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The endometrial immune environment of women with endometriosis

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BACKGROUND: Endometriosis, a common oestrogen-dependent inflammatory disorder in women of reproductive age, is characterized by endometrial-like tissue outside its normal location in the uterus, which causes pelvic scarring, pain and infertility. While its pathogenesis is poorly understood, the immune system (systemically and locally in endometrium, pelvic endometriotic lesions and peritoneal fluid) is believed to play a central role in its aetiology, pathophysiology and associated morbidities of pain, infertility and poor pregnancy outcomes. However, immune cell populations within the endometrium of women with the disease have had incomplete phenotyping, thereby limiting insight into their roles in this disorder.

OBJECTIVE AND RATIONALE: The objective herein was to determine reproducible and consistent findings regarding specific immune cell populations and their abundance, steroid hormone responsiveness, functionality, activation states, and markers, locally and systemically in women with and without endometriosis.

SEARCH METHODS: A comprehensive English language PubMed, Medline and Google Scholar search was conducted with key search terms that included endometriosis, inflammation, human eutopic/ectopic endometrium, immune cells, immune population, immune system, macrophages, dendritic cells (DC), natural killer cells, mast cells, eosinophils, neutrophils, B cells and T cells.

OUTCOMES: In women with endometriosis compared to those without endometriosis, some endometrial immune cells display similar cycle-phase variation, whereas macrophages (Mø), immature DC and regulatory T cells behave differently. A pro-inflammatory Mø1 phenotype versus

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anti-inflammatory Mø2 phenotype predominates and natural killer cells display abnormal activity in endometrium of women with the disease. Conflicting data largely derive from small studies, variably defined hormonal milieu and different experimental approaches and technologies.

WIDER IMPLICATIONS: Phenotyping immune cell subtypes is essential to determine the role of the endometrial immune niche in pregnancy and endometrial homeostasis normally and in women with poor reproductive history and can facilitate development of innovative diagnostics and therapeutics for associated symptoms and compromised reproductive outcomes.

Key words: endometrium / endometriosis / immune populations / immune cell markers / inflammation

Introduction

Endometriosis and immune system dysfunction

Endometriosis is a common oestrogen-dependent inflammatory disease that affects nearly 200 million women and teen girls worldwide, up to 50% of women with chronic pelvic pain and 30–50% of women with infertility (Bulun, 2009; Burney and Giudice, 2012; Giudice, 2010). It is characterized by endometrial-like tissue outside its normal location lining the uterus, which is found mainly on pelvic structures and rarely at sites distant from the pelvis (Giudice, 2010). While its pathogenesis is poorly understood, genetics and the environment are key drivers, and the immune system is believed to play a major role in its pathophysiology and symptomatology (Burney and Giudice, 2012; Patel *et al.*, 2018; Zondervan *et al.*, 2018). The most commonly accepted theory of the origin of endometriosis, proposed by Sampson (1928), states that at menses, endometrial cells and tissue fragments reflux through the fallopian tubes, survive and attach to and invade pelvic structures. In so doing, they undergo neuroangiogensis and elicit a local inflammatory response, fibrosis/scarring and pain (Burney and Giudice, 2012; Patel *et al.*, 2018). Infertility is due to the distorted anatomy due to scarring, local inflammatory effects on oocyte quality and early embryo development, and an inhospitable endometrial environment for embryo nidation. While there is 90% prevalence of retrograde menstruation in cycling women, the prevalence of endometriosis is 10%, suggesting hereditary or acquired abnormalities in the eutopic endometrium, hereditary or acquired abnormalities in the peritoneal epithelium and/or defective immune clearance of the sloughed tissue in the pelvic cavity (Bianco *et al.*, 2012; Burney and Giudice, 2012; Patel *et al.*, 2018; Zondervan *et al.*, 2018). What these aberrations are and why they occur are poorly understood but are increasingly a focus of study to understand endometriosis risk and effective therapies. The immune component is addressed herein.

Both the peripheral immune system and the immune status of endometrium within the uterus are altered in women with endometriosis and likely contribute to infertility, early pregnancy failure and abnormal tissue homeostasis in affected women (Agic *et al.*, 2006; Lessey *et al.*, 2013; Patel *et al.*, 2018; Saraswat *et al.*, 2017). Transcriptomic analysis of endometrium of women with endometriosis has revealed altered steroid hormone signalling and up-regulation of pathways involving lymphocyte activation, antigen presentation, cytokine induction and inflammation (Houshdaran *et al.*, 2016; Tamaresis *et al.*, 2014). Specific mining of endometrial whole tissue immune and inflammation gene signatures in women with endometriosis suggests abnormalities in endometrial stromal fibroblast cellular apoptosis and progesterone-induced differentiation (decidualization), the latter being essential for pregnancy establishment and maintenance (Ahn *et al.*, 2016). Endometrium of women with endometriosis also exhibits altered gene expression and altered global DNA-methylation profiles (Houshdaran *et al.*, 2016; Penna *et al.*, 2008; Sbracia *et al.*, 2016). Cellular components in the endometrial niche that display these immune activation and pro-inflammatory profiles have not yet been determined, although there are several candidates, including resident endometrial and migratory immune cells, as well as endometrial stromal fibroblasts (Aghajanova *et al.*, 2010; Agic *et al.*, 2006; Bruner-Tran *et al.*, 2013; Burney and Giudice, 2012; Lessey *et al.*, 2013; Weiss *et al.*, 2009). Overall, data acquired in the past decade support an altered pro-inflammatory immune environment in the endometrium of women with compared to those without the disease, among other abnormalities in non-immune cells in this tissue, which are believed to contribute to endometriosis establishment, its pathophysiology and known adverse reproductive outcomes (Saraswat *et al.*, 2017).

Immune cells in normal cycling endometrium

The menstrual cycle consists of several phases: proliferative, secretory, menstrual and regenerative (Fig. 1A). The morphology of the endometrium, proliferation and differentiation of its cellular components and trafficking of immune cell populations change throughout the cycle, largely under the influence of ovarian-derived estradiol and progesterone and potential interactions with other endometrial cellular components. Early in the cycle (Days 1–4), the tissue begins repair and regeneration independent of estradiol. Thereafter, throughout the proliferative phase, estradiol induces mitosis of all cellular constituents, including luminal and glandular epithelium, stromal fibroblasts and vascular components. After ovulation, progesterone induces secretory transformation of the epithelial cells and stromal fibroblast differentiation (decidualization), preparing for embryo nidation. In the absence of implantation, with the decline of steroid hormones, desquamation of the tissue ensues, resulting in menses, followed anew by repair and regeneration from endometrial stem cells (Hawkins and Matzuk, 2008) (Fig. 1A). Structurally, human endometrium is composed of two layers, the basalis and the functionalis, the latter of which is shed during menstruation and regenerated in the absence of pregnancy (Fig. 1B).

Most immune cells in endometrium are tissue-resident cells, with some migrating from the peripheral circulation (Lee *et al.*, 2015). They are scattered throughout the stromal compartment and between epithelial cells in the functionalis layer (Fig. 1B) and in lymphoid aggregates in the basalis. Lymphoid aggregates develop during the proliferative phase and are composed of a core of B cells surrounded

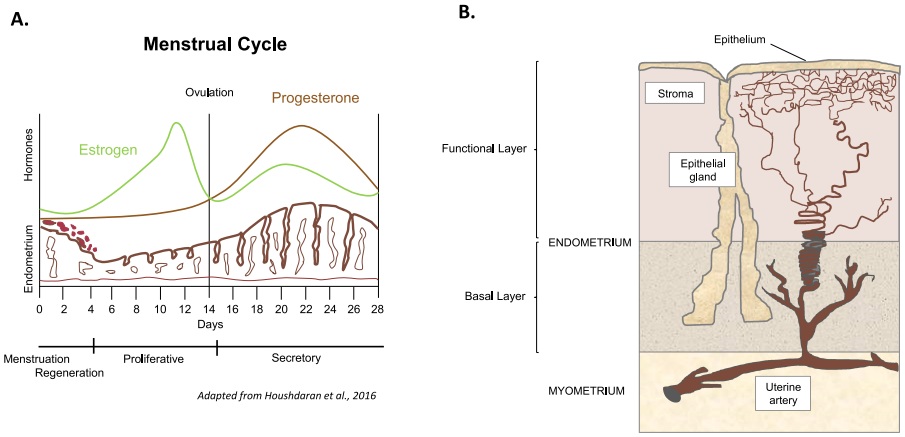

Figure 1 The menstrual cycle (A) and the structure of the endometrium (B). A) Menstrual cycle. The menstrual cycle consists of several phases: menstrual, regenerative, proliferative and secretory. The morphology of the endometrium changes throughout the cycle largely under the influence of ovarian-derived estradiol and progesterone. **B**) Endometrium structure. Structurally, human endometrium is composed of two layers: the basalis and the functionalis, the latter of which is shed during menstruation and regenerated in the absence of pregnancy. The endometrium is composed of two compartments: epithelium and stroma. Tubular glands reach from the endometrial surface through to the base of the stroma.

by a population of CD8+ T cells, which, in turn, is surrounded by macrophages (Mø) (Yeaman *et al.*, 2001). The number, type and activation state of immune cells in the functionalis are highly dependent on the hormonal milieu and reflect their diverse roles. For example, regulatory T cells (Treg cells) promote fetomaternal immune tolerance, uterine natural killer (uNK) cells regulate trophoblast invasion and enhance vascular remodeling by the extravillous trophoblast and macrophages and dendritic cells (DC) and their subtypes protect against infection (Achache and Revel, 2006; Erlebacher, 2013; Lee *et al.*, 2011). Leukocytes, expressing the surface marker leukocyte common antigen (CD45), account for 10–20% of all endometrial cells and increase in the functionalis layer in the secretory and menstrual phases compared to the proliferative phase (Evans and Salamonsen, 2012; Flynn *et al.*, 2000; Givan *et al.*, 1997; King and Critchley, 2010; Wira *et al.*, 2015; Yeaman *et al.*, 2001). In normal endometrium, T cells represent the majority of leukocytes in the proliferative phase, followed by uNK cells and Mø (Lee *et al.*, 2015; Wira *et al.*, 2015). In contrast, in the late secretory phase, uNK cells comprise approximately 70–80% of total leukocytes, Mø about 30%, and T cells decrease to less than 10% (Flynn *et al.*, 2000; Wira *et al.*, 2015). Given the dynamic involvement of the endometrial immune niche in endometrial function, pregnancy establishment, tolerance of the semi-allogenic fetus (as sentinels of infection) and overall endometrial tissue homeostasis, abnormalities in this niche can have severe consequences. This review focuses mainly on immune abnormalities in eutopic endometrium and briefly ectopic endometriosis lesions in women with endometriosis and the relationships with disease establishment and symptomatology. To our knowledge, this is the first review that comprehensively compares all immune populations, activation states and variations across the menstrual cycle between eutopic endometrium from healthy women and women with endometriosis and ectopic lesions. To accomplish these goals, we have conducted a narrative review of the literature from 1982 to 2018 and have stated gaps in knowledge, controversies in findings and times where studies concur. Most of the studies conclude that the immune niche in women with endometriosis is compromised, and a major opportunity prevails for further investigation in robust and well-defined cohorts, pursuing advanced technologies for immune cell phenotyping and development of targeted therapeutics to improve the immune niche for endometrial homeostasis and pregnancy success.

