Ovarian steroid hormones: effects on immune responses and *Chlamydia trachomatis* infections of the female genital tract

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Female sex hormones are known to regulate the adaptive and innate immune functions of the female reproductive tract. This review aims to update our current knowledge of the effects of the sex hormones estradiol and progesterone in the female reproductive tract on innate immunity, antigen presentation, specific immune responses, antibody secretion, genital tract infections caused by *Chlamydia trachomatis*, and vaccine-induced immunity.

INTRODUCTION

A critical function of the unique mucosal immune system of the female genital tract (FGT) is to identify and eliminate potentially pathogenic viral and bacterial agents and to provide protection against sexually transmitted diseases. Globally, it has been estimated that in adults between 15 and 49 years of age there were 105.7 million cases of new *Chlamydia trachomatis* sexually transmitted infections (STIs) in 2008.¹ Significant disease sequelae following chlamydial infections of the FGT include pelvic inflammatory disease, tubal infertility, and ectopic pregnancy.^{2–4}

The FGT comprises several immune compartments found in the upper genital tract (UGT; endocervix, ovaries, fallopian tubes, and uterus) and lower genital tract (LGT) (ectocervix and vagina). The UGT with Type I mucosa⁵ is lined with a single layer of columnar epithelium and the LGT with Type II mucosa is lined with stratified squamous epithelium (reviewed in Pudney *et al.*⁶). The transitional zone where squamous epithelium changes to columnar epithelium is the most immunologically active site of the FGT. Although currently documented (relative) microbial sterility is found in the uterus and fallopian tubes, commensal vaginal flora such as *Lactobacillus crispatus* are found in the LGT and are under hormonal influences at this site.⁷

The human FGT contains components of the innate and adaptive mucosal immune responses that are found in various distributions at different sites throughout the tract. **Figure 1**

depicts the FGT anatomy and the location and relative abundances of innate immune cells at this mucosal site.^{6,8–15}

In the FGT, the major lymphocyte components are natural killer (NK) cells and T lymphocytes, including cluster of differentiation (CD) 3 + T lymphocytes that are present in all the tissues of the tract. In the LGT, the CD8 + and CD4 + are dispersed throughout the stroma while lymphoid aggregates of these cells are formed in the uterus.¹⁶ Granulocytes are present and these are principally located in the fallopian tubes. Finally, relative to T lymphocytes, smaller numbers of monocytes and B lymphocytes are found throughout all tissues of the FGT.¹⁷

Immune cells at this site express both surface and cytosolic pattern recognition receptors (PRRs), including among others Toll-like receptors (TLRs) and the nucleotide-binding oligomerization domain (NOD)-like receptors. TLRs 2 and 4 are highly expressed in the UGT (fallopian tubes and cervix) when compared with expression levels in the LGT (endometrium and ectocervix) while TLRs 7, 8 and 9 and NOD-1 (which is involved in sensing *Chlamydia* infection) and NOD-2 are expressed at similar levels throughout the tract.^{18–20}

In addition to providing a physical barrier for protection, the genital tract epithelial cells (ECs) lining the FGT are immunologically active cells. For example, they act as key initiators of innate and adaptive immunity by secreting cytokines and chemokines, including interleukin (IL)-1b, tumor necrosis factor α (TNF- α), IL-6, IL-8, and GM-CSF

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Figure 1 The female genital tract: anatomy and innate immune cells of the genital mucosa. This figure depicts non-immune and innate immune cells found throughout the female genital tract, including macrophages, dendritic cells, neutrophils and natural killer (NK) cells. Immune cell receptors are shown on immune cells. Note that epithelial cell receptors CD4, C-C motif chemokine receptor 5 (CCR5), C-X-C motif chemokine receptor 4 (CXCR4) and GaIC on endometrial and endocervical cells are not shown in this figure. TLR, Toll-like receptor; NKG2D, an activating receptor expressed on NK cells that binds to MHC class 1 chain-related protein A and B (MICA and MICB).

(granulocyte-macrophages colony-stimulating factor), in response to human chlamydial infections.²¹

Host protection of the FGT is afforded by two arms of defense comprising innate and acquired immunity. The cellular components of specific immune responses at this site are T lymphocytes expressing $\alpha\beta$ - and $\gamma\delta$ -T-cell receptors (TCR) and immunoglobulins (Igs) producing B lymphocytes.²² Effectors of non-specific responses include ECs, monocytes, macrophages, granulocytes (neutrophils, eosinophils, and basophils), dendritic cells (DCs), and NK cells that confer protection due to chemokines, cytokines, and phagocytosis. Cells of both the innate and adaptive immune systems are found in fallopian tubes, uterus, cervix, and vagina.²³ Localization of T-lymphocyte populations in the female UGT and LGT over the menstrual cycle is summarized in **Table 1**.^{10–15,24–40}

Innate host defense, particularly in the LGT, is provided by the vaginal microbiota maintained in a healthy equilibrium (reviewed in Farage *et al.*⁴¹) as well as by the myriad of antimicrobial peptides and protease inhibitors of cervicovaginal fluid (Cole⁴² and reviewed in Farage *et al.*⁴¹ and Wira *et al.*⁴³). The antimicrobial peptides and PRR TLRs are key intermediaries of innate immunity in the FGT.^{43–46}

The recruitment and function of immune components of the FGT are precisely regulated by hormonal changes during the menstrual cycle by the sex steroids that co-ordinate cell trafficking and immune activation at this mucosal site. Female sex hormones estrogens, progestins, and androgens are produced by ovarian cells with levels of estradiol (E2 or 17 β -estradiol) and progesterone or P4 (pregn-4-ene-3, 20-dione) varying in accordance with fluctuations in the menstrual cycle.²² In the menstrual/ovarian cycle of humans, the endometrium develops and follicles grow until ovulation in the Proliferative/Follicular phase. The Secretory/Luteal phase is characterized by high levels of progesterone from the corpus luteum to maintain the endometrium. Finally, corpus luteum regression and menstruation occurs during the menstrual phase. At menstruation, serum E2 levels measure typically $<50 \text{ pg ml}^{-1}$. Gradually, increasing amounts of E2 are found in the follicular (proliferative) phase of the adult female menstrual cycle with serum levels at a pre-ovulatory stage (day 14) ranging from $110\text{--}410 \text{ pg ml}^{-1}$ and dropping briefly at ovulation. Levels of E2 ($20\text{--}160 \text{ pg mol}^{-1}$) and P4 ($>5 \text{ ng ml}^{-1}$) are present during the luteal (secretory) phase from days 14–28 of the menstrual cycle typically peaking at day

Table 1 Localization of T lymphocytes in the female upper (UGT) and lower (LGT) genital tracts: effects of menstrual cycle stage and chlamydia infection

