptor subfamily 3, group C, member 1) expressed on ovarian surface epithelial cells (Rae *et al.* 2004). The pro-inflammatory cytokine interleukin-1 (IL-1), which would be produced during ovulation, increases the expression of the enzyme hydroxysteroid (11- β) dehydrogenase 1 (HSD11B1) in ovarian surface epithelium, which converts cortisone to anti-inflammatory cortisol (Rae *et al.* 2004). Consequently, cortisol, rather than cortisone, binds to the NR3C1 and inactivates anti-inflammatory signalling pathways (Rae & Hillier 2005).

More recently, murine cumulus and granulosa cells have been shown to express a range of innate immune-related genes important for cell survival and surveillance during ovulation (Shimada *et al.* 2006). These include toll-like receptors (TLRs; discussed below) and scavenger receptors. Moreover, Shimada and colleagues demonstrated that cumulus and granulosa cells exhibit phagocytic activity against bacterial particles. These studies illustrate that not only does ovulation exhibit the classic features of an inflammatory reaction, but also that cumulus and granulosa cells appear to exhibit immune surveillance activity in their own right. The implications of toll receptor signalling and immune surveillance are discussed further in relation to uterine epithelial cells (see below).

The uterus as a unique immune/mucosal organ

The uterine mucosa is unique amongst other mucosal sites, since it undergoes regular changes in structure under the influence of sex steroids (Lea & Clark 1989, Quayle 2002). The lower reproductive tract must tolerate commensal flora but be able to respond against infectious agents. In addition, the presence of sperm and the semi-allogeneic blastocyst must be tolerated in a way that does not compromise uterine immune surveillance against bacteria, yeast and viruses (Quayle 2002, Entrican & Wheelhouse 2006).

The presence of seminal plasma in the mouse, pig and human female reproductive tract has been shown to induce local inflammatory changes that are believed to protect against infectious agents introduced during intercourse (Robertson & Seamark 1990, O'Leary *et al.* 2004, Sharkey *et al.* 2007). Thus, while sperm presents an immunologic challenge, seminal plasma appears to skew local inflammatory mechanisms *in utero* for the benefit of maternal reproductive health. Key factors induced by seminal plasma include colony-stimulating factor 2 (CSF2, granulocyte-macrophage-CSF) and IL-6, and this occurs in murine and porcine endometrial tissue

that would normally be exposed to semen following coitus (Robertson & Seamark 1990, O'Leary *et al.* 2004). Recent studies in the human in which semen is deposited in the cervix and vagina indicate that the cervix is highly responsive to seminal plasma in the induction of cytokines (Sharkey *et al.* 2007). It follows therefore that the establishment of pregnancy relies not only on the influence of ovarian steroid hormones on endometrium but also on seminal fluid-induced changes in specific regions of the female reproductive tract.

The process of implantation, particularly in species with invasive haemochorial placentation, involves increasing contact between semi-allogeneic trophoblast and maternal immune cells either in uterine tissue or in blood. This would seem an immunologically disastrous situation and yet a majority of mammalian pregnancies continue through to term. The mechanisms that underlie these unique properties of the reproductive tract have been the focus of numerous immune and endocrine studies over many decades, and during this period, the separation of these two disciplines has become increasingly difficult.

Immune surveillance and response to pathogens in the non-pregnant reproductive tract

In order to understand how uterine reproductive function is regulated by the sex steroids, it is critical to review the key cell populations of the uterus both prior to implantation and during the implantation process. The first uninterrupted barrier to infectious agents, sperm and trophoblast *in utero* is the uterine epithelium. Recent studies have shown that this cell population exhibits a number of immune properties placing this cell type in a central immune surveillance role (Wira *et al.* 2005). Beneath the epithelial layer lies the stroma, which contains a number of immune cells most of which show temporal and spatial changes across the cycle and into pregnancy: an indicator of sex steroid regulation. Proliferative phase endometrial stroma contains 10% leukocytes and this increases to 20% in the proliferative phase and 30% in early pregnancy decidua (Bulmer *et al.* 1991). The predominant immune cell type is the natural killer cell (NK), which makes up 70% of endometrial/decidual leukocytes. Macrophages comprise 20% of uterine leukocytes and the remaining cells consist of specialised $\gamma\delta$ T-cells, polymorphonuclear leukocytes and B-cells (Lea & Calder 1997, Yeaman *et al.* 1997, Critchley *et al.* 2001, Lea 2001).

Since the epithelial cells occupy the first barrier to the implanting blastocyst and/or infectious agents, they have evolved a number of specialised immunological functions. Epithelial cells throughout the female reproductive tract express receptors that recognise conserved pathogen-associated molecular patterns (PAMPs) present on micro-organisms. These TLRs function as an immune sensory mechanism and comprise a family of pattern

recognition receptors that are expressed throughout the reproductive tract (Wira *et al.* 2005).

Thirteen TLRs have been reported in mammals that between them detect a wide range of pathogenic stimuli with subsequent activation of intracellular pathways and expression of genes with immune function (Albiger *et al.* 2007). TLRs 1–9 have been identified in human uterine endothelial cells by RT-PCR and TLRs 1–6 by immunohistochemistry (Fazeli *et al.* 2005, Schaefer *et al.* 2005). Evidence is now emerging that these receptors are regulated by sex steroids since TLRs 2–6, 9 and 10 are significantly increased during the secretory phase of the cycle (Aflatoonian *et al.* 2007). Moreover, the expression of TLRs in murine vaginal epithelium is also modulated by the oestrous cycle as well as exposure to a pro-gestogen (Yao *et al.* 2007). TLRs are also expressed on uterine NK cells indicating that these cells may also respond against PAMPs, particularly at menstruation when the epithelial layer is broken and uNK cells are the predominant endometrial uterine leukocyte (Sentman *et al.* 2007).

Another key strategy of the reproductive tract to limit infection is through the production of natural antimicrobial peptides, which also modulate host innate immunity. These antimicrobials can be divided into two major families, namely the defensins and whey acidic protein (WAP) containing proteins (King *et al.* 2003a). Defensins have antibacterial, antiviral and antifungal activity and fall into two structurally different categories: α -defensins, found in neutrophils and epithelial sites, and β -defensins found mainly at epithelial surfaces (Huttner & Bevins 1999). The WAPs, which contain the WAP motif, comprise a family of factors including the protease inhibitors, secretory leukocyte peptidase inhibitor (SLPI) and peptidase inhibitor 3 (PI3, elafin; Bouchard *et al.* 2006).