Specific immune cells in normal eutopic endometrium and endometriosis

Macrophages

Mϕ in normal eutopic endometrium

Mø are key effector cells in both innate and humoural immunity as they recognize and phagocytose pathogens, act as antigen-presenting cells (APC) to activate T cells and have a role in tissue regeneration (Jensen *et al.*, 2012), all of which are important in their functions in endometrium (Fig. 2A). In normal endometrium, these cells are found within the luminal epithelium and the sub-epithelial stroma of the functionalis layer and in lymphoid aggregates and adjacent to the glandular epithelium in the lamina basalis (Kaldensjö *et al.*, 2011). They comprise approximately 10% of the total endometrial immune cell population in the proliferative phase (Givan *et al.*, 1997; Salamonsen *et al.*, 2002; Starkey *et al.*, 1991; Wira *et al.*, 2015), being the second most abundant endometrial leukocyte population after T cells (Ning *et al.*, 2016). They change in numbers in different menstrual cycle phases (Fig. 3), suggesting regulation by estradiol and progesterone (Berbic *et al.*, 2009; Bonatz *et al.*, 1992; Deloia *et al.*, 2002; King, 2000; De and Wood, 1990). Mø comprise 1–2% of endometrial cells in the proliferative phase, 3–5% in the secretory phase and the 6–15% in the menstrual phase (Salamonsen *et al.*, 2002). Their increased numbers during menses may be attributed to their phagocytotic properties and role in clearing cell debris and apoptotic cells during endometrial shedding (Khan *et al.*, 2005). They may also facilitate endometrial

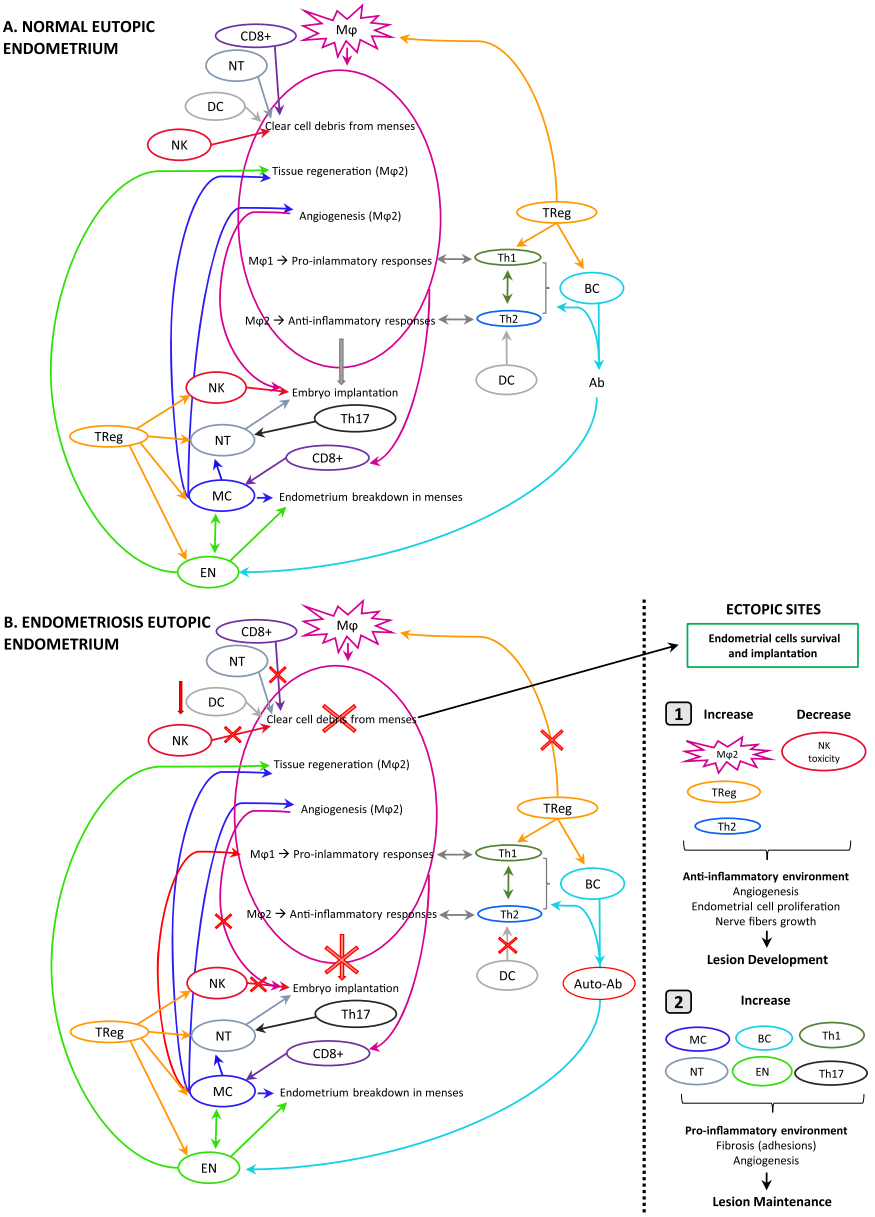

Figure 2 Schematic of interactions of immune populations in normal (A) and endometriosis eutopic endometrium with possible effects in ectopic sites (B). In normal conditions, Mø are involved in clearing cell debris during menses, tissue regeneration and angiogenesis. In addition, Mø1 have pro-inflammatory properties and Mø2 have anti-inflammatory properties. It has been described that Mø2 predominate in normal endometrium, indicating that eutopic endometrium in healthy women has an anti-inflammatory environment, which allows embryo implantation. In endometriosis, it has been suggested that the main population of Mø is Mø1, indicating that the environment is more pro-inflammatory, which affects embryo implantation. In addition, Mø do not increase in the secretory phase, as well as DC, which implies that cell debris can survive and migrate to the peritoneal cavity, implant and develop the ectopic lesions. In addition, Treg cells also promote an anti-inflammatory environment in normal eutopic endometrium. However, they are deregulated in women with endometriosis, which leads to a more pro-inflammatory environment. Another important aspect to consider is that NK are less cytotoxic in women with endometriosis, which affects embryonic implantation. In general, we can observe that in endometriosis eutopic endometrium, the anti-inflammatory functions are inhibited by the aberrant function of some immune populations. In addition, this affects the survival of endometrial cells, which can migrate to ectopic sites and develop the endomeriotic lesions. The peritoneal cavity could initially be an anti-inflammatory environment (increase of Mø2, Treg cells and Th2 and defective NK), allowing the implantation of endometrial cells in the ectopic sites. Upon lesion development, activation of pro-inflammatory immune populations, such as MC, NT, EN, Th1 and Th17, may occur, allowing the maintenance of the endometriotic lesion by promoting angiogenesis, fibrotic adhesions and an hospitable inflammatory environment preventing other immune cells from clearing ectopic lesions. However, all these interactions between immune cells need further investigation. Abbreviations: Mø1, macrophages 1; Mø2, macrophages 2; DC, dendritic cells; NK, natural killer cells; MC, mast cells; EN, eosinophils; NT, neutrophils; B cells, B lymphocytes; T cells, T lymphocytes; T helper cells (Th1, Th2 and Th17); Treg cells, regulatory T cells.

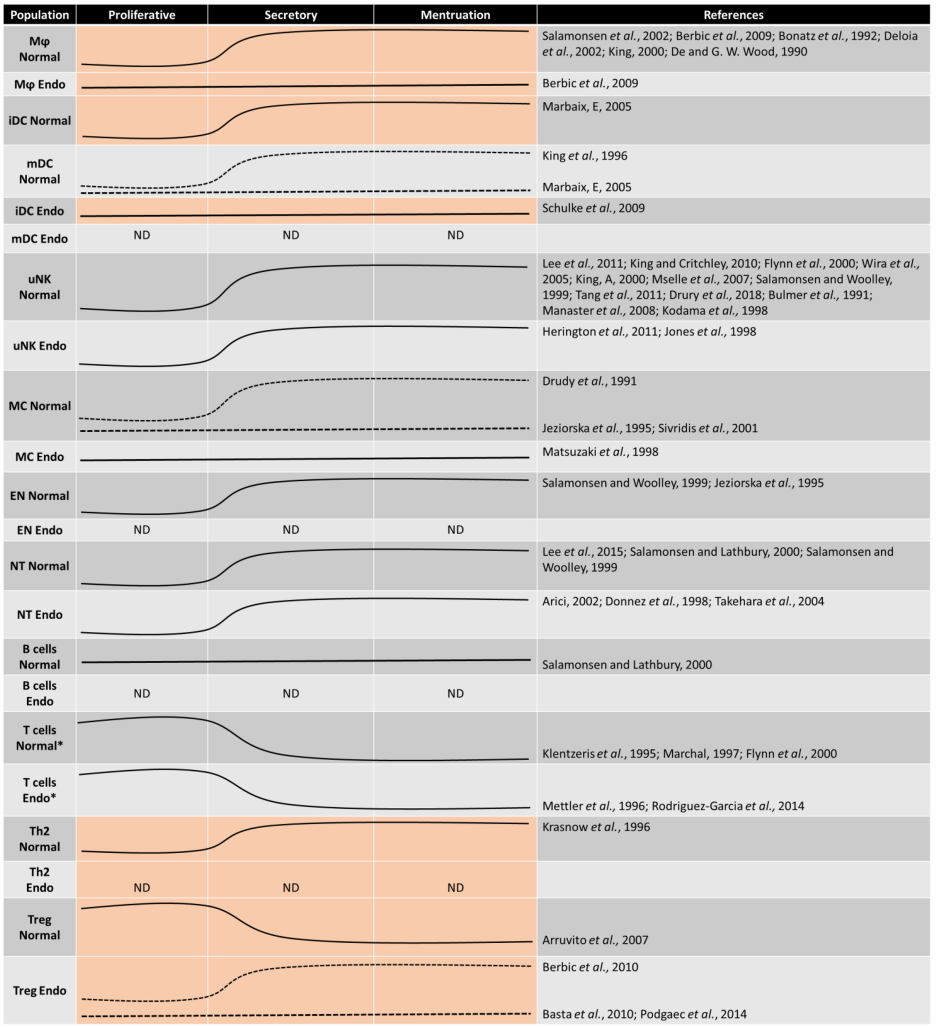

Figure 3 Fluctuation of immune populations throughout the menstrual cycle in normal eutopic endometrium and eutopic endometrium of women with endometriosis. Solid lines indicate increase/decrease or constant presence of each population in each menstrual cycle phase. Dotted lines indicate that populations with divergent behaviours have been reported in the literature. Orange rows indicate that the population behaves differently in eutopic endometrium of women with versus those without disease. *All T cells except Th2

regeneration, as they have a role in angiogenesis and wound healing in other tissues (Mantovani *et al.*, 2004; Stewart *et al.*, 1998).

Fluctuations of endometrial tissue-resident Mø numbers across the menstrual cycle may be direct or indirect effects of estradiol and progesterone. Whether Mø express the cognate steroid hormone receptors, oestrogen receptor-α (ERα), ERβ and progesterone receptor (PR) is controversial. One study, using immunohistochemistry and RT-PCR, detected ER and PR expression in endometrial Mø *in situ* and in suspension with endometrial stromal fibroblasts *in vitro* (Fujishita *et al.*, 1997; Khan *et al.*, 2005). Another study, using immunohistochemistry, reported no endometrial Mø steroid receptor expression (Stewart *et al.*, 1998). Thus, Mø numbers may be influenced by factors secreted by other endometrial cell types whose functions are directly regulated by steroid hormones. The discrepant observations are likely due to technical differences and antibody clones and await further validation.

Depending on activation state and surface markers, Mø are classified as 'classically activated' Mø (Mø1) or 'alternatively activated' Mø (Mø2) (Table I). This plasticity in phenotype is due to environmental cues (Gordon and Taylor, 2005). For example, tumour-associated Mø can make a bidirectional transformation between Mø1 and Mø2 phenotypes leading to pro- or anti-inflammatory sequelae, depending on their environment (Mantovani *et al.*, 2005; Ning *et al.*, 2016). Mø1 secrete pro-inflammatory factors, whereas Mø2 are involved in angiogenesis, anti-inflammatory processes and coordination of tissue repair (Jensen *et al.*, 2012; Mantovani *et al.*, 2004) (Fig. 2A). Activated Mø also express nitric oxide synthase (NOS) and cyclooxygenase 2 (COX-2) and secrete IL-1, IL-6, IL-8 IL-10 and IL-13, as well as TNFα and angiogenic factors (e.g. platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF)) (Ning *et al.*, 2016). Activation of Mø1 involves antigen presentation and production of IL-12, IL-23 and NOS, which activate T helper 1 (Th1) T cells and lead to a pro-inflammatory cascade. In contrast, Mø2 are involved in angiogenesis and tissue remodelling and secrete IL-10, which leads to an immunosuppressive phenotype and activation of Th2 cells (Ning *et al.*,

Table I Markers of immune cell populations described in endometrium.