T lymphocytes	Upper genital tract (UGT)			Lower genital tract (LGT)			Menstrual cycle stage (ovarian phase)		References
	Ovaries	Fallopian tubes	Uterus/endometrium	Endocervix	Ectocervix	Vagina	Proliferative (follicular)	Secretory (luteal)	
Serum estradiol							220 pmol l ⁻¹	< 130 pmol l ⁻¹	
Progesterone							< 2 ng ml ⁻¹	> 5 ng ml ⁻¹	
<i>T helper cells</i>									
CD45 +, CD3 +, CD4 + Th1, Th2, Th3, Th17 or TFH		CD45 + cells CD3 + CD8 + > % than CD3 + CD4 +	CCR5 + CD4 + CD3 + CD45 +	CD3 +, CD4 +	CD3 + CD4 +	CD4 + (30–40% of vaginal T cells) CD3 +	CCR5 + (+) CD45 + leu- kocytes (+) (mid-luteal)	CCR5 + (+) CD45 + leu- kocytes (+) (mid-luteal)	Endocervix: CD4 + cells (+ + + +) Endometrium CCR5 and CD103 cells (+) Cervix CD3 + CD4 +
									Kaldensjö ²⁴ Hamad ²⁵ Shaw <i>et al.</i> ²⁶ Dissep <i>et al.</i> ²⁷ Ficarra ²⁸ Johansson <i>et al.</i> ²⁹ Kamat & Isaacson ³⁰ Starkey <i>et al.</i> ³¹ Vassiliadou & Bulmer ³² Agrawal <i>et al.</i> ³⁵
<i>Cytotoxic T cells</i>									
CD45 +, CD3 +, CD8 +	+	CD8 + CTL activity	CD3 + CTL CD8 + CTL activity	+	+	+	↑ (uterus)	↓ (uterus) ↓ CTL activity (no change in CD8 + cell number)	White <i>et al.</i> ³³ White <i>et al.</i> ³³ Li <i>et al.</i> ³⁴ Ficarra ²⁸ Johansson <i>et al.</i> ²⁹ Agrawal <i>et al.</i> ³⁵
		CD8 + CTL		CTL activity		CTL activity	Measurable	Measurable	White <i>et al.</i> ³⁶ Yeaman <i>et al.</i> ¹⁶
			CD8 + CD4 –						
<i>Treg cells</i>									
Natural CD4 +, CD25 + FoxP3 + Adaptive Tr1 and Th3		+					↑ Levels correlating with E2 (uterus) ↑ Levels (E2 at physiological doses)	↓ Levels significant after ovulation ↓ Levels (E2 at physiological doses)	Arrivito <i>et al.</i> ³⁷ Tai <i>et al.</i> ³⁸ Marks <i>et al.</i> ³⁹

Table 1 Continued

	Upper genital tract (UGT)			Lower genital tract (LGT)		Menstrual cycle stage (ovarian phase)		References
	Ovaries	Fallopian tubes	Uterus/endometrium	Endocervix	Vagina	Proliferative (follicular)	Secretory (luteal)	
T lymphocytes								
$\gamma\delta$ T cells								
Intraepithelial lymphocytes (IELs)			IELs stromal CCR5 + CD4 +					Kaldensjö <i>et al.</i> ²⁴
Memory T cells								Kelly <i>et al.</i> ⁴⁰
								Endocervix: $\alpha 4\beta 7$ + cutaneous lymphocyte antigen (CLA) +

CCR, C-C motif chemokine receptor; CD, cluster of differentiation; CTL, cytotoxic T lymphocyte; TFH, T follicular helper; Th, T helper. (+) symbol indicates the presence of T cells in the UGT and LGT over the menstrual cycle with increasing numbers of (+) symbols indicating increased cell numbers.

21 of the cycle. At the end of the secretory phase, E2 levels return to their menstrual levels.

The sex hormones E2 and P4 are key contributors to susceptibility and innate and acquired immune responses to bacterial and viral infections of the FGT⁴⁷ (reviewed in Bouman *et al.*,²² Rakasz *et al.*⁴⁸ and Butts *et al.*⁴⁹). It is also known that these hormones contribute to the sex-based differences both in innate and in acquired immunity and infectious diseases with estrogens affecting levels of chemokine receptors by T cells and the higher CD4 + T-cell populations in women undergoing significant changes during the ovarian cycle (reviewed in Fish⁵⁰). Differences in E2 levels between male and female mice have also been reported as impacting on the expression of inducible nitric oxide synthase (iNOS)⁵¹ and hence the production of nitric oxide (NO), a gas that modulates production of pro-inflammatory cytokines, such as IL-6 and TNF- α .⁵²

Innate and adaptive mucosal immunity in the FGT are regulated by the female sex hormones E2 and P4 and have been reviewed in Bouman *et al.*²² and Butts and Sternberg.⁵³ Hormones regulate the transport of Igs, the levels of cytokines, the expression of TLR genes,^{54,55} and the distribution of immune cells and antigen presentation in the genital tissues during the reproductive cycle^{23,44} **Figure 2** depicts the menstrual cycle phases, sex steroid hormone levels, and immune cells present in the FGT.

The afferent and efferent arms of uterine, cervical, and vaginal immune responses are separately regulated and the control by sex hormones is distinct and species-specific in vaginal immune compartments.⁴⁸ In humans, uterus/cervix cellularity and CTL activity is menstrual cycle-dependent with abundant IgA-secreting cells and secretory component expression in the endocervix. In the vaginal immune compartment, cellularity and CTL activity is not menstrual cycle-dependent, CD8 + T cells dominate, (and these are vital in a response to chlamydial infection),⁴⁸ there is only a minor population of IgA plasma cells and low levels of secretory component expression, and IgG dominates with peri-ovulatory antibody peaks.⁴⁸

This review summarizes our current knowledge of the effects of sex hormones E2 and P4 in the FGT on innate and adaptive immunity, antibody secretion, genital tract infections caused by *C. trachomatis*, and on vaccine-induced immunity.

HORMONAL EFFECTS ON INNATE IMMUNITY

There are many components of innate immunity operational in the vagina and these have recently been reviewed^{45,56} as have components of innate immunity in the ovary and endometrium.⁵⁷ These include soluble factors (such as mannose-binding lectin, complement components, defensins, secretory leukocyte protease inhibitor (SLPI), and nitric oxide), membrane-associated components such as Toll-like-dependent and -independent receptors, as well as phagocytic cells. The innate immune system of the FGT is also comprised of many cells, including macrophages, DCs, neutrophils, NK cells, and ECs. It is now becoming increasingly apparent that all aspects of innate immunity, including secretion of protective

Figure 2 Menstrual cycle phases, sex steroid levels and effects on innate immune cells in the female genital tract. This figure depicts the immune cell types and their relative abundances across the E2 dominant (proliferative) and P4 dominant (secretory) phases of the menstrual cycle.