Both defensins and WAPs are produced by uterine epithelial cells and uterine leukocyte populations, and expression varies across the menstrual cycle indicative of a degree of sex steroid regulation. In brief, the expression of human defensin, β 103A (DEFB103A: human β -defensin-3, HBD3) in human endometrium is highest during the secretory phase of the cycle, whereas HBD-4 is highest during the proliferative phase (King *et al.* 2003c). Moreover, in this study, the expression of both DEFB103A and HBD4 was altered by exposure to the combined oral contraceptive pill *in vitro* but not to progesterone alone. Thus, there is some degree of sex steroid regulation of these factors (King *et al.* 2003c). In addition, SLPI is produced by endometrial glandular epithelial cells at the period of uterine receptivity, and in the human, expression is up-regulated by progesterone (King *et al.* 2000, 2003b).

In terms of antimicrobial production by endometrial leukocytes, levels will largely mirror changes in cell numbers. Thus, neutrophils infiltrating into endometrium at the time of menstruation provide antimicrobial protection through the expression of α -defensins and

PI3. Other uterine immune cells are likely to express similar antimicrobial factors.

The endocrine control of intrauterine inflammatory activity

In the non-pregnant reproductive tract, the physiological role of progesterone is to prepare an oestrogen-primed endometrium for implantation. Consequently, there is much interest in the mechanisms by which progesterone promotes uterine receptivity. A key component of this effect is the control of uterine inflammatory activity by sex steroids. Oestrogen has pro-inflammatory effects in the uterus and has been linked to an influx of leukocytes at oestrous in the mouse (De & Wood 1990). In addition, increased levels of pro-inflammatory cytokines in the mouse uterus, particularly IL-1, occur immediately before implantation and have been associated with the pre-implantation surge of oestrogen (De *et al.* 1993). There is some evidence that this effect is cell type specific, since in the mouse oestradiol is reported to induce pro-inflammatory cytokine production from mast cells but not from macrophages (Hunt *et al.* 1997). In contrast, progesterone has been associated with anti-inflammatory activity. Indeed, ovariectomised mice treated with oestrogen exhibit an influx of leukocytes into the uterus that is blocked by co-administration of progesterone and this effect is ablated in progesterone receptor (PGR) knockout mice (Tibbetts *et al.* 1999). These findings illustrate that progesterone acting through its receptor is crucial for its anti-inflammatory role.

Another endocrine mediated anti-inflammatory mechanism is thought to be mediated through the NR3C1 (glucocorticoid receptor), which is expressed in the human endometrium across the menstrual cycle and in first trimester decidua (Henderson *et al.* 2003, McDonald *et al.* 2006). Since endometrial HSD11B1 mRNA levels increase at the time of menstruation, cortisol binding to the NR3C1 may induce a similar anti-inflammatory response to that described in the ovary, and this may be an important component of tissue remodelling (McDonald *et al.* 2006).

In the sheep and other species with less invasive placentas, the importance of controlled intrauterine immune regulation is less clear. Nevertheless, in the ovine uterus, low concentrations of progesterone can block tissue graft rejection (Majewski & Hansen 2002, Padua *et al.* 2005), and this has been associated with the induction of a uterine immunoregulatory protein belonging to the serpin family of proteinase inhibitors (see later). The accumulation of macrophages and $\gamma\delta$ T-cells in the ovine uterus during pregnancy also appears to be partly under sex steroid regulation (Tekin & Hansen 2004).

In the human, data on the role of progesterone in the regulation of uterine immune cells have been obtained from a series of studies looking at the effects of

progesterone withdrawal. Physiological progesterone withdrawal, occurring with the demise of the CL at the end of the menstrual cycle, triggers a series of immune-related events, which ultimately leads to menstrual bleeding in primates, or morphological changes consistent with the start of the next cycle in other species. When implantation occurs and progesterone levels are maintained, a different set of local immune events ensures that pregnancy continues.

Sex steroid regulation of chemokines in the uterus

Sex steroids influence the processes of menstruation and implantation largely through the regulation of inflammatory mediators. Coincident with the decline of circulating progesterone prior to menstruation is the up-regulation of specific chemokines, defined by their ability to recruit leukocytes to a specific site in a tissue (homing) and to activate them (Fig. 1). The binding of a chemokine to its receptor on a specific leukocyte

up-regulates the expression of adhesion molecules, thus promoting leukocyte adhesion to the endothelium and extravasation and chemotaxis along a concentration gradient (Le *et al.* 2004). There are over 50 chemokines subdivided into four major families based on the number and position of their cysteine residues (C, CC, CXC and CX3C), where C is the number of cysteine residues in the N-terminal and X is the number of intervening amino acids between the first two cysteines (Le *et al.* 2004).

A key concept relevant to chemokines in endometrium and other tissues is that specific leukocytes are recruited by the combined action of multiple chemokines (Le *et al.* 2004, Dimitriadis *et al.* 2005). In support of this, nine chemokines are abundantly expressed in the human endometrium along with a range of chemokine receptors. Moreover, there is a clear influence of the menstrual cycle on the variability of chemokine expression indicative of a direct and/or indirect regulatory effect of sex steroids (Jones *et al.* 2004). Jones and colleagues showed that several chemokines (CX3CL1, CCL7, CCL14 and CCL4)

Figure 1 Modulation of immune activity in the non-pregnant reproductive tract. Ovarian-derived oestradiol (pro-inflammatory) and progesterone (anti-inflammatory) have paracrine actions on the ovary and endocrine actions on the uterus. In the ovary, the sex steroids exhibit paracrine effects on cytokine and prostaglandin production from immune and follicular cells, and these factors are critical for follicle development, ovulation and CL function. Similarly, in the uterine endometrium, the sex steroids exhibit endocrine effects on cytokine and prostaglandin production by epithelial, stromal and resident immune cells. These factors play a regulatory/developmental role in cyclic changes to the endometrium, menstruation, implantation and placental development. Receptors associated with immune surveillance (e.g. toll receptors) are also expressed by ovulatory ovarian follicular cells and the uterine epithelium. Sex steroids influence the ability of epithelial cells to respond to microbes and likely have similar effects at the follicular level.

were up-regulated in midsecretory phase endometrium and maintained in early pregnancy. These factors were identified as chemoattractants for macrophages and NK cells, and thus equate with the accumulation of these cells at the implantation site (Jones *et al.* 2004). Uterine NK cells also express chemokine receptors and are able to respond to specific chemokines, e.g. CCL21. Premenstrually, a localised up-regulation of IL-8, CX3CL1 (fractalkine), CCL11 (eotaxin) and CCL22 (ADAM metallopeptidase domain 11) occurs in vascular endothelium, and this fits with previous reports of individual chemokines across the cycle, e.g. IL-8, CCL2 (monocyte chemoattractant protein-1, MCP-1) and CCL8 (MCP-2) and the recruitment of neutrophils and monocytes (Critchley *et al.* 2003). During the process of menstruation, activated immune cells release matrix metalloproteinases and their action on extracellular matrix is central to endometrial breakdown (Salamonsen & Woolley 1999). These data therefore indicate that the specific recruitment of immune cells for menstruation or implantation is controlled by the expression of chemokines and receptors. Evidence for the regulation of chemokines and their receptors by progesterone has recently been obtained by gene array of endometrial tissue exposed to the antiprogesterin RU486 (Catalano *et al.* 2007; Table 1).