Population	General markers	Tissue resident markers	Activation markers	Blood markers	Secretion molecules
Mϕ1	CD68, TLR4, CD14, HLA-DR^{High}, CD80, CD86	SAMHD1, CD4	CD80		IL-1, IL-6, IL-8, PGE2, HGF, IL-12, IL-23, NOS
Mϕ2	CD68, CD14^{Low}, TLR4, CD163, HLA-DR^{Low}, CD80^{Low}, CD86^{Low}, CD40, DC206	CD4	CD163		IL-10
iDCs	CD1a, CD11, HLA-DR^{Low}, CD209				MMP, IL-6, IL-10, IL-12, TNFα, RANTES, MCP-1
mDCs	CD1a, CD11, HLA-DR^{High}, CD83				MMP
NK	CD56	CD56^{High}, CD16^{Low}, CD9, CD94, CD69, CD151, HLA-DR^{High}, NKp30^{-}, NKp44^{-}	CD16, NKp46	CD56^{Low}, CD16^{High}	VEGF, ANG2, TNFα, TGFβ, IL-2, LIF, GM-CSF, CXCL10, CXCL12, IL-17
MC	CD2, CD25, CD35, CD88, CD203c		CD117		Heparin, Histamine, Tryptase, Chymase, IL-4, IL-5, IL-6, TNFα, LTC4, LTB4, PGD2, PAF, GM-CSF, IL-17
EN			EPO		IgE, EPO, IL-15, eotaxin, RANTES
NT	CD11b, CD14, CD15, CD16, CD62L, CD66b				VEGF, INFγ, IL-17
B cells	CD19, CD22, CD20		CD40, CD80, CD86, CD69		
T cells	CD3, TCR	CCR5	CXCR4		
CD8+ T cells	CD3, TCR, CD8				TNFα, INFγ, Granzymes, Perforin
CD4+ T cells	CD3, TCR, CD4				
CD4+ Th17	CD3, TCR, CD4, CD90, CCR6, RORC2				IL-17, IL-21, IL-22, CXCL1, CXCL5, IL-8, CCL2, CCL7, IL-10, IL-23
CD4+ Th1	CD3, TCR, CD4				IL-2, INFγ, Lymphotoxin, TNFα
CD4+ Th2	CD3, TCR, CD4				IL-4, IL-5, IL-6, IL-10
Treg cells	CD3, TCR, CD4, Foxp3, CD25, CD127^{-}				IL-10, TGFβ
NK T cells	CD3, CD56				
γδT cells	CD3, CD8^{-}, CD4^{-}, TCRγδ				IL-17

Abbreviations: Mø1, macrophages 1; Mø2, macrophages 2; iDC, immature dendritic cells; mDC, mature dendritic cells; NK, natural killer cells; MC, mast cells; EN, eosinophils; NT, neutrophils; B cells, B lymphocytes; T cells, T lymphocytes; CD8^{+} T cells, cytotoxic T cells; CD4^{+} T cells, T helper cells (Th1, Th2 and Th17); Treg, regulatory T cells; NK T cells, natural killer T cells; γδT cells, gamma-delta-T cells.

2016). Both subsets of Mø express the intracellular marker CD68, and although this marker is enriched in Mø, it is also expressed by other cell types including NK cells, DC, basophils, endothelial cells and fibroblasts in a variety of tissues (Jensen *et al.*, 2012). Mucosal Mø also express sterile alpha motif (SAM) and histidine aspartic (HD) domain containing deoxynucleoside triphosphate triphosphohydrolase 1 (SAMHD1) (Quillay *et al.*, 2015), which acts in response to viral infections by blocking virus replication. Furthermore, endometrial and decidual Mø also express the HIV-1 receptor molecule CD4, characteristic of T cells (Quillay *et al.*, 2015), suggesting this cell population may play a role in HIV transmission via the upper female reproductive tract (Quillay *et al.*, 2015).

Mø express CD14, as do monocytes and some granulocytes (Ziegler-Heitbrock and Ulevitch, 1993). The Mø cell surface pattern recognition receptor Toll-like receptor 4 (TLR4), a co-receptor of CD14, recognizes lipopolysaccharides (LPS), which are major cell surface components of gram-negative bacteria, and this interaction precipitates Mø-mediated bacterial phagocytosis and cell death (Ziegler-Heitbrock and Ulevitch, 1993). Moreover, Mø1 express higher CD14 than Mø2, although 30% of Mø2 also express CD14 and TLR4 (Jensen *et al.*, 2012). In contrast, CD163 is exclusively expressed in Mø2 and monocytes (Buechler *et al.*, 2000; Komohara *et al.*, 2006). Mø2 have low levels of major histocompatibility complex II (MHC-II or HLA-DR) as well as low levels of the activation markers CD80 and

CD86, in contrast to Mø1. In addition, Mø2 express high CD40, a co-stimulatory receptor (Jensen *et al.*, 2012) and the mannose receptor CD206 (Cominelli *et al.*, 2014).

In normal endometrium (Fig. 2A), the majority of Mø are $CD163^{+}CD14^{Low}$, corresponding to alternatively activated macrophages (Mø2) (anti-inflammatory phenotype) (Cominelli *et al.*, 2014; Jensen *et al.*, 2012). Mø2 can be further phenotyped depending on their surface markers and activation status: Mø2a, Mø2b and Mø2c (Jensen *et al.*, 2012). All these phenotypes secrete the anti-inflammatory interleukin IL-10. While Mø2a and Mø2c secrete low levels of pro-inflammatory cytokines, Mø2b produce high levels of inflammatory cytokines, including IL-1, TNFα and IL-6 (Mantovani *et al.*, 2004). Mø2b polarization is critically dependent on exposure to TLR or IL-1R agonists and is characterized by production of C-C motif ligand 1 (CCL1). The consequence of this activation is recruitment of Treg and immunoregulation (Mantovani *et al.*, 2004).

Mø in endometriosis eutopic endometrium

Mø have been widely studied in endometriotic lesions (see below), but little is known about them in endometrium of women with disease and how they (and other immune cells) contribute to disease establishment and the inhospitable environment for embryo implantation leading to increased risk of infertility and adverse pregnancy outcomes in women with disease (Vannuccini *et al.*, 2016). This is particularly true with regard to factors such as SAMHD1 involved in viral replication and the detailed activation status of Mø1 and characterization of Mø2 surface markers and activation status (Mø2a, Mø2b and Mø2c) compared with normal endometrium, as described above. Figure 2B depicts a model of likely interactions among endometrial immune cells in women with endometriosis, based on the available literature.

Mø abundance across the menstrual cycle in endometrium of women with endometriosis has mainly been evaluated using immunohistochemistry or flow cytometry (Cominelli *et al.*, 2014; Ning *et al.*, 2016; Vannuccini *et al.*, 2016). Three groups reported greater abundance of Mø in endometrium of women with endometriosis in the proliferative phase (Berbic *et al.*, 2009) or across all cycle phases and without cycle dependence, compared with normal controls (Khan *et al.*, 2010; Takebayashi *et al.*, 2015), a finding that was not confirmed by another group (Ding *et al.*, 2002). In women without endometriosis, increased Mø in the mid-late secretory phase initiate apoptosis of endometrial cells and phagocytose cell debris during endometrial shedding (Fig. 3). Since this increase does not occur in women with endometriosis (Berbic *et al.*, 2009), incomplete endometrial disruption and shedding could result, e.g. in different antigens displayed in desquamated tissue in the peritoneal cavity contributing to compromised clearance therein. Interestingly, eutopic endometrial cells in women with endometriosis are less susceptible to apoptosis (Ding *et al.*, 2002; Gebel M *et al.*, 1998; Liu *et al.*, 2006a). The lack of increased numbers of Mø during menses in women with disease reported by one group (Berbic *et al.*, 2009), along with aberrant secretion of pro-inflammatory factors, could, moreover, enhance survival of shed and refluxed endometrial cells, enabling them to establish endometriosis lesions in the pelvic cavity. Of note, eutopic endometrial cells from women with endometriosis have more invasive and adhesive phenotypes (Matarese *et al.*, 2003), consistent with their propensity to lesion formation.

While in healthy endometrium, Mø2 are the predominant phenotype (Cominelli *et al.*, 2014; Jensen *et al.*, 2012; Takebayashi *et al.*, 2015), in women with endometriosis, the endometrial Mø2 population is lower in all cycle phases compared with controls (Takebayashi *et al.*, 2015). These limited data in endometrium from women with the disease suggest that there is pro-inflammatory predominance in this tissue: a conclusion that warrants further validation. This is particularly important, as Mø activation can result in secretion of numerous pro-inflammatory cytokines and angiogenic and growth factors (see above), unfavourable to embryo nidation (Mor *et al.*, 2011). Activation of the nuclear factor kB (NFkB) pathway is a hallmark of Mø1 activation, and while it is characteristic of peritoneal Mø of women with versus women without endometriosis (Lousse *et al.*, 2008; see below), this has not been reported in endometrial Mø in women with disease. Notable is the −94 insertion/deletion ATTG polymorphism in the NFKB1 gene promoter in eutopic endometrium that correlates with increased endometriosis risk (Zhou *et al.*, 2010).

Interestingly, it has been suggested that endometrial infection may contribute to this altered phenotype, as *Escherichia coli* and endotoxin have been reported in menstrual and peritoneal fluid of women with endometriosis in contrast with controls (Khan *et al.*, 2010). These observations raise the possibility of an infectious component of endometriosis pathogenesis, and this and the pathogens eliciting the reported endometrial Mø1 dominance in endometriosis women warrant further study.

Several studies underscore differences in gene expression, angiogenesis, immune components, cytokine production and presence of nerve fibres in eutopic endometrium of women with and without endometriosis, although cells of origins of these factors have not been clearly established (Aghaey Meibody *et al.*, 2011; Bulun, 2009; Burney and Giudice, 2012; Ota *et al.*, 1996; Sharpe-Timms, 2001; Tamaresis *et al.*, 2014; Tokushige *et al.*, 2006). IL-1, IL-6 and IL-8 (Bondza *et al.*, 2009; Cao *et al.*, 2005) and hepatocyte growth factor (HGF) (Bondza *et al.*, 2009) are increased in endometrium of women with endometriosis compared with controls. Elevated levels of monocyte/macrophage-activating chemoattractant protein-1 (MCP-1) (Jolicoeur *et al.*, 1998) and macrophage inhibitory factor (Akoum *et al.*, 2006) further promote a pro-inflammatory environment in endometrium of women with disease. Interestingly, nerve fibres are increased in the functionalis of women with endometriosis (Al-Jefout *et al.*, 2007; Tokushige *et al.*, 2006). It is known that Mø play a role in nerve fibre growth, development and repair (Liuzzi and Tedeschi, 1991; Wu *et al.*, 2017). Nonetheless, it has been shown that Mø play an active role in pain by the production of cytokines, prostaglandins and neurotrophins, which can activate nerve fibres in some tissues (Ji *et al.*, 2016; Miller *et al.*, 2009). However, whether they promote such in endometrium of women with endometriosis is currently unknown and warrants further investigation.

Women with endometriosis have higher risk of infertility due in part to endometrial abnormalities (Bonci *et al.*, 2017; Lessey *et al.*, 2013) and of ectopic pregnancy and miscarriage (Bulleti *et al.*, 2010). Whether they are at higher risk of placental-related disorders (e.g. pre-eclampsia, antepartum hemorrhage), when pregnancy is achieved, is controversial (Maggiore *et al.*, 2016; Saraswat *et al.*, 2017; Stephansson *et al.*, 2009). The impact of the pro-inflammatory endometrial environment, including abnormal tissue-specific Mø, along with aberrations in other immune populations, such as uNK cells and Tregs,

on pregnancy outcomes of women with endometriosis awaits further study. Further phenotyping of the Mø populations within the endometrium of women with the disease is of paramount importance.

Mø in ectopic endometriosis

Endometriosis has been referred to as 'a disease of the Mø', as Mø are abundant in lesions where they are recruited and undergo alternative activation (Capobianco and Rovere-Querini, 2013). Mø2 predominate in lesions and are increased in the peritoneal cavity and peritoneal fluid of women with versus without endometriosis (Bacci *et al.*, 2009; Khan *et al.*, 2004). This anti-inflammatory environment may be permissive to lesion formation, activating T cell–mediated responses and promoting angiogenesis (Capobianco and Rovere-Querini, 2013). Other contributors to lesion formation include reduced phagocytosis functionality of peritoneal Mø in women with the disease (Chuang *et al.*, 2010) and Mø production of the potent angiogenic factor, VEGF (Capobianco and Rovere-Querini, 2013). Blood monocytes, which differentiate into tissue Mø, enhance endometrial cell proliferation when obtained from women with endometriosis, whereas monocytes from women without the disease suppress endometrial cell proliferation (Braun *et al.*, 1994), suggesting that circulating monocytes in women with endometriosis also contribute to endometriotic lesion development (Braun *et al.*, 1994).

Peritoneal Mø likely contribute to infertility and pelvic pain in women with endometriosis. They secrete specific cytokines associated with reduced fertility, which can compromise oviductal function, oocyte quality and early embryo development (Beste *et al.*, 2014). Pelvic pain in endometriosis patients correlates with nerve hypertrophy and density in lesions (Wu *et al.*, 2017), and higher Mø density in the lesions and peritoneal fluid correlate with lesion nerve fibre density (Morotti *et al.*, 2015). As Mø secrete factors (e.g. IL-1 and TNFα) that stimulate nerve growth factor, they may indirectly promote development and survival of nerve fibres (Morotti *et al.*, 2015) and thereby contribute to lesion-based pain.

Mø2 promote tumour development (Mantovani *et al.*, 2004), and although endometriosis is a benign disease, it shares characteristics with neoplastic processes, including inflammation and tissue invasion (Anglesio *et al.*, 2017; Vercellini *et al.*, 2000). Roles for Mø1 in ectopic endometrial cell invasion into the peritoneum and pelvic tissues, however, remain to be determined (Harada *et al.*, 2004; Liu and Lang, 2011).