Table 2 Innate immunity in the human FGT

Lower genital tract (LGT) (ectocervix and vagina)	Proliferative (E2 dominant)	Secretory (P4 dominant)	References
Soluble factors (AMPs, HBD2, elafin)	↓ HBD2 and elafin	↑ SLPI	7,23
Epithelial cells (multilayered, squamous)	Thick vaginal epithelium	Thinner vaginal epithelium	16
Neutrophils	(=)	(=)	53,59
Macrophages	Vagina (=)	Vagina (=)	59
NK cells (CD45 +, CD56 + CD3 – CD16 +)	(=)	(=)	26,31
Upper genital tract (UGT) (Endocervix and endometrium, fallopian tubes, and ovaries)	Proliferative (E2 dominant)	Secretory (P4 dominant)	References
Soluble factors (AMPs, HBDs, elafin)	↑ HBD4 (endometrium) (note: HBD2 and elafin peak during menstruation) SLPI and elafin (fallopian tubes—constitutively expressed)	↑ SLPI, HBD1, HBD3 (endometrium) (maximal expression)	60–62
PRRs (TLR, NOD-1, 2)	TLR10 (fallopian tubes) TLRs 1–7 but not TLR4 (endocervix)	TLRs 2, 3 5, 6, 9, including TLR4 ↑ (endometrium), TLR10 ↑ (fallopian tubes)	55,63–68
Epithelial cells (single layer, columnar)	Thick uterine epithelium. Constitutively express IFN-ε (Type 1 IFN) in luminal and glandular cells highest in the proliferative phase	Thin uterine epithelium	16,69
Neutrophils	(=)	Endometrial ↑	54,59,70
Macrophages		Endometrial ↑ (before menstruation)	31
Dendritic cells	CD83 + (=)	CD1a + ↑	71
NK cells CD45 +, CD56 + CD3 – CD16 –, CD69 + CD94 +	uNK ↑ CTL activity	Endometrial NK cells (with NKG2D receptor) ↑ MICA uNK ↓ CTL activity via IL-18	10–15,26,31,40

AMP, antimicrobial peptide; CD, cluster of differentiation; CTL, cytotoxic T lymphocyte; FGT, female genital tract; HBD2, human β defensin 2; IFN, interferon; IL, interleukin; SLPI, secretory leukocyte protease inhibitor; NK, natural killer; NKG2D, an activating receptor expressed on NK cells that binds to MHC class 1 chain-related protein A and B (MICA and MICB); NOD, nucleotide-binding oligomerization domain; PRR, pattern recognition receptor; TLR, Toll-like receptor; uNK, uterine natural killer. The effects of menstrual cycle phase (proliferative or secretory) on the levels (↑, increased; (=), constant; or ↓, decreased) and functions (activity) of innate immune cells, non-immune cells, and soluble factors.

factors and localization and function of innate immune and non-immune cells, and receptor expression on ECs⁵⁸ vary with the release of ovarian steroid hormones over the menstrual cycle. **Table 2**^{7,10–16,23,26,31,40,59–71} summarizes the effects of estrogen (E2) and Progesterone (P4) at the proliferative and secretory phases on the aspects of innate immunity in the FGT.

The innate response is triggered by PRRs (including TLRs and non-TLRs such as NOD-like receptors) expressed predominantly by immune effector cells such as macrophages, neutrophils, and DCs that are found in the genital mucosa. These include TLR4 and its co-receptor CD14 (chlamydial lipopolysaccharide (LPS) and heat-shock protein 60

(HSP60)),^{72–74} TLR2 (chlamydial HSP60),^{75,76} and NOD protein, Nod2 (rudimentary proteoglycan motif produced by *C. trachomatis*).⁷⁷ Expression of TLR genes 2–6, 9, and 10 is reportedly significantly higher in human endometrial tissue during the secretory phase (when P4 is the predominant hormone) compared with all the other phases of the menstrual cycle.^{47,68}

The epithelial layer of the vaginal epithelium (LGT) and endometrium, endocervix, and fallopian tubes (UGT) is induced to proliferate producing a thickened uterine epithelium during the proliferative stage of the menstrual cycle when increased secretion of the E2 hormone is observed. During the secretory phase of the menstrual cycle when P4 levels exceed E2 levels, the epithelial layer is reduced in thickness. The human FGT is not as permeable as the rodent FGT to antigen uptake during the P4-dominated secretory phase as P4 does not significantly reduce the thickness of the vaginal epithelium in humans.⁷⁸

Rodent FGT

In murine models, it has been reported that increased genital infections with *C. trachomatis* are seen in progesterone-treated mice.⁷⁹ Thus the immune responses to chlamydial infections seen in the FGT rodents and humans are likely to be modulated in different ways by these vaginal permeability differences.

HORMONAL EFFECTS ON ANTIGEN PRESENTATION

In the FGT, sex hormones regulate both antigen presentation by DCs and also the numbers of macrophages and antigen-presenting cells (APCs) found within ECs of uterine and vaginal tissues.^{80,81} Human vaginal tissue contains cells that can present antigen to memory T cells,⁸² and ECs from uterine endometrium are known to constitutively express major histocompatibility complex Class II molecules that are required to present antigen to T lymphocytes.⁸³ In the rat, E2 enhances antigen presentation by uterine ECs,⁸⁰ whereas in the mouse E2 inhibits EC antigen presentation.⁸¹ Mature DCs are characterized by high production of IL-12⁸⁴ that facilitates the development of gamma interferon producing Th1 cells.⁸⁵ Estrogen has been shown to modulate the expression of cytokines and chemokines in human monocyte-derived DCs, with mature DCs pre-treated with E2 showing an increased ability to stimulate naive CD4+ T cells.⁸⁶ E2 can therefore regulate DC effector function and can induce and maintain inflammatory responses in the FGT.

The effects of E2 on the differentiation and function of APCs *in vitro* has been reviewed (see Nalbandian and Kovats⁸⁷ and Butts and Sternberg⁸⁸). Much of the current evidence shows that E2 can activate DCs and differentially regulate adaptive immune responses through direct effects on DC functions (see Hughes and Clark⁸⁹) via E2 receptor (ER) ligands (reviewed in Kovats and Carreras⁹⁰). It has been shown that ER-alpha (ER α) is the main receptor regulating E2-dependent DC differentiation in murine bone marrow-derived DCs *in vitro*.⁹¹ At high concentrations typical during pregnancy, E2 plus TNF- α have been reported to induce a distorted maturation of human DCs resulting in an increased capacity to initiate Th2 responses.⁹²

P4 has been shown to inhibit many functions of DCs, e.g., suppressing TLR-mediated cytokine production by some subsets of DCs with a recent study focussing on the effect of this sex hormone on TLR signalling in DCs.⁸⁹ These authors state that P4 inhibition of plasmacytoid DC function may impair specific antiviral responses (e.g., HIV or Herpes simplex virus (HSV)-2) that rely on certain TLR subsets and that this inhibition may have a significant impact on global health. P4 is also a potent inducer of semi-mature tolerogenic DCs and is reported to inhibit mature DC activity, which can ultimately contribute to the induction of pro-inflammatory responses.⁹³ The effect of sex steroids on the induction and function of a recently described CD4 T-cell subset, Th17 T cells that are involved in chronic inflammation in some disease states,⁹⁴ is also an area flagged for important future research, as P4-mediated disease remission in autoimmune diseases may involve impaired DC-regulated induction of Th17 cells.⁸⁹ The inhibitory effects of P4 on immature bone marrow-derived DCs (BMDCs) in female and male rodents has recently been reported to differ and is likely due to differences in P4 receptor protein expression affecting DC function in these animals.⁸⁸