Further evidence for a role of sex steroids on chemokines has been obtained in other species. For example, in the mouse, CSF3 (granulocyte-CSF, G-CSF) predominates in uterine fluid and serum at oestrous (Orsi *et al.* 2007). In the ewe uterus, early pregnancy is characterised by an influx of eosinophils into the uterus that express both CCL2 (MCP-1) and CCL8 (MCP-2) (Asselin *et al.* 2001). Although the exact role of progesterone in this process is uncertain, these preliminary data suggest that chemokine receptor networks exist across species and are likely involved in the recruitment of immune cells to the uterus.

More recently, chemokines have been implicated in the process of trophoblast adhesion and invasion. Indeed, during the implantation window, the chemokines CX3CL1 and CCL7 are expressed along with their receptors (CX3CR1, CCR1, CCR2, CCR3 and CCR5) in both endometrium and first trimester trophoblast populations (Hannan *et al.* 2006). During the peri-implantation period, the expression of CXCL9, CXCL10 and CXCL11 increases in the endometrium, whereas their common receptor (CXCR3) is expressed by the trophoblast (Imakawa *et al.* 2006). The selective expression of chemokines and chemokine receptors on trophoblast indicates that the chemokine receptor network under the influence of progesterone might be critical for the process of implantation. In addition, since the degree of trophoblast invasion and subsequent placental structure shows marked variation between species, there are likely to be species-specific networks of chemokines and chemokine receptors.

Prostaglandin signalling and sex steroids in the uterus

During the implantation window in the human, endometrial prostaglandin levels are elevated and this occurs concurrently with a rise in prostaglandin-endoperoxide synthase 2 (PTGS2: cyclooxygenase 2; Jones *et al.* 1997). This is thought to be an indirect response to the withdrawal of progesterone and oestradiol. Although this suggests a role of PTGS2 in implantation, studies in mice have led to inconsistent results. While one group reported that PTGS2 knockout mice exhibit poor decidualisation and implantation failure (Lim *et al.* 1999), another found no effect on implantation but did find that the rate decidualisation proceeds was delayed (Cheng & Stewart 2003). Although these findings indicate that the precise role of the PTGS2 enzymes and prostaglandins in human implantation requires further clarification, in the non-pregnant endometrium PTGS2 enzyme expression and prostaglandin E2 (PGE2) synthesis are associated with vascular function and levels are maximal premenstrually (Jones *et al.* 1997, Milne *et al.* 2001, Critchley *et al.* 2006). Indeed, elevated levels of endometrial PTGS1 and PTGS2 mRNA as well as prostaglandins have been associated with heavy menstrual bleeding (Smith *et al.* 1981, 2007). In addition, two PGE2 receptor isoforms have been detected in endometrial tissue (E type prostaglandin 2 (EP2) and EP4) and EP4 was expressed at higher levels during the late proliferative stage than in early, late and mid-secretory stage endometrium (Milne *et al.* 2001). Thus, prostaglandin signalling represents another sex steroid-regulated process important for normal endometrial function.

Interestingly, in domestic species, prostaglandin production by the endometrium is important for implantation and early pregnancy and interferon- τ has no direct effect on the expression of PTGS2, and thus prostaglandin production (Kim *et al.* 2003). Table 1 illustrates that PTGS2, necessary for prostaglandin synthesis, is down-regulated by progesterone in human endometrium but up-regulated in ovine endometrium (Charpigny *et al.* 1997, Critchley *et al.* 1999). In the human, prostaglandin production has been highlighted as integral to the inflammatory processes important for menstruation. Consequently, production is inhibited when progesterone is maintained during pregnancy. In the sheep, however, the mode of implantation is markedly different from the human, and this is thought to account for the up-regulation in PTGS2, i.e. prostaglandin may be involved in the establishment of the superficial endotheliochorial placenta (Charpigny *et al.* 1997).

Immunoendocrine signalling and the maternal recognition of pregnancy

The interface between endocrinology and immunology is also manifest in the maternal recognition of pregnancy. This area has been extensively reviewed elsewhere, and

Table 1 Table of putative progesterone or progesterone receptor-sensitive endometrial immunoregulatory genes.