Conclusions

Mø in endometrium of women without endometriosis normally increase peri-menstrually and likely facilitate desquamation of the tissue. In endometrium of women with endometriosis, however, Mø do not fluctuate during the cycle, which can predispose to enhanced cell survival and lesion formation. Importantly, in women with the disease, predominance of the endometrial Mø1 pro-inflammatory phenotype and pro-inflammatory cytokine secretion provide an inhospitable environment for pregnancy. Increased Mø2 in the peritoneal cavity and ectopic lesions suggest this cell population contributes to an anti-inflammatory environment permissible for lesion establishment and growth. Moreover, Mø2 in the peritoneal cavity and lesions may contribute to pain experienced by women with endometriosis by promoting nerve fibre growth. Interestingly, Mø in peritoneal fluid of women with endometriosis exhibit activation of the NFkB pathway, underscoring a pro-inflammatory phenotype in contrast to the anti-inflammatory/permissive environment for establishment of disease. The presence of extensive fibrosis and inflammation in the pelvis of women with endometriosis is a testimony to a mixed phenotype of pro- and anti-inflammatory factors operating in the pelvis, albeit without a comprehensive understanding of the dynamics and timecourse. While the Mø phenotypes in women with versus those without endometriosis are different, further studies are needed to understand the molecular and functional differences between Mø in normal endometrium and eutopic and ectopic endometrium of women with the endometriosis. New single-cell sequencing technologies offer great promise for deep phenotyping different Mø populations and activation status for disease classification and development of novel therapeutics to optimize the immune environment for symptom control and pregnancy.

Dendritic cells

DC in normal eutopic endometrium

DC are specialized APCs that are actively involved in the immune responses in mucosal surfaces, including endometrium (Schulke *et al.*, 2008). Depending on their origin and secreted molecules, DC are classified into plasmocytoid (CD123$^+$, HLA-DR$^+$) (lymphoid origin) and myeloid (CD1a$^+$, CD11c$^+$, HLA-DR$^+$) (hematopoietic origin) (Lee *et al.*, 2015) (Table I). Plasmocytoid DC (pDC) are involved in recognition of viruses and produce interferon (IFN), whereas myeloid DC are involved in T-cell activation (Ueno *et al.*, 2007). Myeloid DC, most relevant to endometrium and endometriosis, are classified by their maturation status. Immature DC (iDC) are present in peripheral tissues where they recognize and process foreign antigens (Schulke *et al.*, 2008). They become mature DC (mDC) in response to inflammatory factors and foreign antigens and then present the antigen to T cells expressing MHC molecules (Schulke *et al.*, 2008). DC can communicate directly and indirectly with T cells, B cells, natural killer (NK) cells and Mø (Banchereau and Steinman, 1998), and thus their normal maturation process and functionality are a key in the immune network within tissues.

DC are present in endometrium in low levels and are located within the luminal epithelium (functionalis layer) and in the basalis layer (Kaldensjö *et al.*, 2011). iDC (CD1a$^+$, CD209$^+$, HLA-DR^{Low}) increase in numbers in the secretory and menstrual phases in the basal layer (Marbaix, 2005) (Fig. 3). There is controversy about whether mDC, expressing CD83 and HLA-DR^{High}, fluctuate throughout the cycle, as one report indicated they do not (Marbaix, 2005) and another found increased mDC cell density in the late secretory and menstrual phase and more in the basalis than in the functionalis (King *et al.*, 1996) (Fig. 3). It has been postulated that influx, but not maturation, of DC in endometrium is regulated by sex hormones (King *et al.*, 1996), although these cells do not express ER or PR (King *et al.*, 1996). Thus, if iDC fluctuate throughout the cycle in the basalis, they may be indirectly modulated by cytokines or chemokines secreted by other leukocytes or endometrial cell populations. Increased iDC during the secretory and menstrual phases suggests a role in menstruation, and, indeed, they produce matrix metalloproteinases (MMPs) that are important in this process (Marbaix, 2005). iDC also produce cytokines and chemokines, including IL-6, IL-10, IL-12, TNFα, RANTES and MCP-1, which could promote menstruation or facilitate embryo nidation and regulate other

lymphocyte populations (Nagorsen *et al.*, 2004; Schulke *et al.*, 2008). Furthermore, iDC are higher in numbers compared to mDC regardless of the menstrual cycle phase (King, 2000; Rieger *et al.*, 2004; Schulke *et al.*, 2008), possibly due to mDC migration outside the uterus to secondary lymphoid organs.

DC in endometriosis eutopic endometrium

In contrast to normal eutopic endometrium wherein iDC increase during the menstrual phase, this pattern is not observed in endometriosis (Schulke *et al.*, 2009) (Figs 2B and 3) and has been suggested to contribute to inefficient clearance of endometrial debris shed during the menses and facilitating establishment of endometriotic lesions. The density of iDC is higher than mDC (Schulke *et al.*, 2009) and greater in the basalis (but not the functionalis) in the proliferative phase in women with the disease compared with controls (Schulke *et al.*, 2009). Furthermore, mDC density is significantly reduced in both endometrial layers of women with the disease (Maridas *et al.*, 2014; Schulke *et al.*, 2009), suggesting defective DC maturation in women with endometriosis.

DC in ectopic endometriosis

DC are directly involved in coordinating immune responses, and alterations in these cells could affect the progression and pathogenesis of endometriosis. iDC are present in lesions and are significantly increased in adjacent peritoneum compared with control peritoneum (Schulke *et al.*, 2009). As with Mø, DC may promote neuroangiogenesis in both eutopic and ectopic endometrium of women with the disease (Schulke *et al.*, 2009). This fact, together with the increase of pro-inflammatory cytokines and chemokines in these tissues, may be important in pain generation and also increased pain perception of women with endometriosis due to the down-regulation of opioid receptors (Schulke *et al.*, 2009). Moreover, in murine models of endometriosis, flow cytometry and immunofluorescence demonstrated that iDC infiltrate endometriotic lesions and increase angiogenesis and lesion growth (Fainaru *et al.*, 2007). Therefore, blocking DC function or interfering with their invasion into lesions may provide a novel therapeutic approach to abrogate lesions and potentially associated pain symptoms.

Notably, iDC are reduced in peripheral blood in the menstrual phase in women without disease but not in endometriosis patients. Moreover, a decrease in mDC in peripheral blood was observed in women with endometriosis as well as in eutopic endometrium (Maridas *et al.*, 2014). To our knowledge, in women with endometriosis, pDC have been only studied in peripheral blood. In women without endometriosis, they decrease from the beginning to the end of the cycle. In contrast, in women with endometriosis, they increase as the cycle progresses (Maridas *et al.*, 2014). The clinical implications of circulating pDC dynamics across the menstrual cycle remain to be determined. This dysregulation of circulating DC in women with disease may contribute to inefficient or aberrant immunological targeting of endometrial fragments shed at menstruation, facilitating their survival and establishment of endometriosis.

Conclusions

In summary, DC, like Mø, can act in ectopic lesions to increase neuroangiogenesis and contribute to lesion growth and pain transmission. Normally, they are increased in endometrium in the secretory and menstrual phases, but they do not fluctuate cyclically in endometriosis endometrium. In general, in lesions and eutopic endometrium of women with endometriosis, iDC are increased compared to eutopic endometrium of women without the disease, suggesting a failure in DC maturation in women with endometriosis, leading to an altered clearance of endometrial cells shed at menstruation.

Natural killer cells

NK cells in normal eutopic endometrium

NK cells are key components of the innate immune system, acting as the first line of defence against viral infections and tumour growth, and are important for normal tissue homeostasis (Thiruchelvam *et al.*, 2016). uNK cells are the predominant leukocyte population in human endometrium (Parkin and Fazleabas, 2016), comprising 30–40% (Manaster *et al.*, 2008) of total leukocytes in the proliferative phase and up to 70% in the secretory phase (Drury *et al.*, 2018; Flynn *et al.*, 2000; King, 2000; King and Critchley, 2010; Lee *et al.*, 2011; Manaster *et al.*, 2008; Mselle *et al.*, 2007; Salamonsen and Woolley, 1999; Tang *et al.*, 2011; Wira *et al.*, 2005) (Fig. 3). This cycle variation suggests steroid hormones affect migration of circulating NK cells into the tissue followed by subsequent alteration of their phenotype by the tissue microenvironment or that steroid hormones directly stimulate proliferation of resident endometrial tissue uNK cells. ERα and PR have not been detected in uNK by RT-PCR or immunohistochemistry (Bulmer *et al.*, 1991; King *et al.*, 1996), although ERβ1 immunoreactivity has been detected (Henderson *et al.*, 2003). Thus, uNK cell proliferation and migration into the endometrium may be regulated by cytokines, chemokines and other factors produced by steroid hormone–responsive endometrial cells and perhaps other immune cells (Lee *et al.*, 2015).

uNK differ from their counterparts in the peripheral blood, which comprise about 1% of all circulating lymphocytes (Vivier *et al.*, 2008). uNK are $CD9^{+}CD3^{-}CD56^{High}CD16^{Low}$, whereas peripheral blood NK cells are $CD9^{+}CD3^{-}CD56^{Low}CD16^{High}$ (King, 2000; Koopman *et al.*, 2003; Lee *et al.*, 2011; Salamonsen and Woolley, 1999; Yang *et al.*, 2011) (Table I). uNK express low levels of CD16 (Mselle *et al.*, 2007), an immunoglobulin superfamily member receptor that induces gene transcription of inflammatory cytokines in NK cells. Expression of CD16 coincides with increased cytotoxic activity of NK cells (Tang *et al.*, 2011), which is regulated by activating receptors such as NKp30 and NKp46 (Vacca *et al.*, 2011) and inhibiting receptors such as killer immunoglobulin-like receptor (KIR) and immunoglobulin-like transcript (Dosiou and Giudice, 2005). Despite overexpression of some activation markers, uNK cytotoxicity is diminished in the secretory phase (Salamonsen and Lathbury, 2000; Souza *et al.*, 2001; Yovel *et al.*, 2001), consistent with their $CD56^{High}CD16^{Low}$ phenotype and lack of activating NKp30 and NKp44 receptors (Manaster *et al.*, 2008). Moreover, uNK cells express CD151 (Mselle *et al.*, 2007) and are $HLA\text{-}DR^{High}$, which are increased in the secretory phase together with CD94 and CD69 (Kodama *et al.*, 1998), confirming that uNK cells increase in this phase.

Although their cytotoxicity is low, uNK mediate anti-microbial functions (Mselle *et al.*, 2007); however, their major function is believed to facilitate embryo implantation and a suitable environment for the developing placenta (Mselle *et al.*, 2007). To this end, uNK cells produce and secrete angiogenic factors, including vascular VEGF and angiotensin 2 (ANG2), that promote maturation of endometrial blood vessels,

important for successful implantation and pregnancy establishment (Li et al., 2015a; Lysakova-Devine and O'Farrelly, 2014). uNK also regulate trophoblast invasion and promote successful placental development by their secretion of cytokines such as TNFα, TGFβ, IL-2, LIF, GM-CSF, CSF-1, CXCL10 and CXCL12 (Li et al., 2015a). Thus, uNK are key players in implantation (Thiruchelvam et al., 2015) (Fig. 2A), and dysregulation of their numbers or functionality could lead to failed embryo implantation or aberrant placentation, as reported in women with endometriosis (Fig. 2B) (Thiruchelvam et al., 2015).

NK cells in endometriosis eutopic endometrium

In endometrium of women with endometriosis, uNK cells increase in the secretory phase, as in women without the disease (Herington et al., 2011; Jones et al., 1996) (Fig. 3) although whether the absolute numbers are the same as in normal endometrium is controversial, probably due to the samples and techniques used (Giuliani et al., 2014; Yang et al., 2011). One group found fewer uNK cells in women with endometriosis versus controls (Yang et al., 2011), and another found no differences (Giuliani et al., 2014). Also, uNK cells in one study were demonstrated to have less cytotoxicity in women with compared to those without endometriosis (Petta et al., 2010), although another found that infertility status was important. Specifically, $CD56^+$ uNK cells from women with infertility versus fertile controls expressed CD16 and higher NKp46, markers of cytotoxicity (Giuliani et al., 2014). Moreover, only women with endometriosis and infertility or recurrent pregnancy loss expressed these markers, while those with endometriosis who were fertile did not (Giuliani et al., 2014). It has been suggested that $CD16^+$ uNK cells may produce cytotoxic factors in response to trophoblast cells, which could contribute to infertility and/or miscarriage, or placentation abnormalities, which are more prevalent in women with endometriosis (Giuliani et al., 2014) (Fig. 2B).

uNK cells have been proposed to originate from hematopoietic stem cells in the endometrium and to exist in four maturational stages: (i) wherein uNK cells express CD10 (an endometrial stromal marker) and CD34 (a hematopoietic stem cell marker); (ii) wherein they express CD34 and CD117 (the latter a hematopoietic stem cell marker); (iii) with the phenotype of $CD34^-CD117^+CD94^-$ (the latter is expressed by NK and $CD8^+$ T cells); and (iv) with the phenotype of $CD34^-CD117^{-/+}CD94^+$ (Giuliani et al., 2014). Interestingly, more immature uNK (Stages I and II) were found in the eutopic endometrium of women with endometriosis compared to controls (Giuliani et al., 2014). Thus, *in situ* development of mature uNK cells appears to be dysfunctional in women with endometriosis, potentially contributing to abnormal endometrial development, abnormal placentation, increased implantation failure and poor pregnancy outcomes, all of which are more common in women with endometriosis.