A recent review of the functions of DCs and subsets of macrophages in the FGT has also addressed the sex hormone regulation of these professional APCs at this mucosal site.⁹⁵ Changes in the populations of DC and macrophage cells in the FGT occur due to (i) varying levels of ovarian steroid hormones at this site,⁹⁶ (ii) the delivery of CpG oligodeoxynucleotide to the murine genital tract,⁹⁷ and (iii) the stages of the menstrual cycle, noting that the frequencies of macrophages and immature DCs are higher in the menstrual phase when compared with the other phases of the cycle.^{98,99}

In a study investigating genital tract infections of C57BL/6 mice with *C. trachomatis* serovar D, it has been reported that in the LGTs of these mice IL-10 (primarily from CD11b+ DC) was the dominant IL, suggesting the presence of Th2 cells in these animals.³⁹ In the UGTs of these same animals, a Th1 subset of cells dominated with increased levels of IFN- γ expression was recorded following genital tract infections of these mice with *C. trachomatis*. This suggests that DCs are major contributors to IL-10 production in the LGT of mice and that production of this IL likely leads to an anti-inflammatory site of poor Th1 immunity at this mucosal site.³⁹

CD16-expressing macrophages and monocytes are also key components of the innate immune system of the FGT and are cells that are capable of producing cytokines and chemokines upon activation. These cells are also known to be profoundly affected by E2 (reviewed in Fish⁵⁰). Studies have shown that E2 uses both ER α and ER beta (ER β) to decrease CD16 expression on monocytes and hence to alter monocytic cytokine release following CD16 receptor activation.¹⁰⁰ More recently, it has been shown that human macrophages predominantly express an N-terminal truncated variant of ER α , ER α 46, and that this is also regulated by E2.¹⁰¹ Removal of endogenous E2 has been shown to reduce expression of TLR4 on the surface on murine macrophages thus decreasing both pro- and anti-inflammatory cytokine releases from these cells. The addition of exogenous

E2, however, corresponded with significantly higher expression of cytokines from these cells highlighting the immuno-enhancing effects of E2 on these cells.¹⁰² TLR4-mediated immune responses in macrophages have recently been reported to be inhibited by P4 via suppression of NF- κ B activation, suggesting a mechanistic explanation for the regulation of innate immune responses of these cells by this sex hormone.¹⁰³

ROLES OF NON-CONVENTIONAL APCs IN UTERINE AND VAGINAL TISSUES IN INNATE IMMUNITY

In the FGT, the underlying stromal cells are known to regulate growth and function of polarized ECs,⁴⁴ and uterine stromal cells mediate TNF- α release from uterine ECs in the presence of E2.¹⁰⁴ It has also been reported that TLRs 1–10 expression levels in murine vaginal ECs were significantly increased during the diestrous phase and expression of TLR2 was significantly decreased in ECs cultured in the presence of stromal cells.¹⁰⁵

The role of ECs and their associated cytokines in innate immunity of the FGT has been well documented¹⁰⁶ (reviewed in Quayle⁹). Sex hormones regulate the estrous cycle in rodents and the menstrual cycle in women to optimize mating and fertility, and they are also involved in regulating innate immunity in the FGT in both rodents and in mammals.¹⁰⁴ Human oviduct ECs express TLR 3, and E2 has been reported to suppress the TLR-3-induced cytokine and chemokine production in human endometrial ECs.¹⁰⁷ Chemokines, including C-C motif chemokine ligand 20/macrophage inflammatory protein 3 α (CCL20/MIP-3 α), have been found to have antimicrobial properties,¹⁰⁸ and CCL20/MIP-3 α is unique in binding exclusively to CCR6 (chemokine (C-C motif) receptor 6) receptor in humans.¹⁰⁹ Expression of CCL20/MIP-3 α has been reported from ECs of the FGT, and secretion occurs constitutively as well as being induced by inflammatory conditions.¹¹⁰

E2 inhibits IL-1 β -mediated responses by uterine ECs, suggesting that an inhibitory effect of E2 may be vital for reducing the pro-inflammatory responses that are mediated by these cells.¹¹¹ E2 also can act on endometrial ECs to suppress TLR3-induced cytokines and chemokines.¹⁰⁷

The effects of E2 and P4 on the release of antibacterial factors by ECs in the rat uterus was studied, and it was reported that in ovariectomized rats treated with E2, antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* increased in uterine secretions, an effect that was reversed with P4 treatments.⁶¹ P4 has been reported to potentiate the E2-stimulated expression of NOS isoforms, including endothelial NOS in endometrial-derived primary and human endometrial surface EC line.¹¹² P4 has also been reported to stimulate the expression of NOS in endometrial ECs, and this may potentially affect infection outcomes at this mucosal site.¹¹³

HORMONAL EFFECTS ON T LYMPHOCYTES IN THE FEMALE UGT AND LGT

The FGTs of humans and mice lack underlying organized lymphoid aggregates,¹¹⁴ contrasting with reports of lymphoid cell aggregates in the mucosa of the simian vagina.¹¹⁵ Present in

the female UGT and LGT are T lymphocytes, specifically CD4+ and CD8+ T cells, along with APCs including macrophages and DCs. Immune mediators of cell-mediated immunity are found distributed within the distinct regions of the female LGT. The cervix is a major inductive and effector site for cell-mediated immunity in the lower human FGT. In women with no current FGT inflammation, macrophages and CD4+ and CD8+ T lymphocytes are most prevalent between the ecto- and endo-cervix, in the so-called cervical transformation zone, and also in the surrounding tissue. Intraepithelial lymphocytes are predominantly CD8+ T cells in the cervical transformation zone. The normal vaginal mucosa contains T cells and APCs with the few immune cells present consisting primarily of CD1a+ DCs and CD8+ intraepithelial lymphocytes.¹¹⁶ The tissues in the UGT have been found to express a greater number Th1-associated chemokines than the tissues in the LGT comprising the cervical-vaginal region.¹¹⁶ The menstrual cycle and menopause have been reported to have no apparent effect on cellular localization or abundance of T-lymphocyte subsets and APCs in any of the LGT tissues.⁶

Migration of T-lymphocyte subsets is vital for adaptive immune responses and is the result of host–pathogen interactions. Effector lymphocytes must be recruited to the FGT after activation in the local iliac lymph nodes.⁶ To eradicate pathogens from the infected genital tract, effector T cells must migrate back to the FGT.^{6,116} Following infection of the mucosal ECs of the FGT, the pathogen induces release of host factors modifying the adhesion cascade. Following chlamydial infection of the murine FGT, increased expression of an adhesion molecule¹¹⁷ and expression of Th-1-associated chemokines¹¹⁸ has been reported. The release of host factors, in turn, influences the recruitment of lymphocytes to the infection site. These host factors act to modify the specific T-lymphocyte subsets recruited to the FGT in response to infection. In humans, women with cervicitis and vaginitis have been reported to have increased numbers of intraepithelial CD8+ and CD4+ lymphocytes and APCs in the vaginal mucosae when compared with women having no symptomatic infections at these mucosal sites.¹¹⁶