Gene name ^a	Symbol	Species	Target cell/tissue	Study	Progesterone effect	Reference
Chemokines and receptors						
Chemokine (C–C motif) ligand 2	<i>CCL2</i>	Human	Endometrium	<i>In vitro</i>	–	Critchley <i>et al.</i> (1999)
		Sheep	Endometrial eosinophils	<i>In vivo</i>	+	Asselin <i>et al.</i> (2001)
Chemokine (C–C motif) ligand 3	<i>CCL3</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Chemokine (C–C motif) ligand 8	<i>CCL8</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Chemokine (C–C motif) ligand 13	<i>CCL13</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Chemokine (C–X3–C motif) ligand 1	<i>CX3CL1</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Chemokine (C–X–C motif) ligand 1	<i>CXCL1</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Chemokine (C–X–C motif) ligand 5	<i>CXCL5</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Chemokine (C–X–C motif) ligand 12	<i>CXCL12</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Chemokine (C–X–C motif) ligand 13	<i>CXCL13</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Chemokine (C–X–C motif) receptor 4	<i>Cxcr4</i>	Mouse	Uterus	<i>In vivo</i>	–	Jeong <i>et al.</i> (2005)
Chemokine orphan receptor 1	<i>CMKOR1</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Chemokine-like factor superfamily 6	<i>CKLFSF6</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Interleukin-8	<i>IL-8</i>	Human	Endometrium	<i>In vitro</i>	–	Critchley <i>et al.</i> (1999)
Small chemokine (C–C motif) ligand 11	<i>Ccl11</i>	Mouse	Uterus	<i>In vivo</i>	–	Cheon <i>et al.</i> (2002)
Cytokines, growth factors and receptors						
Colony-stimulating factor 1	<i>CSF1</i>	Human	T-cells from decidua and peripheral blood	<i>In vitro</i>	+	Piccinni (2006)
Interleukin-4	<i>IL-4</i>	Human	T-cells from decidua and peripheral blood	<i>In vitro</i>	+	Piccinni (2006)
Interleukin-5	<i>IL-5</i>	Human	T-cells from decidua and peripheral blood	<i>In vitro</i>	+	Piccinni (2006)
Interleukin-15	<i>IL-15</i>	Human	First trimester decidual cells	<i>In vitro</i>	+	Kitaya <i>et al.</i> (2000)
Interleukin-1 receptor, type 1	<i>IL1R1</i>	Human	Endometrial stromal cells	<i>In vitro</i>	+	Okada <i>et al.</i> (2003)
	<i>Il1r1</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002)
Interleukin-4 receptor, α	<i>Il4ra</i>	Mouse	Uterus	<i>In vivo</i>	–	Cheon <i>et al.</i> (2002)
Interleukin-13 receptor, $\alpha 2$	<i>IL13RA2</i>	Human	Endometrial stromal cells	<i>In vitro</i>	–	Okada <i>et al.</i> (2003)
			Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
	<i>Il13ra2</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002) and Jeong <i>et al.</i> (2005)
Leukaemia-inhibitory factor	<i>LIF</i>	Human	T-cells from decidua and peripheral blood	<i>In vitro</i>	+	Piccinni (2006)
Myeloid leukaemia factor 1	<i>MLF1</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Small inducible cytokine subfamily E, member 1 (endothelial monocyte activating)	<i>SCYE1</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Transforming growth factor, $\beta 1$	<i>TGFB1</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Tumour necrosis factor receptor superfamily, member 1A	<i>TNFRSF1A</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Tumour necrosis factor receptor superfamily, member 21	<i>TNFRSF21</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Immune cell markers						
CD1d1 antigen	<i>Cd1d1</i>	Mouse	Uterus	<i>In vivo</i>	–	Jeong <i>et al.</i> (2005)
CD24a antigen	<i>CD24a</i>	Mouse	Uterus	<i>In vivo</i>	–	Cheon <i>et al.</i> (2002) and Jeong <i>et al.</i> (2005)
CD69 antigen (p60, early T-cell activation antigen)	<i>CD69</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)

CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen associated)	<i>Cd74</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002)
Cytotoxic T-lymphocyte-associated protein 2 α	<i>Ctla2a</i>	Mouse	Uterus	<i>In vivo</i>	+	Jeong <i>et al.</i> (2005)
Histocompatibility 2, class II antigen A, β 1	<i>H2-Ab1</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002)
Lymphocyte antigen 6 complex, locus A	<i>Ly6a</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002)
Major histocompatibility complex, class II, DO β	<i>HLA-DOB</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Thymus cell antigen 1, θ	<i>Thy1</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002)
Immune/inflammation modulators						
Complement component 3	<i>C3</i>	Mouse	Uterus	<i>In vivo</i>	—	Cheon <i>et al.</i> (2002) and Jeong <i>et al.</i> (2005)
Complement component 1, q subcomponent, β -polypeptide	<i>C1qb</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002)
Galectin 15	<i>LGALS15; OV GAL11</i>	Sheep	Endometrial luminal and superficial glandular epithelium	<i>In vivo</i>	+	Gray <i>et al.</i> (2005) and Gray <i>et al.</i> (2006)
Histidine decarboxylase	<i>Hdc</i>	Mouse	Endometrium epithelial cells	<i>In vivo</i>	+	Paria <i>et al.</i> (1998) and Jeong <i>et al.</i> (2005)
Peptidoglycan recognition protein 1	<i>Pglyrp1</i>	Mouse	Uterus	<i>In vivo</i>	—	Cheon <i>et al.</i> (2002)
Progesterone-induced blocking factor 1 ^b	<i>C13orf24</i>	Mouse, human, sheep	Systemic γ/δ T-cells (mouse and human), urine (human), endometrial cells (sheep)	<i>In vivo in vitro</i>	+	Szekeres-Bartho <i>et al.</i> (1997a, 1999), Polgar <i>et al.</i> (2004), Lea <i>et al.</i> (2005) and Sandra <i>et al.</i> (2005)
Secreted phosphoprotein 1	<i>Spp1</i> <i>SPP1</i>	Mouse Sheep	Uterus Glandular epithelium	<i>In vivo</i> <i>In vivo in vitro</i>	— —	Cheon <i>et al.</i> (2002) Johnson <i>et al.</i> (2000)
Interferon-related proteins						
Interferon-induced transmembrane protein 3 (1–8U)	<i>IFITM3</i>	Sheep	Endometrium	<i>In vivo</i>	—	Gray <i>et al.</i> (2006)
Interferon, α -inducible protein 27	<i>IFI27</i>	Sheep	Endometrium	<i>In vivo</i>	—	Gray <i>et al.</i> (2006)
Interferon, α -inducible protein 6	<i>IFI6</i>	Sheep	Endometrium	<i>In vivo</i>	—	Gray <i>et al.</i> (2006)
Interferon-induced protein with tetratricopeptide repeats 1	<i>IFIT1</i>	Human	Endometrium	<i>In vivo</i>	—	Catalano <i>et al.</i> (2007)
Interferon-related developmental regulator 2	<i>IFRD2</i>	Sheep	Endometrium	<i>In vivo</i>	—	Gray <i>et al.</i> (2006)
Interferon-stimulated gene, 20 KD	<i>Isg20</i>	Human Mouse	Endometrium Uterus	<i>In vivo</i> <i>In vivo</i>	— +	Catalano <i>et al.</i> (2007) Jeong <i>et al.</i> (2005)
Intracellular signalling						
Immunoresponsive gene 1	<i>Irg1</i>	Mouse	Endometrium luminal epithelium	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002), Cheon <i>et al.</i> (2003), Chen <i>et al.</i> (2003) and Jeong <i>et al.</i> (2005)
Janus kinase 1	<i>JAK1</i>	Human	Stroma, endometrial luminal and glandular epithelium	<i>In vitro</i>	+	Catalano <i>et al.</i> (2003)
Lymphocyte cytosolic protein 2	<i>LCP2</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Nuclear factor, IL-3, regulated	<i>Nfil3</i>	Mouse	Uterus	<i>In vivo</i>	+	Jeong <i>et al.</i> (2005)
Pre-B-cell leukaemia transcription factor 3	<i>PBX3</i>	Human	Endometrium	<i>In vivo</i>	+	Catalano <i>et al.</i> (2007)
Signal transducer and activator of transcription 1, 91 kDa	<i>STAT1</i>	Human	Endometrium	<i>In vivo</i>	—	Catalano <i>et al.</i> (2007)
Signal transducer and activator of transcription 5A	<i>STAT5a</i>	Sheep	Endometrium	<i>In vivo</i>	+	Gray <i>et al.</i> (2006)
	<i>Stat5a</i>	Mouse	Uterus	<i>In vivo</i>	—	Cheon <i>et al.</i> (2002)

(continued)

Table 1 Continued.