NK cells in blood and ectopic endometriosis

In endometriosis, most studies have focused on NK cells in peripheral blood or in peritoneal fluid (Dias et al., 2012; Oosterlynck et al., 1994; Thiruchelvam et al., 2015). The majority have found no differences in the numbers of circulating NK cells in women with versus those without disease (Oosterlynck et al., 1994; Thiruchelvam et al., 2015), although one study reported an increase of peripheral NK cells in women with deep infiltrating endometriosis (Dias et al., 2012). Moreover, higher numbers of immature peripheral NK cells have been reported in women with endometriosis compared to normal controls, similar to eutopic endometrium of women with the disease (Kikuchi et al., 1993; Thiruchelvam et al., 2016). Interestingly, after surgical removal of disease, peripheral differentiated (mature) NK cell numbers increased, suggesting that endometriosis and its associated chronic disease status affect NK differentiation (Kikuchi et al., 1993).

NK cells in peritoneal fluid of women with endometriosis have lower cytotoxicity than uNK cells in women with disease or in eutopic endometrium of controls (Thiruchelvam et al., 2015). KIR2DL1, an inhibitory factor of KIR (which is a cytotoxic activation factor), is upregulated in peritoneal fluid NK cells in women with compared to those without endometriosis (Matsuoka et al., 2005), likely contributing to the observed lower cytotoxicity of uNK in women with endometriosis. In addition, the expression of platelet-derived TGFβ1 suppresses the expression of NKG2D ligands (sMICA and sMICB), resulting in reduced cytotoxicity of NK in women with disease (Du et al., 2017; Guo et al., 2016). This decreased cytotoxic activity could lead to reduced endometrial fragment clearance by NK cells in the peritoneal cavity, thereby potentiating the implantation of ectopic implants.

Conclusions

In summary, in normal endometrium, uNK cells have low cytotoxic activity. In endometrium of women with endometriosis, their activity is even more reduced than in normal endometrium, but when the endometriosis patient is also infertile and/or presents with recurrent miscarriage, uNK cytotoxic activity is increased. Thus, it appears that uNK cells from women with endometriosis are immature and that uNK cytotoxic activity could be an indicator of endometriosis-related infertility and recurrent miscarriage. These findings warrant further patient stratification and deep characterization of uNK cell phenotypes, given the centrality of uNK cells in the process of pregnancy establishment and maintenance. Moreover, with regard to the lesions, NK cells in these have blunted cytotoxic activity, which could favour endometrial cells and fragments establishing peritoneal disease. This is an area of great potential for novel therapeutic development.

Mast cells (MC)

MC in normal eutopic endometrium

MC are granulocytes that play a pivotal role in fibrosis, angiogenesis, inflammation, wound healing and tissue remodelling (Galli, 2000, 1993). When activated, they release heparin and histamine, which can also promote fibroblast and smooth muscle cell contraction (Yamamoto et al., 2000). MC also release chemotactic factors for eosinophils (EN) (Jeziorska et al., 1995). In endometrium, they are activated prior to menstruation and participate in shedding of the tissue through a combination of ischemic vasospasm of the spiral arteries and induction of matrix degrading enzymes in the stromal fibroblasts (Zhang et al., 1998). They are also involved in tissue regeneration (Mori et al., 1997), have a role in neoangiogenesis (Galli, 2000) and are markedly decreased in endometrium of menopausal women, consistent with their role in menstruation (Galli, 2000).

Low levels of MC are present in the functionalis and basalis levels throughout the menstrual cycle (Jeziorska et al., 1995; Sivridis et al., 2001). Some studies suggest no cycle variation, although their activation status increases pre-menstrually (Jeziorska et al., 1995; Sivridis et al., 2001) (Fig. 3), suggesting regulation by sex steroid hormones (Xu et al., 2007). MC activation triggers a cascade of matrix-degrading

enzymes (Berbic *et al.*, 2014), essential for menstruation. However, one study reported an increased MC activation throughout the cycle, peaking pre-menstrually in healthy endometrium and in women with dysfunctional uterine bleeding (Drudy *et al.*, 1991). These discrepancies are likely attributable to technical factors involved in determining MC activation and perhaps patient selection. To our knowledge, whether MC express either ER or PR has not been reported, so their fluctuation across the menstrual cycle could be an indirect effect of factors secreted by other cells.

MC express CD2, CD25, CD35, CD88, CD117 and CD203c (Table I). The mast/stem cell receptor CD117 (cKIT) is an activation marker and a receptor tyrosine kinase protein. MC have a heterogeneous phenotype depending on their tissue location (Tainsh *et al.*, 1991) and granule content of serine proteases. MC containing only tryptase is in the functionalis layer, whereas MC containing tryptase, chymase, carboxypeptidase and cathepsin G-like protease is predominantly in the basalis layer and myometrium (Saunders *et al.*, 2017). Recently, three distinct MC subtypes were described: (i) MC tryptase-positive, which express ERα; (ii) MC chymase-positive; and (iii) MC tryptase−/chymase-positive. Moreover, endometrial MC express ERβ1. There is agreement among studies that MC positive for tryptase and chymase are higher in the myometrium and in the endometrial basalis layer in all phases of the cycle (Saunders *et al.*, 2017). We suggest their function in basalis endometrium is to support tissue regeneration after menstruation, as MC are also involved in wound healing and angiogenesis in multiple tissues (Galli, 2000; Galli, 1993).

MC in endometriosis eutopic endometrium

There is controversy in the literature about MC cycle fluctuation and activation states in endometrium of women with endometriosis. One group found no cycle dependence (Matsuzaki *et al.*, 1998) (Fig. 3). Others have reported increased infiltration of MC in endometrium in women with compared to those without disease and increased activation of MC in ectopic lesions, while activated MC in eutopic endometrium was rarely detected (Sugamata *et al.*, 2005). Soluble products such as IL-4, IL-5, IL-6 and TNFα secreted by MC (Jeziorska *et al.*, 1995) stimulate the production of collagenase and PGE2 by fibroblasts (Abbas *et al.*, 2008) and stimulate Mø, which, in turn, may produce pro-inflammatory factors within the endometrium. MC also secrete LTC4, LTB4, PGD2, PAF, IL-5 and GM-CSF, which are chemoattractant for EN and neutrophils (NT) (Jeziorska *et al.*, 1995). These observations support that MC have a role in the influx into endometrium of other inflammatory cells, such as NT, EN and Mø, which are also increased in the menstrual phase of women with endometriosis (Jeziorska *et al.*, 1995) (Fig. 2B). Functionalis/basalis layer partitioning of MC described above in normal endometrium has not been studied in women with endometriosis and is worthy of further study.

MC in ectopic endometriosis

MC are greatly increased in peritoneal endometriosis, compared with eutopic endometrium of women with disease (Matsuzaki *et al.*, 1998). Moreover, degranulated (i.e. activated) MC in the stroma of endometriotic lesions are greater than in eutopic endometrium of women with and without endometriosis (Sugamata *et al.*, 2005). As MC activation is implicated in the formation of fibrous adhesions, it is likely that MC play a major role in endometriosis lesion formation and accompanying scarring and fibrosis (Sugamata *et al.*, 2005). Moreover, a significant increase in the number of chymase-positive cells and endogenous chymase expression were observed in the endometriotic lesions, further suggesting that MC have a role in the development of the disease (Paula *et al.*, 2015). Furthermore, an overexpression of kynurenine by endometriotic cells activates the aryl hydrocarbon receptor, a transcription factor that responds to environmental toxins and endogenous compounds and is present in MC. These cells respond to the receptor activation by producing IL-17 and reactive oxygen species, which increase inflammation in ectopic lesions (Mariuzzi *et al.*, 2017).

Conclusions

In summary, in normal endometrium, MC are involved in the menstrual process, facilitating tissue desquamation and attracting other immune populations into the tissue to participate in this process. In contrast, eutopic endometrium of women with endometriosis, MC do not fluctuate across the cycle (although not all studies agree), suggesting aberrant regulation and/or function of these cells in women with disease. Moreover, MC are increased in endometriosis lesions, where they can exacerbate inflammation by releasing pro-inflammatory factors that, in turn, can increase infiltration of other immune populations to the lesion. Further studies are needed to fully elucidate MC function in endometriosis pathogenesis and pathophysiology.

Eosinophils

EN in normal eutopic endometrium

These leukocytes display specialized phenotypes regulated largely by MC, monocytes and T cells (Fig. 2A). They participate in inflammation in human diseases such as asthma, skin disorders and heart disease. EN bind to immunoglobulin E (IgE), which is involved in initiation of inflammatory responses (Gleich and Adolphson, 1986). In general, their mediators have vasoactive effects and promote histamine release by MC and activation of Mø and endothelial cells (Jeziorska *et al.*, 1995). Usually, their degranulation marker, eosinophil peroxidase (EPO) (Table I), identifies EN. Moreover, other chemoattractant molecules, not specific to EN, have been used to determine their presence in tissues, including IL-5, eotaxin and RANTES (Blumenthal *et al.*, 2000).

EN represent 3–5% of the total endometrial cell population in menstrual phase (Salamonsen and Woolley, 1999). They are absent between Cycle Days 5–26 and appear on Days 27 and 28 and persist during menstruation (Jeziorska *et al.*, 1995) (Fig. 3), suggesting a role for them in menstrual shedding and endometrial tissue regeneration (Hornung *et al.*, 2000). Notably, EN secrete MMPs, a key to these processes (Salamonsen and Woolley, 1999). Furthermore, EN increase fibroblast proliferation and collagen production (Birkland *et al.*, 1994), supporting a role for them in endometrial regeneration. To our knowledge, it has not been described that EN express ER or PR.

EN in endometriosis eutopic endometrium

To our knowledge, there is no information regarding EN in eutopic endometrium of women with endometriosis. However, an EN-related protein, eotaxin, a chemoattractant for EN, is expressed in endometrium of women with disease and may have a role in endometriosis-associated infertility by leading to endometrial or

blastocyst dysfunction and impaired implantation (Hornung et al., 2000). Specifically, endometrial epithelial cells secrete eotaxin (Zhang et al., 2000), a chemokine, and we have demonstrated that it is overexpressed by a specific set of cells (LGR5$^+$ cells) in eutopic endometrium of women with deep infiltrating endometriosis (Vallvé-Juanico et al., 2017). Thus, this protein may be related to reproductive outcomes and/or to aggressiveness of the disease (Vallvé-Juanico et al., 2017). As a chemoattractant for EN, overexpression of eotaxin in eutopic endometrium of women with endometriosis would suggest higher levels of EN in women with versus without endometriosis. However, this awaits future confirmation.

EN in ectopic endometriosis

EN degranulation promotes fibrous adhesions (Blumenthal et al., 2000), which are a hallmark of endometriosis. It has been postulated that infiltration and degranulation of EN are due to MC activation (Sugamata et al., 2005), as MC activation is characteristic of endometriotic lesions (Paula et al., 2015); a potential role for activated EN in adhesion formation in the disease is plausible although unproven. High levels of eotaxin are present in peritoneal fluid of women with severe endometriosis compared with those with minimal or mild disease or no disease (Hornung et al., 2000), likely produced by endometriotic lesion epithelial cells. Unfortunately, there is a lack of EN data, and further studies are needed to address their role(s) in ectopic lesion pathophysiology.

Conclusions

The above observations support a role for EN in menstruation normally, and in promoting a pro-inflammatory environment in endometrium of women with endometriosis, contributing to their poor reproductive outcomes (Fig. 2). There is a need for further investigation of this immune cell population in eutopic endometrium of women with (and without) endometriosis.

Neutrophils

NT in normal eutopic endometrium

Neutrophils, or polymorphonuclear leukocytes (PMN), are the most abundant circulating leukocytes in humans and have an essential role in the innate immune response to foreign pathogens (Kobayashi and Deleo, 2009). CD11b, CD14, CD15, CD16 and CD62L have been used individually or in combination to identify NT (Elghetany et al., 2004), although they are not specific markers for NT. Recently, a flow cytometry panel of 374 CD markers revealed NT consistently express CD11b, CD16 and CD66b, independent of cell location, level of activation and disease state (Lakschevitz et al., 2016) (Table I).