In mice, investigations into the distribution and function of T lymphocytes in the FGT have reported that 70–90% of the lymphocytes in the uterus and upper parts of the cervix of C57BL/6 mice are extrathymically derived CD3+ α BTCR+ CD4-CD8- T cells.¹¹⁹ In the murine vaginal tract, 30–40% of the T lymphocytes have been reported as being mostly CD4+ T cells (see mini-review by Hamad²⁵). Hormonal control of cytokine secretion by vaginal and uterine lymphoid cells has been investigated in two strains of mice (Balb/c and C57BL/6), and it was reported that the numbers of IFN- γ -secreting vaginal and uterine lymphoid cells increased at diestrous in both strains of mice.¹²⁰ Female C57BL/6 mice were also used to show that estrogen-conditioned bone marrow-derived DCs (BM-DC) could enhance regulatory T-cell (Treg) activity and also directly inhibit effector T cells in the absence of Treg cells.¹²¹

In humans, expansion of human CD4 + CD25 + Tregs has been reported to occur in the late follicular (proliferative) phases of the menstrual cycles of fertile women.³⁷ This increase in Tregs was correlated not only with normal serum levels of E2 in these women but also with an increased expression of the Foxp3 gene.³⁸ Interestingly, E2 is reportedly responsible for the elevated immune-suppressive potential of Tregs.¹²²

Increased expression of FoxP3 + mRNA was seen primarily in the FGT of mice following infections of the genital tracts of these animals with *C. trachomatis* serovar D.³⁹ It is generally accepted that Treg cell expansion is modulated by E2³⁷ and may be associated with reduced cell-mediated immune responses. Thus it seems that increased levels of P4 during the menstrual cycle (and progestins delivered via hormonal contraceptives) may have profound effects on the outcomes of bacterial infections of the FGT, including those caused by *Chlamydia*, by increasing Treg frequencies and lowering cell-mediated immunity responses in these infected populations.¹²³ Infections of the human FGT with *C. trachomatis* are, however, less likely to occur in P4-dominant conditions. This is due to the fact that, as previously mentioned, the vaginal epithelia of humans is not as permeable to antigen uptake during the P4-associated luteal phase of the menstrual cycle, as P4 does not significantly thin this mucosal epithelium.

Decreases in the numbers of Tregs have been reported in endometrial samples of 15 progestin-treated patients with atypical endometrial hyperplasia. In these same patients, progestin treatment was associated with significant increases in cytotoxic T-cell activity, suggesting that sub-populations of lymphocytes can be affected by progestins.¹²⁴ P4 and, to a lesser extent, E2 have both also been reported to reduce Foxp3 + Treg cells in peripheral blood mononuclear cells in *in vitro* systems that represented the second trimester of human pregnancies.¹²⁵ T-cell activation signalling (by CD3zeta, Janus-associated kinase 2 (JAK2), and JAK3 proteins) and cytokine production in cells exposed to hypoestrogenic (4 pg ml⁻¹ of E2) postmenopausal levels of E2 were shown to diminish significantly, and this correlated with reduced IL-2 production in Jurkat 6.1 T cells.¹²⁶ This latter finding highlights the fact that a reduction in levels of E2 after menopause can and does contribute to immunosenescence.

T-lymphocyte activity in the human uterus is also influenced by the menstrual cycle; e.g., CD3 + CD8 + CTL activity is detectable during the proliferative phase but absent during the secretory (postovulatory) phases of fertile women.³³ In postmenopausal women, strong CD3 + T-cell cytolytic activity is detected in all compartments of the FGT. These findings suggest that the high levels of E2 and P4 present during the proliferative phase of the menstrual cycle can downregulate CTL activity in the uterus. Studies show that E2 greatly increases the activity of the IFN- γ promoter in human T-cell lines, suggesting that E2 may favour Th1 responses. Receptors for E2 have also been shown on both CD8 and CD4 T-cell populations.¹²⁵

Hormonal fluctuations during the estrous cycle that affect cell-mediated immunity in the upper FGT may also potentially

have a major part in making this mucosal site distinctively susceptible to chlamydial infections.^{127,128}

The FGT of humans and of rodents differs in many ways that can modulate the immune responses at this mucosal site. Both human and rodent genital tracts have large numbers of DCs; however, only the human FGT contains large numbers of CD4 +, CD8 + T cells, B cells, and macrophages. Leukocyte subpopulations are found throughout the human FGT and include T helper cells (Th1, Th2, Th17), cytotoxic T cells, Treg cells, NK cells, and $\gamma\delta$ T cells. In contrast to rodents, the numbers of T cells in the vagino-cervix mucosae of women does not change during the various phases of the menstrual cycle. Cytotoxic T-cell activity in the cervix and vagina of women is demonstrated during all phases of the menstrual cycle.

Thus, both the human LGT and UGT are immunologically active throughout the normal menstrual cycles of women. Localization of T-lymphocyte populations in the human female UGT and LGT and the effects of menstrual cycle phases and of chlamydial infections on these immune cells is summarized (Table 1).

HORMONAL REGULATION OF IMMUNOGLOBULIN SECRETION

In humans, the predominant antibody isotype in female cervicovaginal secretions is IgG,¹²⁹ although secretory IgA in the polymeric form is also present.¹³⁰ Levels of IgG and IgA antibodies in FGT fluids are modulated by the stage of the menstrual cycle in women and the estrous cycle in rodents.

It is known that the neonatal Fc receptor, FcRn, in the genital epithelium mediates transport of IgG into the FGT and that local levels are affected by stage of the murine estrous cycle, with highest levels being recorded at diestrus.¹³¹ The transport of IgG into the fallopian tubes and uterus is also significantly decreased by P4 treatment, and this is likely correlated with FcRn expression.¹³²

Mucosa-associated epithelial chemokine CCL28 can recruit IgA plasma cells to the uterus of the murine reproductive tract following mucosal (intranasal or vaginal) immunizations, and this migration is dependent upon E2.¹³³ Interestingly, the IgA plasma cells induced by mucosal vaccination were recruited to the uterus but not to the cervix or the vaginal canal. E2 also upregulates the *in vitro* expression in the FGT of secretory component increasing transport of IgA into the lumen,¹³⁴ and transport of polymeric IgA (pIgA) into FGT tissues is significantly decreased by ovariectomy, although this decline can be reversed by the addition of E2 but not of P4.¹³²

The levels of Igs in genital tract secretions of women vary during the menstrual cycle, with the lowest levels of IgG occurring around the time of ovulation in women with human papillomavirus (HPV)-16 vaccine-specific antibodies with total IgG and IgA titers in cervical fluid highest in the proliferative phase of the cycle.^{135,136} In normal cycling female macaques, it has been reported that the frequency of Ig-secreting cells (ISC) was significantly higher in tissues collected from animals in the peri-ovulatory period of the menstrual cycle than in tissues collected from other stages of the

cycle and that this effect was apparently due to the action of estrogen on CD8 + T cells.¹³⁷ The effects of ovarian hormones on (ISC) function in healthy women were also investigated, and it was reported that ISC frequency in PMBC was highest during the peri-ovulatory stage of the menstrual cycle.¹³⁸