Gene name ^a	Symbol	Species	Target cell/tissue	Study	Progesterone effect	Reference
Suppressor of cytokine signalling 6	<i>SOCS6</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Prostaglandins and lipid metabolism						
Arachidonate 15-lipoxygenase	<i>Alox15</i>	Mouse	Uterus	<i>In vivo</i>	+	Cheon <i>et al.</i> (2002)
Prostaglandin-endoperoxide synthase 2	<i>PTGS2</i>	Human	Endometrium	<i>In vitro</i>	–	Critchley <i>et al.</i> (1999)
	<i>PTGS2</i>	Sheep	Endometrium luminal and glandular epithelium	<i>In vivo</i>	+	Chapigny <i>et al.</i> (1997) and Gray <i>et al.</i> (2006)
Proteinase inhibitors						
Serine (or cysteine) proteinase inhibitor, clade A (α -1 antitrypsin, antitrypsin), member 1	<i>SERPINA1</i>	Human	Endometrium	<i>In vivo</i>	–	Catalano <i>et al.</i> (2007)
Secretory leukocyte peptidase inhibitor	<i>SLPI</i>	Human	Endometrial glandular epithelial cells	<i>In vitro</i>	–	King <i>et al.</i> (2000, 2003c)
Uterine milk protein precursor A (family of SERPine Proteinase INhibitors; SERPIN)	<i>UTMP</i>	Sheep	Uterine glandular epithelium	<i>In vivo</i>	+	Ing <i>et al.</i> (1989) and Hansen (2007)

^aThe names of the genes are presented according to the HUGO nomenclature (<http://www.gene.ucl.ac.uk/nomenclature/>). The term 'uterus' has been used when the whole tissue (myometrium and endometrium) has been subjected to gene analysis. Papers referring to microarray analyses are shown in roman. When the gene expression and regulation have been corroborated using a single gene approach, references are shown in bold. ^bProgesterone-induced blocking factor 1 (C13orf24) has been placed as an 'immune/inflammation modulator', since the structure of the protein is not classically recognised as a growth factor or a cytokine.

therefore, only the key features will be described here (Moffett & Loke 2006, Spencer *et al.* 2007). In the human and many primates, blastocyst-derived chorionic gonadotrophin binds to receptors in the CL, thus maintaining the secretion of progesterone, and therefore its continued impact on endometrial/decidual and trophoblast function. In cattle and sheep, however, the signalling process is initiated by the release of large quantities of interferon- τ by the elongating conceptus (Spencer & Bazer 2004). The antiluteolytic activity of interferon- τ is manifest through the inhibition of oestrogen receptor (ESR) gene expression in the uterine epithelium. This, in turn, prevents the induction of oxytocin receptor expression (OXTR). Since the binding of oxytocin (OXT) to its receptor induces the luteolytic pulses of PGF2a, these pulses no longer occur and luteolysis is inhibited (Spencer *et al.* 2007). In these species, we therefore have a situation where ovarian endocrine function is regulated by a maternal-derived prostaglandin, the production of which is indirectly regulated by a conceptus-derived cytokine (Martal *et al.* 1998). Progesterone also affects the expression of interferon-related genes other than interferon- τ ; however, the biological importance of these changes are uncertain (Jeong *et al.* 2005, Gray *et al.* 2006, Catalano *et al.* 2007; Table 1).

Another interesting variation is the pig, where the secretion of oestrogen from the conceptus appears to be the initiating signal with interferon- γ release occurring a bit later (La Bonnardiére 1993). Comparison of the various mechanisms that underlie the maternal recognition of pregnancy across species provides a number of examples of both direct and indirect interactions between the endocrine and immune systems (Lee & DeMayo 2004).

Sex steroid receptors in the non-pregnant reproductive tract

Sex steroids induce their effects by acting through nuclear receptors, the expression of which varies across the menstrual cycle. Given the complexity of the endometrium and decidua in terms of the variety of immune and non-haematopoietic cells present, it is important to establish which cell types express the steroid receptors and the downstream consequences of ligand binding or blocking with antigestogens.

Oestrogen and PGRs belong to the nuclear receptor superfamily of the ligand-inducible transcription factors. The binding of the ligand–receptor complex to the hormone response element in the DNA then triggers the transcription of oncogenes and genes encoding growth factors and cytokines. Two subtypes of ESR, classical ESR1 (ER α) and novel ESR2 (ER β), have been identified in the mouse, rat and human (Okada *et al.* 2005, Saunders 2005). In the uterus, the uterotrophic effects of oestradiol operate through the ESR1 receptor, which has a higher

affinity for oestradiol than ESR2. Both ESR1 and ESR2 are expressed in the luminal epithelium and stroma of the immature uterus and, *in utero*, ESR2 appears to be a negative regulator of ESR1-mediated gene transcription (Weihua *et al.* 2000). This suggests that the ratio of ESR1 to ESR2 has important functional implications. Oestrogen, acting through the ESR1 receptor, differentially regulates the expression of the PGR (Weihua *et al.* 2000).

There are two isoforms of the PGR (PGRA and PGRB) both of which are expressed in the reproductive tract in different ratios, an observation that is believed to relate to progesterone action on specific tissues (Wang *et al.* 1998). In the human, PGRA/PGRB show cyclical variation in the glandular epithelium with a decline from the proliferative to the secretory phase (Mylonas *et al.* 2007). In this study, PGRB exhibited some variation in the stroma, as did both ESRs; however, the cell types were not identified.

A relatively recent and controversial development relevant to the progesterone regulation of reproductive function has been the discovery of membrane progesterone receptors (Losel & Wehling 2003). These receptors are thought to account for the non-genomic actions of progesterone, i.e. those that do not influence gene expression but drive more rapid effects such as activation of signalling cascades and inhibition of transmembrane Ca^{2+} entry. In vertebrate species, three membrane progesterone receptors have been described (mPGR α , β and γ) with mPGR α predominant in reproductive tissues (Zhu *et al.* 2003). Endometrial mPGR α is up-regulated during the luteal phase and down-regulated in the myometrium with progesterone withdrawal (Fernandes *et al.* 2005). Since mPGRs are present in ovary as well as the uterus, they may also influence local immune mediators at this site. In support of this possibility, membrane progesterone receptors have been implicated in T-cell immunosuppression during pregnancy (Ehring *et al.* 1998).