NT represent 6–15% of the total endometrial cells at menstrual phase (Lee et al., 2015; Salamonsen and Lathbury, 2000; Salamonsen and Woolley, 1999) (Fig. 3). They express high levels of VEGF, as do endometrial epithelial and stromal cells (Mueller et al., 2000), and are more abundant in the secretory and menstrual versus proliferative phases (Donnez et al., 1998; Mueller et al., 2000; Song et al., 1996; Takehara et al., 2004). Their increase in menstrual endometrium likely derives from chemotactic factors secreted by MC (Jeziorska et al., 1995), Mø (e.g. IL-8) and other cells. Although NT do not express ER or PR (King et al., 1996; Mueller et al. 2000), their cycle variation suggests indirect regulation by other steroid hormone–responsive endometrial cells. These observations suggest an important role for NT in regulating cyclic endometrial vascular proliferation and tissue repair.

Endometrial NT also produce IFNγ (Yeaman et al., 1998), a cytokine that regulates cellular proliferation, differentiation and immune responses important in implantation and/or maintenance of pregnancy (Tabibzadeh, 1994). Roles of IFNγ in human endometrial function await further study.

NT in endometriosis eutopic endometrium

In endometrium of women with endometriosis, IL-8, chemoattractant to NT and secreted by epithelial cells, is elevated in the late secretory and early proliferative phases (Arici, 2002), suggesting NT are higher in endometrium of women with versus without disease (Fig. 3). In women with endometriosis, VEGF and NT are increased in pre-menstrual endometrium (Donnez et al., 1998; Takehara et al., 2004), leading to the postulate that NT in endometrium of women with the disease overexpress VEGF and thus contribute to lesion establishment from the shed tissue.

NT in ectopic endometriosis

VEGF is the major angiogenic factor overexpressed in ectopic endometrium and produced by the epithelial and stromal cells (Osuga et al., 2016; Taylor et al., 2002). Thus, NT-derived VEGF may contribute to angiogenesis in endometriosis lesions. Of note, IL-17A produced by Th17 T cells in endometriosis is chemotactic to NT, believed to be important in establishment of lesions, in part, due to robust angiogenesis involving VEGF and other factors (Osuga et al., 2016). The number of NT is increased in the peritoneal cavity of women with endometriosis. NT produce angiogenic factors such as VEGF and pro-inflammatory cytokines, including IL-8 and CXCL10, and also reactive oxygen species,which may promote disease progression (Izumi et al., 2018). Highly supportive of this postulate are studies using a mouse model of endometriosis in which depletion of NT decreased the number of endometriotic lesions (Takamura et al., 2016). In addition, neutrophil extracellular traps are increased in the peritoneal fluid of women with endometriosis (Berkes et al., 2014). In particular, they are higher in deep infiltrating endometriosis and thus may be involved in aggressive disease pathophysiology (Munrós et al., 2019). These data demonstrate a role for this cell type in disease establishment and underscore novel approaches for developing innovative targeted therapies for endometriosis.

Conclusions

Angiogenesis is a hallmark of endometrial physiology, and VEGF is the major angiogenetic factor in vascularization of this tissue throughout the cycle, after menstrual shedding and during tissue regeneration. NT, one of the three main endometrial VEGF-expressing cells, is a strong candidate for participation in this process normally. While elevated VEGF and NT in eutopic endometrium of women with endometriosis suggest a role for NT in endometriosis pathophysiology, further study on NT in cycling endometrium of women with disease is warranted, with a focus on associated heavy bleeding, infertility and poor pregnancy outcomes. NT are likely also involved in neoangiogenesis in endometriosis lesions. Advanced single-cell technologies and animal models are anticipated to provide more detailed NT phenotyping and functionality in endometrium in women with and without endometriosis and in the disease lesions per se.

B cells

B cells in normal eutopic endometrium

B cells are responsible for the humoural immune response by producing antibodies to foreign antigens. They express specific markers, CD19 (Kurosaki, 2011), CD22 and CD20 (Kaminski *et al.*, 2012) that interact with foreign antigens, resulting in their activation. Early cell-surface changes associated with B-cell activation include up-regulation of CD40, CD80, CD86 and CD69 (Kaminski *et al.*, 2012) (Table I). Activated B cells differentiate either into plasma cells capable of antibody secretion or memory cells that can provide long-lived protection against secondary infection (Harwood and Batista, 2010). B cells represent 1–2% of total leukocytes in normal eutopic endometrium and do not exhibit cycle dependence (Salamonsen and Lathbury, 2000). Whether endometrial B cells express ER and/or PR has not been reported.

B cells and autoantibodies in endometriosis eutopic endometrium

Four studies have characterized B cells in eutopic endometrium of women with endometriosis compared to controls without the disease. Two found no differences in the number of B cells (Klentzeris *et al.*, 1995; Witz *et al.*, 1994), and two found increased numbers (Antsiferova *et al.*, 2005; Scheerer *et al.*, 2016) versus controls. Since it has been postulated that endometriosis could be an autoimmune disease, different authors have also measured the presense of autoantibodies in serum and endometrial secretions (Mathur *et al.*, 1982; Klentzeris *et al.*, 1995; Witz *et al.*, 1994). Passive haemagglutination and immunofluorescence assays revealed that serum antibody titres to endometrium, ovary, theca cells and granulosa cells were significantly higher in women with versus without endometriosis (Mathur *et al.*, 1982). Immunofluorescent antibody assays of biopsied endometrial tissue (and also sera) from women with the disease revealed that the antibodies were mainly IgG and IgA (Mathur *et al.*, 1982). Interestingly, no differences were observed regarding CD22 (a surface marker of mainly mature B cells that prevents overactivation of the immune system and development of autoimmune diseases) between eutopic (and ectopic) endometrium of women with compared to those without endometriosis (Klentzeris *et al.*, 1995; Witz *et al.*, 1994). Further studies are warranted to distinguish B cell numbers, cycle dependence and autoantibody production in endometrium of women with and without the disease, to determine possible specific auto-immunity to eutopic endometrium in the setting of disease. Far more information is available regarding these factors in ectopic endometriosis lesions (see below).

B cells in ectopic endometriosis

In ectopic endometriosis lesions, peritoneal fluid and serum, B cells produce autoantibodies, or antibodies against endometrial epitopes (Straub, 2007; Gajbhiye *et al.*, 2008). For this reason, among others, it has been postulated that endometriosis is an autoimmune disease (Nothnick, 2001). It is unclear whether the number of B cells in peritoneal fluid or in peripheral blood differs in endometriosis patients compared to controls. An increase (Antsiferova *et al.*, 2005; Badawy *et al.*, 1989; Berbic *et al.*, 2013; Chishima *et al.*, 2000; Gleicher *et al.*, 1987; Hever *et al.*, 2007; Lachapelle *et al.*, 1996; Odukoya *et al.*, 1996a; Odukoya *et al.*, 1996b; Riccio *et al.*, 2017; Scheerer *et al.*, 2016), a decrease (Gagné *et al.*, 2003; Oosterlynck *et al.*, 1993) or no significant differences (Christofolini *et al.*, 2011; Gebel *et al.*, 1993; Klentzeris *et al.*, 1995; Nomiyama *et al.*, 1997; Witz *et al.*, 1994; Yeol *et al.*, 2015) in B cell numbers in peritoneal fluid and in the circulation have been reported in patients with endometriosis compared to controls. It is difficult to compare the results of these various studies because samples analysed included ectopic endometriosis lesions, peritoneal fluid (reflecting the peritoneal cavity environment) and blood or serum, where the findings are systemic. Moreover, the methodologies used are diverse and have different levels of sensitivity, including immunohistochemistry, immunofluorescence, flow cytometry, ELISA, PCR, avidin–biotin immunoperoxidase (ABC) technique and immunobead rosette technique (IBT). Some (e.g. ABC and IBT) are no longer used, and some have been improved over time. Despite these confounders, there are some conclusions that can be drawn about how B cells behave in disease lesions, the peritoneal cavity and systemically in women with endometriosis. These are described below.

Enhanced IgG autoantibody levels and activation of B cells ($CD23^+$) in blood and peritoneal fluid have been reported in women with endometriosis, including those with mild disease, demonstrating that even early disease promotes a B-cell response (Odukoya *et al.*, 1996a). Moreover, lower levels of IgG in advanced stage disease suggest that early disease is more immunologically active than later stage disease (Gebel *et al.*, 1993). While concentrations of immunoglobulin types in eutopic or ectopic endometrium were similar between endometriosis patients and controls (Nomiyama *et al.*, 1997), increased concentrations of IgG and IgA in peritoneal fluid were reported in women with the disease (Riccio *et al.*, 2017). Yeol *et al.* (2015) also studied key transcription factors in B-cell function, such as B lymphocyte inducer of maturation program (Blimp-1), involved in cell differentiation, and its antagonist B-cell leukemia lymphoma-6 (Bcl-6) in B cells in peritoneal fluid. Blc-6 mRNA and Blimp-1 mRNA levels were significantly decreased and increased, respectively, in the endometriosis group, with significant correlations with immunoglobulins IgG and IgA and cytokines (Yeol *et al.*, 2015), indicating that peritoneal immune responses in patients with endometriosis may be due to increased IgG and IgA concentrations, as well as to changes in expression of pro-inflammatory cytokines and transcriptional factors, regulating cellular function. Ectopic lesions express high levels of cytokines that activate B cells, such as B lymphocyte stimulator (BLyS) (Hever *et al.*, 2007), that is also overexpressed in autoimmune diseases. Thus, it could be a potential target in the treatment of diseases with B-cell defects.

Conclusions

There is much controversy regarding the role(s) of B cells in endometriosis. Although several studies have demonstrated aberrant production of endometrial autoantibodies in endometriosis, there is no consensus about the concentration of B cells (in eutopic and ectopic endometrium, circulating blood and/or peritoneal fluid) and their roles in this disorder. Anti-endometrial antibodies may be partially responsible for failure of implantation leading to infertility by affecting endometrial cellular function for embryo receptivity. Identification of specific targets would facilitate understanding the pathophysiology of endometriosis and could also contribute to discovery of a non-invasive test for diagnosis of endometriosis. The development of non-invasive tools based on autoantibodies measured in tissue and serum could greatly advance clinical management of this disease. However, advanced technologies are anticipated to identify and characterize different phenotypes of B cells in the lesions as well as in eutopic

endometrium and in the systemic circulation of women with and without disease and clarify their role and/or contribution in the development and pathophysiology of endometriosis. It would be of interest to study different molecules responsible of the activation of B cells, such as BLyS, and evaluate their potential for the treatment of the disease.

T cells

T cells in normal eutopic endometrium

In general, T cells (or thymocytes) are effector cells of the adaptive immune system that affect the activity of other immune cells through a repertoire of T cell–secreted cytokines (Raphael *et al.*, 2016) (Fig. 2). They are activated by cell populations of the innate immune system and protect the host against invading pathogenic microorganisms. The recognition of foreign pathogens is very specific, in contrast to that which occurs with cells from the innate immune system, but T cells do not work alone, as they produce cytokines that also activate innate immune cells (White *et al.*, 1997).

There are different subsets of mature T cells depending on cell surface markers and function: cells expressing CD8 (cytotoxic T cells), cells expressing CD4 (T helper cells) and cells that do not express either CD8 or CD4. The $CD4^+$ population includes T helper cells (Th1, Th2, Th9, Th17 and Th22 cells; follicular T helper cells) and Treg cells. Each subset is characterized by expression of specific cytokines and chemokines with wide-ranging effects (Raphael *et al.*, 2016). All T cells express T-cell receptors (TCR). Depending on cell surface glycoproteins (CD8 or CD4), they recognize MHC I or MHC II, respectively.

T cells comprise 1–2% of total endometrial cells (Salamonsen and Woolley, 1999). They vary throughout the menstrual cycle and comprise 40–60% of the total leukocyte population in the proliferative phase (Klentzeris *et al.*, 1995; Marchal, 1997) and <10% in the late secretory phase (Flynn *et al.*, 2000; Marchal, 1997) (Fig. 3). Although they vary throughout the cycle, we did not find literature regarding their expression of ER and/or PR. During the proliferative phase, T cells reside mainly in lymphoid aggregates in the basalis layer (Yeaman *et al.*, 1997). As a whole, T cells are characterized by the expression of the CD3 receptor. Endometrial T cells also express CCR5 and CXCR4, which are chemokine receptors and HIV co-receptors, with upregulation of CCR5 during the secretory phase and lower CXCR4 expression compared with peripheral blood T cells (Shanmugasundaram *et al.*, 2014; Alkhatib, 2009). There are several different types of endometrial T cells present, which are discussed individually below.