In murine studies, not surprisingly, it has been shown that E2 influences Ig levels in post-pubertal female C57BL/6 (B6) mice, with these animals expressing genes involved in adaptive immune responses, including IgA, IgG1, IgG2, and B-cell receptor gene involved in B-cell signalling.¹³⁹ In female BALB/c mice, E2 strongly stimulated the production of bovine serum albumin (BSA)-specific antibodies (mostly of the IgG1 isotype) in the sera of BSA-immunized mice.¹⁴⁰ A study of the effects of sex hormones on migration of antibody-secreting cells into the genital tracts of female BALB/c mice revealed that the mRNA expression from adhesion molecules and chemokines intercellular adhesion molecule 1, P-selectin, and JAM-1 (junctional adhesion molecule 1) was significantly increased in ovariectomized mice and that these changes were restored to normal levels by E2 treatment.¹⁴¹

The maturation, selection, and activation of B cells is also known to be affected by estrogen treatment, with E2 shown to alter the later stages of B-cell development (reviewed in Grimaldi *et al.*¹⁴²). Recently, it has been reported that in female B-cell-deficient mice that do not express IgM, E2 increased the frequency of IgA-producing plasma cells, suggesting that E2 can influence an alternative B-cell pathway found in these mice.¹⁴³

IgA-secreting plasma cells typically produce dimeric IgA (dIgA), and once secreted into the lamina propria, dIgA binds the polymeric Ig receptor (pIgR) at the basolateral surface of mucosal ECs forming secretory IgA. It is known that dIgA:pIgR complexes can neutralize intracellular pathogens such as HIV,¹⁴⁴ although the precise effects of these complexes on chlamydial infections is presently unknown. The pIgR is ubiquitously expressed by ECs in the human FGT, with high expression observed in the UGT.¹⁴⁵ Innate (mediated by TLR3) and adaptive immune responses (including IFN- γ) to infection are known to regulate pIgR expression. Additionally, the sex hormones E2 and P4 also have regulatory roles in the expression of pIgR. In female rats, E2 is known to upregulate the pIgR expression,¹⁴⁶ although in primates ovulation coincides with a decrease in mucosal antibodies.¹³⁴ The sex hormone P4 downregulates pIgR expression in rats,¹⁴⁷ and although the specific role of the pIgR in chlamydial infections is debatable, the protective role of IgA and the pIgR may indeed be of importance in the development of subunit vaccines against *C. trachomatis*.

SEX HORMONES AND GENITAL TRACT INFECTION

It is becoming increasingly well documented that sex hormones E2 and P4 regulate host susceptibility, as well as innate and adaptive immune responses, to sexually transmitted pathogens infecting the mucosal surfaces of the FGT. STD pathogens that infect this site include viruses (HIV-1, HSV-2, HPV) and bacteria such as *Neisseria gonorrhoeae* and *C. trachomatis*.

There are numerous reports in the literature on studies in women and in animal models that report the effects of sex hormones on genital tract infections caused by these mucosal pathogens (e.g., for HIV review, see Hel *et al.*¹⁴⁸).

In this review, we will specifically focus our discussions on *C. trachomatis* as this organism is the perennial target for much of our own research.

C. trachomatis

The columnar ECs of the FGT are target cells for chlamydial infection, and although natural immunity to infection with this mucosal pathogen does occur, it is serovar-specific and short-lived. Local host defence against *Chlamydia* involves the mucosal barrier comprised of ECs and mucus and innate, humoral, and cell-mediated immune responses (**Figure 3**). Decreased levels of SLPI in vaginal secretions are reportedly inversely related to infections caused by *C. trachomatis*,¹⁴⁹ but SLPI expression is reportedly induced following *C. trachomatis* infection of the FGT¹⁵⁰ and elafin mRNA expression is upregulated in a *Chlamydia*-infected oviduct EC line⁶² (see **Table 3**^{150–153}).

Infection of the FGT with live replicating *C. trachomatis* is innately recognized by surface PRRs TLR2 and TLR4 that are highly expressed in the UGT tissues.⁴⁵ The myeloid differentiation primary response gene (MyD)88 is essential for nuclear factor- κ B (NF- κ B) signalling and transcription of proinflammatory cytokines. Along with TLR2, MyD88 specifically co-localizes with the intracellular chlamydial inclusion.¹⁵⁴ Additional to the TLRs the NOD-like receptor, NOD1, also senses chlamydial infection of endocervical ECs. Signals transduced from these TLRs lead to induction of IFN- γ and cytokine genes that contribute to resolving chlamydia genital infection in an IFN- γ -dependent manner, noting also that TLR2 is also responsible for severe tubal immunopathology in a murine model and that the chlamydial plasmid has a crucial role in TLR2 activation.^{155,156} In response to chlamydial infection, ECs secrete pro-inflammatory cytokines/chemokines such as IL-6 and IFN- γ that are hypothesized activators of pathology in the cellular paradigm of chlamydial pathogenesis.¹⁵⁷

C. trachomatis infection of the female LGT stimulates innate immune cells by activation of TLR2/TLR4 (reviewed in Schaefer *et al.*¹⁵⁸). TLRs 2 and 4 have been found at the highest expression levels in endometrium and fallopian tube tissue,¹⁵⁹ with the predominant expression of TLR4 and its co-receptor CD14 in the fallopian tubes proposed to have an important role in the innate host defence mechanism against ascending *C. trachomatis* infections.^{158,160} In a recent study, *C. trachomatis*-infected women have been reported to express higher TLR2, TLR4, and iNOS in their cervical monocytes compared with controls. It is thought that TLR4 initiates the innate immune response to chlamydial infection, activation of TLR2 leads to expression of inflammatory cytokines while iNOS contributes to chlamydial clearance from this mucosal site.¹⁶¹

Studies into *C. trachomatis* infections of the FGT have reported many effects of hormones on infection and immunity