In terms of stromal immune cell populations, ESR2 has been co-localised to CD68-positive macrophages, and isolated endometrial macrophages have been shown to express both oestrogen and progesterone receptors (Khan *et al.* 2005). The combined effect of oestrogen and progesterone therefore likely explains the increase in macrophage numbers during the secretory phase peaking prior to menstruation (Milne *et al.* 2005). Moreover, the mechanism underlying this effect likely involves the chemokine–chemokine receptor network described earlier.

The role of uterine NK cells

Since their characterisation in 1989, uterine NK cells have been a major focus of attention in reproductive immunology (King *et al.* 1989). They predominate in late secretory phase endometrium and early pregnancy decidua and are phenotypically distinct from circulating NK cells. For example, they have a high level of CD56 (CD56 bright), lack the CD16 cell surface marker (low

affinity receptor for IgG complexes) and a range of other cell surface markers including CD57. They also express a more restricted array of chemokine receptors than circulating NK cells (Dosiou & Giudice 2005, Wira *et al.* 2005). Changes in uNK cell numbers occur across the menstrual cycle, and during the mid to late secretory phase, the CD56+, CD16– cells are localised to leukocyte aggregates close to endometrial glands and spiral arteries. In the absence of an implanting blastocyst, there is evidence for their demise through apoptosis and when implantation occurs the resident CD56+ cells continue to proliferate as the decidual reaction continues (Trundley & Moffett 2004).

Since 10% of circulating NK cells are CD56 bright, it is thought that the uNK cells may be derived from this population. However, there are still significant differences between these two populations in terms of phenotype and the array of genes expressed (Moffett-King 2002, Koopman *et al.* 2003). Although the uterine NK cells are thought to originate from circulating NK cells, the variation in numbers has been attributed to proliferation *in utero* (King & Loke 1991, Moffett-King 2002). In terms of function, uterine NK cells have been associated with menstruation, decidualisation and implantation. With respect to the latter, uNK cells are thought to be vital in regulating the maternal immune response to the semi-allogeneic trophoblast. Indeed, a majority of uNK cells express one or more inhibitory NK cell receptors (killer inhibitory receptors) such as KLRD1/KLRC1 (killer cell lectin-like receptor subfamily D, member 1/killer cell lectin-like receptor subfamily C, member 1; Ponte *et al.* 1999). These receptors are thought to interact with the unique repertoire of non-classical major histocompatibility complex antigens expressed by invading trophoblast (HLA-G, HLA-E and HLA-F; Moffett & Loke 2006). Since aggregates of decidual uNK cells have been localised close to spiral arterioles, the cells have also been implicated in vascular remodelling. In addition, uNK cells can make a variety of cytokines implicated in the regulation of trophoblast invasion and the regulation of endometrial/decidual angiogenesis (Jokhi *et al.* 1997, Moffett & Loke 2006).

Recent studies have shown that uNK cells express ESR2 but not ESR1 or the progesterone receptors (Henderson *et al.* 2003). This suggests that there may be some degree of oestrogen regulation. In the absence of progesterone receptors, progesterone may indirectly affect NK cell activity through the induction of cytokines from neighbouring cells. Uterine NK cells also express the NR3C1, which is co-localised with HSD11B1 (McDonald *et al.* 2006). This suggests that cortisol may have an anti-inflammatory effect on this cell population. Another possibility is that progesterone may act through NR3C1 (glucocorticoid receptor) or may affect NK cells via a non-receptor mechanism (Miyaura & Iwata 2002, Dosiou & Giudice 2005).

Progesterone as an immunomodulator

Progesterone has been associated with immunomodulatory function for many years and has been described as nature's immunosuppressant (Siiteri *et al.* 1977). Studies in sheep and hamsters illustrate that progesterone at concentrations found at the maternal–foetal interface can inhibit tissue graft rejection *in utero* (Moriyama & Sugawa 1972, Majewski & Hansen 2002). In terms of local effects of progesterone on endometrial/decidual uNK cells, the majority of studies suggest that the immunomodulatory activity may be indirect.

Sex steroids may induce NK cells to produce immunomodulatory proteins or may alter the cytokine environment, which then alters uNK function. This may occur through the action of progesterone on T-cell differentiation into Th1 and Th2 cells. Cytokines produced by Th1 cells are pro-inflammatory (e.g. interferon (IFN)- γ , IL-2), whereas Th2 cell-derived cytokines tend to be anti-inflammatory and associated with humoral immunity (e.g. IL-4, IL-5, IL-6, IL-10). The two T-cell subtypes are mutually inhibitory (Lea 2001). Successful pregnancy has been associated with the predominance of Th2-like cytokines at the maternal–foetal interface and a down-regulation of Th1 cytokines (Lin *et al.* 1993). Although this is now generally considered as an over-simplification, the paradigm continues to prove useful in terms of steroid regulation of cytokine production (Chaouat *et al.* 2004). Since progesterone can induce T-cell differentiation towards the Th2 pathway, it has been associated with the induction of such a cytokine profile *in utero* (Piccinni 2006). However, since non-haematopoietic cells such as trophoblast and epithelial cells can also produce cytokines, it is unclear how such a Th2 bias may be induced (Lea 2001, Hauguel-de Mouzon & Guerre-Millo 2006). Progesterone may act on progesterone receptor-positive cells such as macrophages and glandular epithelial cells or may affect other cell types via the routes discussed above.

Putative progesterone target genes with immune function: an overview of transcriptomic and candidate gene studies

Historically, genes sensitive to endocrine disturbance have largely been identified by candidate gene studies. In more recent years, a complementary approach has been the use of transcriptomics where large numbers of genes are screened simultaneously using broad range or targeted gene arrays. In terms of the sex steroids, both empirical approaches have proved useful in determining the impact of tissue exposure or withdrawal. The use of gene array with no corroborative experiments to investigate gene changes is currently the subject of much debate, and indeed, reported gene changes using transcriptomics alone must be treated with an element of caution (Rockett & Hellmann 2004). Both technical and computational concerns have been raised and many

journals are reviewing policy on including experiments to corroborate changes observed by gene array (Rockett & Hellmann 2004). For this reason, some of the genes listed in Table 1 are 'putative' rather than 'definitive' in terms of their sensitivity to progesterone and some of these genes and/or groups are discussed below.

Table 1 summarises progesterone-sensitive genes associated with immunity, and genes have been divided into groups based on immune function or presence of immune cell surface markers. Overall, Table 1 clearly shows the considerable impact that progesterone has on genes associated with chemokines and their receptors, interferons, cytokines and growth factors, prostaglandins, lipoxins, intracellular signalling molecules, immune modulators, specific immune cell populations and proteinase inhibitors. The chemokines and prostaglandins have already been discussed, and thus the following overview is focussed on some of the other genes in Table 1, where the weight of evidence supports progesterone regulation.