T cells in endometriosis eutopic endometrium

More $CD3^+$ T cells are in endometrium in the proliferative phase and equivalent numbers in the secretory phase of the cycle in women with disease compared to controls (Bulmer *et al.*, 1998; Mettler *et al.*, 1996). However, in addition to numbers of cells, activation status is a critical part of assessing T-cell function, and this has been considered in some but not all studies, as described below.

$CD8^+$ T cells

$CD8^+$ T cells in normal eutopic endometrium. $CD8^+$ T cells are also called cytotoxic T cells, cytolytic T cells, T-killer cells or $CD8^+$ effector cells. Their role is to monitor all the cells of the body, ready to destroy those that may threaten the integrity of the host (White *et al.*, 1997). TCR expressed by these cells recognize MHC I molecules that are presented by APC cells such as DC that capture antigens from 'foreign' cells such as virally infected cells or tumour cells. Once the TCR-MHC I recognition is achieved, $CD8^+$ T cells become active and destroy the cells containing the antigen. In order to properly recognize the MHC I molecules, TCR must partner with CD8 (the latter giving rise to the name of this class of T cells) (White *et al.*, 1997). $CD8^+$ T cells have different mechanisms to destroy and/or lyse the recognized cells: by releasing cytokines (e.g. TNFα or IFNγ) that have anti-tumour and anti-microbial effects by releasing cytotoxins, (e.g. granzymes and perforin) that perforate the cell membrane or by activating the Fas/Fas ligand pathway, leading to apoptosis (White *et al.*, 1997) (Table I).

In normal endometrium, $CD8^+$ T cells are scattered in the stromal compartment and are intra-epithelial and in lymphocyte aggregates (Yeaman *et al.*, 2001). $CD8^+$ T cells are twice as abundant compared to $CD4^+$ T cells in endometrium (66% and 33% of thymocytes, respectively), in contrast to peripheral blood wherein $CD4^+$ T cells predominate in all menstrual cycle phases (Flynn *et al.*, 2000; Rodriguez-Garcia *et al.*, 2014). The total subset of $CD8^+$T cells is increased in the early proliferative phase compared to the secretory phase (Mettler *et al.*, 1996). $CD8^+$ T cell cytolytic or cytotoxic activity is high in the proliferative phase and very low in the secretory phase (Mselle *et al.*, 2007; Salamonsen and Lathbury, 2000; White *et al.*, 1997; Xu *et al.*, 2007). As $CD8^+$ T-cell proliferation and activation are elevated in the proliferative phase, regulation by estradiol is suspected. Lack of cytolytic activity is tissue specific (Yeaman *et al.*, 2001), and inhibition of cytotoxic activity during the secretory phase is mediated by progesterone, presumably to minimize a toxic environment during nidation (White *et al.*, 1997).

$CD8^+$ T cells in endometriosis eutopic endometrium. Endometrial lymphocyte subsets show equal quantity and distribution in endometriosis patients and in women without the disease. After a peak in the early proliferative phase, the absolute number of T cells decreases, while a predominance of $CD8^+$ T cells compared to $CD4^+$ T cells occurs towards the end of the menstrual cycle (Mettler *et al.*, 1996). However, activation status of endometrial T cells in women with the disease has not been characterized.

$CD8^+$ T cells in ectopic endometriosis lesions. $CD8^+$ T cells in ectopic lesions are higher in number compared to eutopic endometrium of women with disease (Witz *et al.*, 1994) and do not vary with hormonal milieu (Bulmer *et al.*, 1998). In addition, in peripheral blood from women with endometriosis, $CD8^+$ T cells do not fluctuate across the cycle, while they do in healthy controls (Slabe *et al.*, 2013). These differences could reflect a lack of regulation in $CD8^+$ T-cell activity in women with endometriosis. Interestingly, Melioli *et al.* (1993) proposed that T-cell cytolytic activity could be restored with administering recombinant IL-2. They found that *in vitro* activated human T cells from peripheral blood were able to lyse autologous endometrial cells efficiently, but not keratinocytes, suggesting that non-specific damage would be minimal for normal cells in the peritoneal cavity.

Conclusions. In conclusion, $CD8^+$ T-cell activity is decreased in normal endometrium in the secretory phase coincident with the invasive phase of implantation. While numbers of endometrial CD8+ T cells do not differ between women with and without endometriosis, whether their activation phenotypes do remains to be determined.

CD4+ cells

CD4+ T cells in normal eutopic endometrium. CD4+ T cells (also called T helper-inducer cells) when activated by APC initiate a specific immune response by secretion of a cascade of cytokines. The cytokines profiles dictate classification of these cells as Th1, Th2, Th17 or Treg cells, among others. In normal endometrium, the global subset of CD4+ cells is most abundant in the proliferative phase and decreases thereafter (Mettler *et al.*, 1996). However, specific subtypes of CD4+ cells do not always follow this pattern (see below) (Fig. 3). CD4+ T cells overall are less abundant than CD8+ T cells in endometrium (Flynn *et al.*, 2000; Rodriguez-Garcia *et al.*, 2014).

CD4+ T cells in endometriosis eutopic endometrium. As in women without disease, CD4+ T cells in endometrium of women with endometriosis are more numerous in the proliferative phase (Mettler *et al.*, 1996) (Fig. 3). However, in contrast to normal endometrium, CD4+ T cells are more abundant than CD8+ T cells in endometrium of women with disease (Witz *et al.*, 1994), suggesting that endometrium of women with endometriosis has less cytotoxic activity than that of controls due to the presence of fewer CD8+ T cells.

CD4+ T cells in ectopic endometriosis. CD4+ T cells are increased compared to CD8+ T cells in ectopic lesions, in peripheral blood and peritoneal fluid of women with endometriosis compared with women without disease (Szyllo *et al.*, 2003). Depending on the subset of CD4+ cells, the environment can promote a pro- or an anti-inflammatory environment. The spatiotemporal dynamics of an anti-inflammatory or pro-inflammatory milieu during lesion establishment, survival, neuroangiogenesis and scarring likely depend on the repertoire of immune cells and endometrial cells participating in these processes. With regard to CD4+ Th17 cells (see below), these could contribute to a pro-inflammatory environment; in contrast, Treg cells, which have anti-inflammatory effects and are also increased in lesions (see below), may facilitate lesion implantation and survival. These are key concepts in the understanding of endometriosis pathogenesis and and pathophysiology and warrant extensive study in the future. The different subsets of CD4+ cells and their phenotypes and effects are presented below.

Th17 CD4+ T cells

CD4+ Th17 cells in normal eutopic endometrium. Th17 cells are located in tissues, especially in those with microbial contact, where they mediate host defence mechanisms against infection and maintain integrity of epithelial surfaces (Sallusto *et al.*, 2012). Th17 cells derive from naive CD4+ T cells in the presence of transforming growth factor alpha (TGFα), IL-6 (Gogacz *et al.*, 2016) and IL-23 (Andreoli *et al.*, 2011). Normal endometrial CD4+ T cells highly express CD90 (Rodriguez-Garcia *et al.*, 2014), especially in Th17 T cells. CD4+ Th17 cells express CCR6 and the RORC2 transcription factor and are characterized by production of IL-17 (Annunziato *et al.*, 2012), which induces PGE2, COX2, MMP3, ICAM1 and MCP-1 expression in target cells (MC, B cells, Mϕ and NK). They secrete IL-17, IL-21 and IL-22 (Albanesi *et al.*, 1999) and produce CXCL1, CXCL5, IL-8, CCL2 and CCL7, which are involved in recruitment of NT in sites of inflammation (Schwarzenberger *et al.*, 2000) (Table I).

CD4+ Th17 cells in endometriosis eutopic endometrium. While there is an extensive literature about Th17 cells in ectopic lesions (summarized below), we did not find any reports regarding Th17 in eutopic endometrium of women with endometriosis. This would be of great value to investigate further, given the effects of cytokines secreted by Th17 cells on a variety of cells.

CD4+ Th17 cells in ectopic endometriosis. In women with endometriosis, Th17 cells are increased in peritoneal fluid and peripheral blood compared to those without disease. Moreover, Th17 cell abundance is higher in peritoneal fluid of women with severe versus mild endometriosis (Gogacz *et al.*, 2016). Greater numbers of Th17 cells in endometriotic lesions may derive from their expressing CCR6, a receptor for the CCL20 ligand that is produced by endometriotic stromal cells and is chemotactic to Th17 cells (Hirata *et al.*, 2010). Also, IL-17A, expressed by Th17, is highly expressed in endometrial lesions compared with the matched eutopic endometrium, and surgical removal of lesions results in reduced IL-17A plasma concentrations (Ahn *et al.*, 2015). These data support a role for Th17 cells in progression of endometriosis. Furthermore, IL-17A concentrations in peritoneal fluid of women with endometriosis correlate with disease severity and infertility (Zhang *et al.*, 2005). Minimal/mild endometriosis is associated with higher levels of IL-17A compared with moderate/severe disease or no disease but does not correlate with pain symptoms. However, one study found no variation in IL-17A concentrations in serum or peritoneal fluid among women with and without endometriosis (Andreoli *et al.*, 2011). Reconciliation of these diverse findings requires further assessment and harmonization in future studies of experimental designs, preparation of biospecimens for analysis and assay protocols.

Of note, IL-17A stimulates IL-8 secretion from endometriotic stromal cells, proliferation of these cells and NT migration (Osuga *et al.*, 2016), which could increase inflammation. The data support a role for T17 cells in growth of endometriotic lesions and give plausibility to mechanisms underlying the presence of NT in lesions. However, Th17 are not unique in secreting IL-17, as other immune cells (e.g. NT, MC, NK and $\gamma\delta$T cells) also produce this interleukin, underscoring the complexity of the interplay among immune cell types in the progression of endometriosis (Ahn *et al.*, 2015). Recently, Th17 cells were shown to produce IL-10 that has anti-inflammatory properties, and the increment of IL-10 is observed in patients with advanced endometriosis (Chang *et al.*, 2017).

Conclusions. In summary, the current literature supports a role for Th17 in the development of endometriotic lesions and in producing the pro-inflammatory environment characteristic of the disease. Moreover, these cells appear to contribute to disease severity. It would be of interest to study this population in eutopic endometrium of women with endometriosis in different disease stages and further elucidate their interactions with resident epithelial and stromal cells in this tissue for a comprehensive perspective of paracrine cross-talk within this tissue and the relevance to pregnancy and symptoms of affected women.

Th1 CD4+ T cells

CD4+ Th1 cells in normal eutopic endometrium. Th1 cells are classified by their profile of secreted cytokines, which elicit pro-inflammatory responses. They secrete IL-2, IFNγ and lymphotoxin, which act on effector cells to enhance cell-mediated immunity (Cher and Mosmann, 1987) by promoting the action of NK cells and Mø (Podgaec *et al.*, 2010). Some released cytokines, e.g. IFNγ, inhibit proliferation of Th2. Moreover, cytokines released by Th1 are associated with pregnancy loss and infertility (Yeaman *et al.*, 1998). It has been suggested that an

enhanced Th1 response at the maternal–foetal interface is associated with foetal loss, while a predominant Th2 response is associated with foetal survival (Wegmann et al., 1993).

In normal endometrium, the percentage of Th1 cells is highest during the proliferative phase, while Th2 predominate in early pregnancy (Saito et al., 1999) (see below).

CD4+ Th1 cells in endometriosis eutopic endometrium. There is scant information about Th1 cells in endometrium of women with endometriosis in comparison with ectopic lesions (Takamura et al., 2015) and no information on cyclic changes in cell numbers or comparisons with normal endometrial Th1 cells.

CD4+ Th1 cells in ectopic endometriosis and the circulation of women with endometriosis. $CD4^+$ Th1 cells are less abundant in endometriotic lesions compared to endometrium of women with endometriosis (Takamura et al., 2015). In peripheral blood, Th1 cells are significantly more abundant in patients with endometriosis compared with control women (de Barros et al., 2017). Moreover, the Th1 response pattern is associated with disease severity (Podgaec et al., 2010). Specifically, concentrations of pro-inflammatory cytokines (TNFα and IL-2), secreted by Th1 cells, are significantly elevated in peritoneal fluid of women with endometriosis, the majority (65%) of whom had deep infiltrating disease.

Conclusions. Little is known regarding Th1 cells in endometrium of women with endometriosis. Available data suggest a role for Th1 cells in disease severity by promoting an initial pro-inflammatory environment, leading to recruitment of other pro-inflammatory immune populations and development and growth of the ectopic lesions. More extensive phenotyping of this cell type in women with and without endometriosis is an opportunity for further study.