Figure 3 Immune protection against chlamydial infection in the female genital tract. Innate, humoral, and cell-mediated immunity act in concert to protect against *C. trachomatis* infection of non-immune host genital epithelial cells and local innate immune cells in the female genital tract. The hormones estradiol and progesterone regulate innate and adaptive immunity against genital *Chlamydia* by promoting mucus secretions, regulating immune and non-immune cell numbers and activities (**Figure 2**), and by altering cell receptor expression at this mucosal site. **Innate:** The epithelial barrier is relatively ineffective at protecting against *Chlamydia* as this mucosal pathogen has a myriad of mechanisms to evade barrier protection. A mucus layer containing a variety of antimicrobial factors and endogenous microbiota contributes towards regulating the pH of the FGT to protect against genital tract pathogens. Innate immune cells constitutively secrete an array of soluble antimicrobials, including secretory leukocyte protease inhibitor (SLPI), human β-defensin 2 (HBD2), lysozyme, lactoferrin, Elafin, cathelicidins. Chlamydial infection of columnar epithelial cells and local genital tract immune cells, including neutrophils, macrophages, and natural killer (NK) cells, produces soluble antimicrobials, chemokines, and pro-inflammatory cytokines that selectively prevent bacterial infection of target host cells. (e.g., interleukin (IL)-1 released from infected epithelial cells promotes T helper type 17 (Th17) differentiation). Recruitment and activation of adaptive immune cells (B and T cells) are also orchestrated by the release of these secreted soluble antimicrobial factors from epithelial cells, dendritic cells, and macrophages. **Humoral:** Antibodies potentially can prevent infection by *Chlamydia*. Immunoglobulin G (IgG) is the predominant antibody in the FGT. Antibodies released from plasma cells (P IgG and P IgA) inactivate extracellular chlamydial elementary bodies. **Cell-mediated:** CD4+ T-cell production of interferon (IFN)-γ contributes to host defence by inhibiting intracellular chlamydial replication. Cell-mediated immunity induces apoptosis of infected cells by cytotoxic CD8+ T cells.^{6,20,35,39,40,62,63,77,117–119,150–157,172} CTL, cytotoxic T lymphocyte; GM-CSF, granulocyte-macrophage colony-stimulating factor; MMP, matrix metalloproteinase; GRO-α, growth related oncogene-α; TNF, tumor necrosis factor; Treg, regulatory T cell.

to the pathogen at this mucosal site. For example, significant increases in the sensitivities of cervical ECs to infection with *C. trachomatis* occur in the later stages of the female menstrual cycle.¹⁶² It has been reported that chlamydial infection occurring in the early E2-dominant phases of the cycle was a significant predictor for development of salpingitis.¹⁶³ Clinical findings have also reported on the enhancing effects of E2 on chlamydial infection and disease sequelae in the infected FGT. It has been reported that women are more susceptible to chlamydial infection under the influence of E2.³⁵ In women with fertility disorders ($n = 115$) and in women with *C. trachomatis* mucopurulent cervicitis ($n = 86$), a significantly positive correlation was recorded between chlamydial load and E2 levels.²¹

Hormones can also affect the immune responses to chlamydial infections of the FGT in mice. A recent seminal study has reported that IFN-ε^{-/-} mice showed significantly increased levels of chlamydial growth in the UGTs at 30 days post intravaginal infection with *C. muridarum*. The mice also showed more severe clinical signs of disease following intravaginal infections with sub-lethal doses of *C. muridarum*. IFN-ε expression is also hormonally regulated. The presence of high levels of E2 (proliferative phase) coincided with the highest expression levels of IFN-ε, and E2 administration to ovariectomized mice induced a six-fold increase in IFN-ε expression. Thus, IFN-ε constitutively expressed by luminal and glandular ECs of the endometrium can protect against *Chlamydia* without aggravating disease—the first report that

Table 3 *Chlamydia trachomatis* and antimicrobial peptides (AMPs)

Study	Experimental findings
<i>Chlamydia stimulates AMP expression</i>	
King <i>et al.</i> ⁶²	<i>In vitro</i> —infection of oviductal epithelial cell line results in increased elafin expression but no effect on SLPI
Wheelhouse <i>et al.</i> ¹⁵⁰	<i>In vitro</i> —infection of HeLa (cervical epithelium) and JEG-3 (trophoblast) cells results in increased SLPI expression
<i>AMP activity against Chlamydia</i>	
Pal <i>et al.</i> ¹⁵¹	<i>In vivo</i> —MMP-7 knockout mice (with reduced α -defensin activity) were unaffected in their ability to clear chlamydial infection
Oberley <i>et al.</i> ¹⁵²	<i>In vitro</i> —surfactant protein D protein inhibits the infection of HeLa cells (an endocervical epithelial cell line by <i>C. trachomatis</i>)
Yasin <i>et al.</i> ¹⁵³	<i>In vitro</i> —rabbit defensin (NP-1), HNP-2, and porcine leukocyte Protegrin reduced chlamydial infection

HNP-2, human neutrophil peptide 2; JEG-3, human placental choriocarcinoma cell line; MMP-7, matrix metalloproteinase 7; NP-1, neutrophil peptide 1; SLPI, secretory leukocyte protease inhibitor.

indicates a Type 1 interferon can protect and not worsen a chlamydial genital tract disease.⁶⁹

Chemokine receptors direct T lymphocytes to the site of mucosal infection, including infection of genital tract ECs with *C. trachomatis*, and these receptors can be modulated by E2. In a study examining samples from human patients, it was reported that CCR5 expression increased followed *C. trachomatis* genital infections.²⁸ It is also now known that CXCR3 (chemokine (C-X-C motif) receptor 3) and CCR5 are the predominant receptors that are crucial for T-lymphocyte access to the genital tract during a *C. trachomatis* infection in a murine model.¹⁶⁴ Therefore, the protective capacity of *Chlamydia*-specific T cells in the genital tract can be modulated by the sex steroid E2.

Reproductive hormones may potentially act with cytokines to regulate immune responses in the FGT to chlamydial genital tract infections *in vivo*. Significant negative correlation has been reported between E2 levels in women with primary chlamydial genital infections and cervical wash concentrations of IL-10, IL-1 β and IL-6 cytokines. Significant negative correlation was also recorded between IL-1 β cytokines and P4 levels in women with recurrent chlamydial infections.¹⁶⁵ Thus it would appear that chlamydial infection of the human FGT is modulated, in part, by the combined actions of cytokines with E2 and P4. More recent investigations reveal that levels of E2 were significantly higher in *Chlamydia*-positive women with fertility disorders when compared with fertile women, suggesting that this sex hormone contributes to the development of disease sequelae to *C. trachomatis* infection of the FGT.³⁵

Results of studies into the course of genital chlamydial infections in animal models have also revealed the effects of reproductive hormones on immune responses and disease sequelae. Results of these studies have validated much of the clinical data for chlamydial genital infections in women. In

mice, significantly more chlamydiae were recovered from the uterine horns and oviducts when animals were infected during periods of increased levels of P4 and E2 (secretory phase) rather than when infections occurred during the follicular phase.¹⁶⁶ Rank *et al.*¹²⁷ reported that high E2 levels in guinea pigs corresponded with a significantly greater percentage of *Chlamydia*-infected animals developing oviduct pathology. It was also reported that high doses of P4 did not alter the course of genital infection in guinea pigs,¹²⁸ but other reports using different animal models showed that these doses increased the rate of infection, e.g., using a rat model¹⁶⁷ and also that high doses of P4 resulted in more intense *C. muridarum* infections of mice.¹⁶⁸