Cytokines and growth factors

Cytokines, growth factors and their receptors represent a broad group of factors with a range of functions. Since progesterone withdrawal or persistence influences inflammatory processes associated with menstruation and implantation, the genes identified through a screening approach will depend on the type and stage of uterine tissues examined. Seven of the fifteen cytokine/growth factor genes listed have been identified by candidate gene studies. CSF1 and leukaemia inhibitory factor (LIF) have both been associated with implantation success and have been the focus of many reviews (Stewart *et al.* 1992, Pollard 1997). In brief, CSF1 (a haematopoietic cytokine) and its receptor (CSF1R) have been localised to the reproductive tract in the mouse, human, pig and cow. Indeed, the progesterone induced expression of CSF1 by uterine epithelial cells and the subsequent regulation of CSF1R-positive endometrial macrophages has been highlighted as one key endocrine–immune axis associated with implantation success (Pollard 1997, Lee *et al.* 2003). Another key progesterone-sensitive cytokine important for implantation is LIF. Studies carried out on isolated human endometrial T-cells indicate that LIF is positively regulated by progesterone (Piccinni *et al.* 2001). In the mouse, however, uterine LIF appears to be primarily regulated by oestrogen (Bhatt *et al.* 1991, Chen *et al.* 2000, Kimber 2005). Although progesterone had no effect in this study, the use of whole uterine mRNA may not have allowed for effects on individual immune cell populations.

Decidua and peripheral blood T-cells exposed to progesterone secrete IL-4 and IL-5 (Th2 cytokines), and this supports the paradigm that progesterone induces a local Th2 bias *in utero* (Piccinni 2006). In two separate

gene array studies, IL-13 receptor $\alpha 2$ has been reported to be down-regulated by progesterone in human endometrium (Catalano *et al.* 2003, Okada *et al.* 2003). Since IL-13 is expressed by human endometrium and since IL-13 receptor $\alpha 2$ (IL13Ra2) has been implicated as a negative regulator of IL-13, progesterone inhibition of the receptor may allow endometrial IL-13 to promote anti-inflammatory activity important for endometrial function and/or implantation (Chegini *et al.* 2002, Chiamonte *et al.* 2003). Interestingly, two separate array studies in the mouse have shown the opposite effect, i.e. progesterone up-regulates IL13ra2 (Cheon *et al.* 2002, Jeong *et al.* 2005). The reason for this between-species difference is uncertain but does illustrate the complex nature of intrauterine cytokine profiles and the need for further corroborative studies. The cytokine IL-15 is also positively regulated by progesterone and is thought to be important in the stimulation of uNK cells in late secretory endometrium and first trimester decidua (Kitaya *et al.* 2000). Although the other genes in the cytokines/growth factor category of Table 1 relate to individual array studies, some of the changes reported further support the Th2/anti-inflammatory paradigm associated with progesterone action, e.g. down-regulation of TNF receptors and up-regulation of TGF β 1 (Catalano *et al.* 2007).

Immune responsive gene 1

Under the category of 'intracellular signalling factors' in Table 1, immune responsive gene 1 was down-regulated by array analysis and subsequent studies have shown it to be expressed in mouse luminal epithelium during the implantation window (Chen *et al.* 2003, Cheon *et al.* 2003). In the mouse, blocking Irg1 with antisense oligonucleotides is reported to reduce the number of implanting embryos by 80% (Cheon *et al.* 2003). Since inflammatory activity is important during the implantation window, it is possible that this may be an example of progesterone acting in a pro-inflammatory manner but to the benefit of the implanting embryo. Cheon and colleagues also point out that Irg1 has a glycosaminoglycan attachment site important for proteoglycan binding, and that Irg1 may be sequestered to cell surface proteoglycans to be later released as an active factor following proteolysis.

Immunomodulatory factors

In terms of progesterone-sensitive factors implicated as key immunomodulators during pregnancy (Table 1), LGALS15 (galectin 15), SPP1 (secreted phosphoprotein 1, osteopontin), uterine milk protein precursor A (UTMP, uterine serpin), histidine decarboxylase and C13orf24 (progesterone-induced blocking factor, PIBF) have been

highlighted by a range of experimental approaches. The following is a brief discussion of each of these factors:

LGALS15 (galectin 15)

LGALS15 has been identified as a key ovine uterine factor, produced by the endometrial luminal epithelium and ductal glandular epithelium of the uterus (Gray *et al.* 2005). LGALS15 is thought to be important in the regulation of implantation and placentation. Indeed, in sheep in which uterine gland development is inhibited by neonatal progestin exposure (the uterine gland knockout phenotype), the failure of sheep blastocysts to elongate or survive past 12 to 14 days has been associated with a reduction in LGALS15 (Gray *et al.* 2000, 2001, 2006). In terms of immune function, other galectin family members are known to modulate innate and adaptive immune responses as well as immune cell activation and differentiation (Rabinovich *et al.* 2002, Young & Meeusen 2004). Thus, it is possible that LGALS15 may have an immunomodulatory role *in utero*.

SPP1 (secreted phosphoprotein 1, osteopontin)

SPP1 is a complex multifunctional glycoprotein found in uterine histotroph that is classified both as a cytokine and as a component of extracellular matrix (Johnson *et al.* 2003). During implantation and early pregnancy, SPP1 is produced by uterine glandular epithelial cells and immune cells present in the endometrium and placenta. A number of studies have demonstrated that epithelial cell-derived SPP1 is progesterone dependent (Johnson *et al.* 2003). In contrast, immune cell-derived SPP1 does not appear to be progesterone regulated (Johnson *et al.* 2000). Nevertheless, since SPP1 from either source can influence the activity of many other immune cells, including those present at the maternal-foetal interface, it represents a key link between progesterone and the activity of intrauterine immune cells (Johnson *et al.* 2003).

UTMP, uterine serpin

UTMP is both a proteinase inhibitor and a progesterone-sensitive immunomodulatory factor (Table 1). UTMP is produced from uterine glandular epithelial cells and is a member of the serpin superfamily of proteinase inhibitors that exhibit weak antiproteinase activity but inhibit a wide range of lymphocyte functions (Ing *et al.* 1989, Peltier & Hansen 2001). Consequently, UTMP is thought to be important in the inhibition of maternal immune responses directed against antigens expressed on the foeto-placental unit (Hansen 2007). Although UTMP has also been detected in cattle, pigs and goats, it has been most characterised in the sheep and shown to exhibit anti-proliferative activity on the pre-implantation embryo and on tumour cell lines (Tekin *et al.* 2005). This implies that UTMP may not only be immunoregulatory but may also regulate cell proliferation possibly including trophoblast.

Histidine decarboxylase

Histidine decarboxylase is a progesterone-sensitive pro-inflammatory mediator identified by the candidate gene approach. However, in the uterus, this factor is produced by the uterine epithelium rather than resident mast cells, and thus histamine may have non-immunological activities at the time of implantation (Paria *et al.* 1998, Wood *et al.* 2000).

Progesterone-induced blocking factor (C13orf24)

During pregnancy in humans, circulating $\gamma\delta$ T-lymphocytes express progesterone receptors (Szekeres-Bartho *et al.* 1999, Barakonyi *et al.* 2002). On binding to the lymphoid receptors on the immune cells, progesterone induces the secretion of a highly immunosuppressive 34 kDa molecule designated as C13orf24 (PIBF; Szekeres-Bartho *et al.* 1989). Clinical interest in C13orf24 during human pregnancy arose from studies correlating urinary C13orf24 with pregnancy outcome. Pre-eclamptic women with at least two symptoms (hypertension and toxemia) were reported to have urinary C13orf24 levels lower than those found in normal healthy pregnancies (Polgar *et al.* 2004). In addition, low C13orf24 levels were associated with pre-term delivery and intrauterine growth restriction (Polgar *et al.* 2004).

The biological significance of C13orf24 secretion during pregnancy was originally established from studies in mice. In this species, the neutralisation of endogenous C13orf24 using a PIBF-specific antibody results in a 70% reduction in the number of viable fetuses per mouse (Szekeres-Bartho *et al.* 1997a). Furthermore, the treatment of pregnant mice with the progesterone receptor antagonist RU486 leads to an increase in resorption rate and this is associated with the abrogation of C13orf24 production by spleen cells. Since the increase in resorption rate can be reversed by simultaneous administration of C13orf24, this unique factor appears to have an important anti-abortive function in pregnant mice (Szekeres-Bartho *et al.* 1990). The anti-abortive mechanism of C13orf24 is thought to be primarily mediated through its inhibitory effects on NK activity. In support of this, high resorption rates in mice due to elevated NK activity can be corrected by administration of C13orf24 (Szekeres-Bartho *et al.* 1990). Conversely, increased resorption induced by anti-PIBF is associated with increased splenic NK activity and the former is reversible with anti-NK antibodies (Szekeres-Bartho *et al.* 1997b).

In the human, C13orf24 expression by peripheral pregnancy lymphocytes is inversely related to NK activity (Szekeres-Bartho *et al.* 1995, 1996). The inhibitory effects of C13orf24 on NK cells are thought to be mediated, in part, by altered arachidonic acid release and subsequent downstream effects on prostaglandin and leukotriene production and hence pro-inflammatory activity (Szekeres-Bartho *et al.* 2005).

C13orf24 also skews cytokine production towards Th2-like anti-inflammatory activity and, consequently, it may mediate the effects of progesterone on cytokine profiles. For example, the pro-NK cytokine IL-12 is reduced and this is thought to favour low NK activity conducive to pregnancy success (Szekeres-Bartho *et al.* 2005). Indeed, in mice, *in vivo* and *in vitro* studies have shown that PIBF favours the secretion of IL-3, IL-4 and IL-10, while suppressing pro-inflammatory cytokines such as IL-12 and IFN- γ (Szekeres-Bartho & Wegmann 1996, Szekeres-Bartho *et al.* 1996). C13orf24 is also reported to affect humoral immunity by increasing the production of asymmetric antibodies thought to have a blocking function (Kelemen *et al.* 1996).

In the sheep reproductive tract, we have identified an orthologue of C13orf24 and localised C13orf24 mRNA to endometrium and protein to placental trophoblast (Lea *et al.* 2005). Interestingly, monitoring C13orf24 RNA levels (exons 8–14) during the ovine oestrous cycle and early pregnancy revealed relatively steady-state levels that did not correlate with physiological changes in progesterone (Lea *et al.* 2005, Sandra *et al.* 2005). Although it is recognised that there may be some species differences in C13orf24 expression and function, these recent data suggest that the regulation of C13orf24 expression may only be partially progesterone dependent even during a mammalian pregnancy.

Conclusion

Cyclical changes in the sex steroids influence a variety of genes in the ovary and uterus, which act to protect these tissues against pathogens, while simultaneously preparing them for ovulation, menstruation or implantation. During the period of increased uterine receptivity, epithelial cells exhibit increased toll receptor expression and altered production of specific antimicrobial peptides (e.g. SLPI), thus enhancing the ability to both detect and respond against PAMPs on micro-organisms. During menstruation, toll receptor expression by uterine NK cells is thought to ensure effective immune surveillance when the epithelial layer is broken. Ovulation, menstruation and implantation are largely characterised by changes in the type and distribution of both immune and non-immune cells in the ovary and/or uterus. With respect to immune cells, the sex steroids regulate the expression of a wide range of chemokines and their receptors that act collectively in the selective recruit of leukocytes. This mechanism likely accounts for the increased numbers of NK cells and macrophages at the implantation site in human secretory phase endometrium. The expression of chemokine receptors on trophoblast suggests that the chemokine–chemokine receptor network may also regulate implantation. ESR2 expression by NK cells and macrophages indicates that both cell types are responsive to oestrogen. In addition, progesterone acting on the PGRA/PGRB in glandular

epithelium provides a means for progesterone to influence NK cells and macrophages indirectly. This may occur through the progesterone-induced expression of a range of endometrial and epithelial cytokines and growth factors. For example, progesterone-induced CSF1 is likely to target CSF1R-positive endometrial macrophages. Other progesterone-induced cytokines may contribute to the anti-inflammatory (Th2-like) environment that characterises early pregnancy. In the sheep, LGALS15, SPP1 and UTMP have been identified as key intrauterine immunomodulatory factors. In both mice and humans, C13orf24 (PIBF) is produced systemically by PGR-positive γ/δ T-cells and affects different aspects of immune function including reduced NK activity and the preferential production of anti-inflammatory Th2-like cytokines. Although the sex steroids drive the inflammatory processes associated with ovulation and menstruation, the glucocorticoids appear to play a role in the rapid resolution of inflammation, thus allowing tissue remodelling to occur. This involves the expression of ovarian and endometrial HSD11B1, which converts cortisone to cortisol. The co-expression of HSD11B1 with NR3C1 (glucocorticoid receptor) in the ovary and uterus ensures that cortisol binds to the receptor, thus inducing anti-inflammatory activity. Key areas for future investigation should include further work on the regulation and function of recently identified progesterone target genes and the manipulation of the sex steroid/glucocorticoid-regulated immunomodulatory pathways described for improved reproductive management in humans and animals.

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