Attachment and infectivity of chlamydial elementary bodies *in vitro* reportedly has been enhanced by E2 for HeLa cells,¹⁶⁹ human endometrial ECs,¹⁷⁰ luminal ECs from swine genital tracts,¹⁷¹ and oestrogen-responsive endometrial carcinoma cell lines Ishikawa and HEC1B and breast cancer lines.¹⁷² *In vivo*, ECs have been reported to be more sensitive to *C. trachomatis* serovar E in the E2-dominant (proliferative) stage of the human menstrual cycle than at other stages of the cycle.^{173,174} More recently, preliminary work from our own laboratory has investigated infection of ECC-1 human endometrial cell lines with *C. trachomatis* serovars D and L2 supplemented with 200 pg ml⁻¹ E2 or 20 ng ml⁻¹ P4. We report that P4 upregulates adhesion molecules involved in cell recruitment (CX3CL1, E-selectin, ITGB8, and others) and downregulates IFN and IFN receptor genes. Our results indicate that the gene expression patterns induced by E2 in this system are suggestive of persistent chlamydial infection.¹⁷⁵

Hormonal contraceptive use in women, particularly depo medroxyprogesterone, has also been reported in observational studies to be associated with a fourfold increase in chlamydial genital tract infections,¹⁷⁶ although the biological explanation for increased risk of infections caused by depo medroxyprogesterone has been challenged.¹⁷⁷ A feasibility plan recently has been conducted to pave the way for a future randomized trial to investigate hormonal contraception and the risks of STI acquisition in women.¹⁷⁸

Thus in terms of developing a protective mucosal vaccine for *Chlamydia*, it is, therefore, prudent to consider the influences of sex hormones on infection and immune responses to *C. trachomatis* within the FGTs both of humans and of animals.

SEX HORMONE INFLUENCE ON VACCINE-INDUCED IMMUNITY

Many studies examining the effects of sex hormones on vaccine-induced immunity against sexually transmitted pathogens have been conducted using the murine model with animals that undergo an estrus cycle. The cycle consists of Proestrus and Estrus, in which follicles grow and mature, the endometrium develops, and an estrogen surge occurs in conjunction with ovulation. In Metestrus, the corpus luteum and high levels of progesterone are seen, and during Diestrus the corpus luteum regresses. In estrus, it is also seen that the endometrium is reabsorbed if conception does not occur while

in the human menstrual cycle the endometrium is shed. It is to be noted, however, that with respect to chlamydial infections of the FGT there are many differences between the murine models using *C. muridarum* and the pathogenesis of human *C. trachomatis* endocervical infections. Two examples of these differences are (i) the strain of *Chlamydia* used in the mouse model, which is not a natural pathogen of the murine FGT, and (ii) Th1 immunity to *C. muridarum* in the mouse model that causes significant damage to the UGTs of mice while in endocervical infections of women with *C. trachomatis* UGT damage occurs infrequently.

When E2 prevents the penetration of antigen into the vaginal epithelium by increasing the mucosal barrier thickness, it reduces the antigen-loading of vaginal APCs. E2 thus inhibits the crucial process of CD8 + T-cell priming, although antigen uptake can still occur in the vaginal mucosa following removal of the mucus layer at this site. Interestingly, when antigen is administered orally or intranasally during the E2-dominant phases of the estrus cycle, specific antibodies in the FGT are significantly enhanced.¹⁷⁹ The ability to prime CD8 + T cells after immunization at the progesterone-treated genital tract is strongly influenced by ovarian steroid hormones.¹⁸⁰ Thus, it needs to be remembered that sex steroids can modulate immune responses following intravaginal and intranasal routes of immunization, routes that are more effective at eliciting genital tract antibody responses than parenteral immunization.

Song *et al.*¹⁸¹ investigated the effects of exogenous E2 administration, following intravaginal inoculation of BALB/c mice with *N. gonorrhoeae*, and they found an increase in gonococcal-specific serum antibody in these animals. Others have found that E2 treatment increased the specific antibody response to Dinitrophenyl-keyhole limpet hemocyanin (DNP-KLH), a T-cell-dependent antigen, in BALB/c mice. By experimenting with agonists to each of the ERs (ER α and ER β), they determined that the effects of E2 on antibody production are mediated via signalling pathways involving both ER receptors.¹⁸² It appears that the timing between E2 administration and immunization or challenge doses can affect the ability of the antigen/micro-organism to penetrate the mucosal epithelia; choice of mouse strain may also affect these processes.

Protection against several STIs, including *C. trachomatis*, requires not only the stimulation of IgG and IgA antibodies but also antigen-specific CD4 + T cells in the genital tract. However, our knowledge is rudimentary regarding both T-cell responses to the effects of sex steroids following mucosal immunizations to induce these responses in the FGT. A recent study investigating live chlamydial genital tract infections in the murine model reported that P4 is required for an effective, protective T-cell response in the genital tract following intravaginal immunizations but that E2 prevented a response.³⁹ Thus CD4 + T-cell immunity in the FGT can indeed be induced by local mucosal immunization with appropriate chlamydial immunogens (e.g., major outer membrane protein) and adjuvants (e.g., CTA-DD) as has recently been reported.¹⁸³ Optimal protection at

this site will require knowledge of the ovarian steroid status at the time of immunization in attempts to elicit protective immunity against a live genital tract infection with *Chlamydia*.

In non-human primates, studies have shown that live attenuated lentivirus (simian-human immunodeficiency virus) protects 60% macaques against challenge with intravaginal simian immunodeficiency virus. Depo-provera pre-treatment of the animals resulted in a decrease in this protection,¹⁸⁴ a finding that is similar to that observed in mice.

Finally, only a limited number of studies to date have examined the effects of sex hormones on vaccination outcomes in females. Women who were given vaginal immunizations with bacterial LPS during either the E2-dominant follicular or the P4-associated luteal phases of the ovarian cycle only induced cervical IgA2-restricted antibodies against LPS when immunized during the follicular phase of the cycle.¹⁸⁵ Women who were given intravaginal immunizations with a recombinant Cholera toxin B subunit and immunogen showed no differences in their resulting immune responses to the antigen whether they were using P4-containing intra-uterine devices, on the oral contraceptive pill, or were using no contraception at all.¹⁸⁶ These results indicate that the magnitude of the immune responses elicited in the FGT could be influenced by the phase of the menstrual cycle for intravaginally administered vaccines.

SUMMARY

The FGT is a site of abundant immune processes having an extensive lymphoid system capable of mounting a protective immune response to intracellular pathogens, such as *C. trachomatis*. The effects of ovarian steroid hormones on innate immunity, antigen presentation, T-cell-specific immunity, and Ig production have been examined with some studies that have been highlighted presenting contradictory findings to other studies discussed. The contrary reports are likely due to the variety of cell lines and models used in the experimental investigations and also due to varying levels and combinations of hormones tested in these studies.

This review highlights and updates our current knowledge of the effects of ovarian steroid hormones on various components of immune responses in the FGT and how these responses can modulate susceptibility of this mucosal site to STIs. Future investigations into vaccines to control infections of the female reproductive tract will need to consider the effects both of E2 and of P4 at this mucosal site. Animal models and *in vitro* assays should continue to be refined in design with respect to sex hormone status in attempts to more accurately reflect the potential efficacy of novel vaccine preparations tested in these models that are destined for use in humans.

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