Th2 $CD4^+$ T cells

CD4+ Th2 cells in normal eutopic endometrium. In contrast to Th1 cells, Th2 cells promote an anti-inflammatory environment via secretion of mainly IL-4, IL-5, IL-6 and IL-10. These cytokines are increased in the secretory phase, consistent with regulation by progesterone (Krasnow et al., 1996) (Fig. 3). Th1 cells secrete cytokines that inhibit Th2-cell proliferation, and in turn, IL-4 and IL-10 secreted by Th2 cells, inhibit Th1 cell pro-inflammatory cytokine secretion (Cher and Mosmann, 1987). Th2 cells also activate B cells, triggering humoural immune responses including immunoglobulin production and recruitment and activation of EN, basophils and MC to sites of inflammation (Podgaec et al., 2007). Thus, Th2 cell–induced suppression of a pro-inflammatory environment in normal endometrium could be a mechanism to facilitate embryo implantation and pregnancy maintenance. This is supported by increased Th2 cell numbers in successful early pregnancy (Krasnow et al., 1996). The percentage of Th2 cells is lowest in the proliferative phase and highest in early pregnancy decidua (Saito et al., 1999).

CD4+ Th2 cells in endometriosis eutopic endometrium. Very little is known regarding Th2 cells in endometrium of women with endometriosis. Messenger RNA coding for IL-10 produced in Th2 cells in endometrium of women with the disease is higher than IL-2 mRNA levels, produced by Th1 cells, suggesting that endometrial Th2-cell cytokine secretion is greater than Th1-cell cytokine secretion in women with disease. However, IL-10 protein levels were not higher than IL-2 protein concentrations, indicating that Th2 cells are not overexpressing IL-10 in the endometrium of women with endometriosis compared with that of controls (Antsiferova et al., 2005). In addition, eutopic endometrial tissue from women with endometriosis has higher mRNA and protein levels in epithelial cells of GATA-binding protein 3 (GATA3), a key regulator of T cell and endothelial biology, compared to healthy endometrial tissue (Chen et al., 2012b). GATA3 expression is regulated by oestrogen, and it activates Th2-cell cytokine production (Chen et al., 2016). More extensive protein and cell activation phenotyping of endometrial Th2 cells are important areas of future investigation to understand mechanisms underlying infertility, recurrent pregnancy loss and adverse pregnancy outcomes in women with endometriosis.

CD4+ Th2 cells in ectopic endometriosis. Concentrations of IL-4 and IL-10, products of Th2 cells, are higher in peritoneal fluid and peripheral blood of women with endometriosis, suggesting that Th2 cells predominate in these women compared with control women (Antsiferova et al., 2005; Podgaec et al., 2007). Notably, the higher incidence of Th2-mediated disorders, e.g. asthma and allergies in women with endometriosis, may be attributable to high numbers of these cells in these patients (Podgaec et al., 2007). Furthermore, Th2 produce IL-4 and IL-13, among other cytokines, which mediate differentiation of resident fibroblasts and recruited fibrocytes to myofibroblasts. These observations suggest that Th2 cells may be implicated in fibrosis that is common in the pelvis of women with endometriosis, by the secretion of these interleukins (Borthwick et al., 2012) and perhaps other mediators. Moreover, IL-4 can stimulate production of eotaxin, a chemoattractant for Th2 cells and EN (Podgaec et al., 2007), which may contribute to the progression of disease by recruiting, and thereby increasing, Th2 cells and NT in ectopic sites and promoting angiogenesis therein. As GATA3 induces Th2 cytokine expression in endometriotic lesions (in addition to eutopic endometrium), it may also play a role in promoting endometriosis disease progression (Chen et al., 2016). Nonetheless, it has been postulated that endometriosis is an autoimmune disease (Nothnick, 2001) and endometrial autoantibodies contribute to infertility risk in women with the disease (Gleicher, 1990). High levels of B cell–derived autoantibodies in peritoneal fluid and serum in women with endometriosis may derive from IL-4, produced in abundance by Th2 cells in ectopic sites, which can stimulate differentiation and maturation of B cells to produce these autoantibodies.

Conclusions. Further studies are warranted with regard to the Th2 cells in endometrium of women with and without endometriosis and in ectopic lesions. Th2 cells may provide a link between autoimmune diseases, allergies, atopic disorders and endometriosis (Nothnick, 2001).

Regulatory $CD4^+$ T cells

CD4+ Treg cells in normal eutopic endometrium. Treg cells are potent suppressors of inflammatory immune responses and have a role in preventing autoimmunity. They are mainly produced in the thymus from which they migrate into the circulation and various tissues. They can differentiate from naïve T cells in the periphery (Takamura et al., 2015). Treg cells regulate Mø, MC degranulation, DC, NT, EN, B cells, T cells and NK cell function and proliferation, all of which have roles in menstruation and in endometriosis (Berbic et al., 2014; Evans and Salamonsen, 2012). The most extensively studied Treg-specific marker is the transcription factor Foxp3 (Corthay, 2009), which is restricted to $CD4^+$ cells, although some $CD8^+$ cells also express

it (Liu *et al.*, 2006b). Foxp3 regulates Treg-cell gene expression and thus T-cell activation and function (Marson *et al.*, 2010). Treg are also characterized by $CD25^{High}CD127^{-}$ (Josefowicz *et al.*, 2012; Li *et al.*, 2016; Liu *et al.*, 2006b) (Table I).

In normal endometrium, Treg cells are increased during the proliferative phase and decreased at the end of the secretory phase (Arruvito *et al.*, 2007) (Fig. 3). This increase prior to ovulation suggests they are important in preparing for successful embryo implantation, providing an immune-tolerant environment by inhibiting cytotoxic activity of other immune cells and other cell types through secretion of immunosuppressive cytokines such as IL-10 and TGF-β (Arruvito *et al.*, 2007; Chen *et al.*, 2012a; Sakaguchi *et al.*, 2010; Takamura *et al.*, 2015).

$CD4^+$ Treg cells in endometriosis eutopic endometrium. There is controversy about the abundance of $CD4^+$ Treg cells in eutopic endometrium of women with endometriosis. Most studies agree that $CD4^+$ Treg cells are increased in endometrium of women with versus without disease. One group described increased Treg cells in proliferative phase endometrium of women with endometriosis versus healthy control, and more abundance still with advanced versus early stage disease (Chen *et al.*, 2012a). In contrast, other studies have demonstrated higher numbers of Treg cells in the secretory phase versus other cycle phases (Berbic *et al.*, 2010), and others reported no differences (Basta *et al.*, 2010; Podgaec *et al.*, 2014) (Fig. 3). In addition, the percentage of activated Treg cells is lower in endometriosis endometrium than in healthy endometrium (Tanaka *et al.*, 2017). These discrepancies could be due to different techniques for detecting Treg cells, e.g. flow cytometry or FOXP3 expression by RT-qPCR or immunohistochemistry. In addition, a high proportion of Treg are observed in the peri-implantation endometrium of infertile women with endometriosis versus fertile control women (Chen *et al.*, 2012a), underscoring dysregulation of this cell type relevant to implantation in women with the disease.

$CD4^+$ Treg cells in ectopic endometriosis. In ectopic lesions, Treg cells are abundant, and in peritoneal fluid and in the circulation of women with endometriosis, they are more abundant compared to controls (Basta *et al.*, 2010; Chen *et al.*, 2012a; Olkowska-Truchanowicz *et al.*, 2013; Podgaec *et al.*, 2014). This high abundance of Treg cells in lesions can lead to an anti-inflammatory environment by suppressing the immune response mounted against the lesions in their ectopic location, thus permitting ectopic lesion attachment, survival and growth (Basta *et al.*, 2010). The number of Treg cells in the peritoneal fluid positively correlates with stage of disease (Li *et al.*, 2014). In contrast, Tanaka *et al.* (2017) found that activated Treg cells were lower in ovarian endometriotic lesions, although total Treg cells in peritoneal fluid and peripheral blood were similar between women with and without endometriosis. Depletion of Treg cells in an endometriosis mouse model increased the weight of lesions and enhanced the proliferative capacity of already existing endometriotic lesions as well as the activation of Mø pro-inflammatory secreted products. Therefore, the lack of anti-inflammatory Treg cells may allow the progression of endometriotic lesions by facilitating a pro-inflammatory environment (Tanaka *et al.*, 2017).

Conclusions. In summary, in normal endometrium, Treg cells are abundant in the proliferative phase, different from endometrium of women with endometriosis wherein they are increased in the secretory phase or do not fluctuate across the cycle. The discrepancy is likely due to different techniques used as well as Treg-cell definition. Most of the studies focused on the Foxp3 marker. However, this is an intracellular marker and cannot be used as a marker with all techniques utilized. In addition, whether Foxp3 expression functionally regulates Treg-cell function remains controversial (Li *et al.*, 2015b). In women without disease, Treg cells are upregulated prior to ovulation, likely to produce an immune-tolerant environment for impending embryo nidation. Different numbers of these cells in endometrium in women with endometriosis suggest an abnormal local immune environment that is inhospitable to nidation. Moreover, greater numbers of endometrial Treg cells in the secretory phase in women with disease may predispose to the pathogenesis of advanced endometriosis (Chen *et al.*, 2012a). Support for this conclusion derives from higher numbers of Treg cells in the peri-implantation endometrium of infertile patients with endometriosis compared with healthy fertile women and positive correlations with stage of disease (Chen *et al.*, 2012a).

NK T cells

NK T cells comprise a small subset of T cells that express NK markers and CD3, which is not expressed by NK or uNK. NK T cells reportedly have a role in defence against infection and cancer and may play a role in transplantation through their repertoire of secreted cytokines (Trundley and Moffett, 2004). Interestingly, during pregnancy NK T cells are higher in the decidua compared to peripheral blood (Trundley and Moffett, 2004). Roles for these cells in pregnancy await further elucidation, as well as in normal eutopic endometrium and in the setting of endometriosis.

$\gamma\delta$T cells

$\gamma\delta$T cells account for less than 10% of T cells in peripheral blood, and they also are found in various tissues, including skin, lung, uterus, ovary and small intestine (Keystone *et al.*, 1991; Stewart-Akers *et al.*, 1998). They do not express CD4 or CD8 markers (Groh *et al.*, 1989) and are characterized by expression of TCR with γ and δ chains (most T cells express the α and β chains of these receptors) (Lee *et al.*, 2011). $\gamma\delta$T cells recognize super-antigens or heat shock proteins (Hirsh and Junger, 2008). In eutopic and ectopic endometrium of women with endometriosis, these cells are increased compared to eutopic endometrium of normal controls (Ota *et al.*, 1996). Nothing else is known regarding these cells in endometriosis, but they are markedly increased in eutopic and ectopic endometrium of women with compared with those without the disease, suggesting they may have a role in the dysfunctionality of the eutopic endometrium and in lesion formation and the inflammatory phenotype observed therein. Further studies are needed to elucidate the role of this population in endometrium and in endometriosis.

Overall summary, conclusions and an eye to the future

Despite the high incidence of endometriosis, it is a poorly understood disorder. It is unclear whether the immune system contributes to the development of the disease or if aberrancies in the immune system are a consequence of it. What is clear is that immune components play a major role in the pro-inflammatory phenotype characteristic of the disease at the level of the lesions and within the eutopic endometrium. Specific immune populations are actively involved in pregnancy establishment and maintenance including tolerance of the

allogeneic conceptus, and immune dysfunction can lead to infertility and abnormal pregnancy outcomes, including miscarriage, foetal growth restriction and placentation disorders such as pre-eclampsia and preterm birth (Stewart-Akers *et al.*, 1998; Trundley and Moffett, 2004). Thus, we propose that efforts be aimed at two major aspects of involvement of the immune system in endometriosis pathogenesis and pathophysiology: (i) elucidation of immune cell numbers and activation status and immune mediators within eutopic endometrium of women with various stages of disease compared with normal controls in an effort to elucidate effects on pregnancy outcomes and overall tissue homeostasis and (ii) elucidation of the role of immune cells and immune mediators promoting lesion establishment and survival, the pro-inflammatory environment in lesions and in the pelvis (and other locations where disease exists), fibrosis and pain. These approaches are anticipated to lead to a better understanding of the disease pathophysiology and to enable development of novel biomarkers for diagnosis and prognosis, as well as new treatments for associated subfertility/infertility and pelvic pain.

Herein, we have reviewed the different immune cell populations in eutopic endometrium of healthy women and those with endometriosis and their possible involvement in the pathophysiology of the disease. Moreover, we have given a general view of each immune cell population in eutopic endometrium compared to controls and compared to ectopic endometriosis lesions, to better understand how these populations behave in different environments and locations and their potential roles in disease pathogenesis and pathophysiology. As discussed above, in healthy controls and in women with endometriosis, there are conflicting data on numbers, markers, activation states and cycle dependence of endometrial immune cells (Ahn *et al.*, 2014; Herington *et al.*, 2011; Jerman and Hey-Cunningham, 2015; Klentzeris *et al.*, 1995; Takebayashi *et al.*, 2015), likely due to different methods of immune cell isolation and characterization, small sample numbers, different cohorts, heterogeneity of the disease and inconsistently documented cycle phase, disease stage and definition of controls.

We propose some approaches for consideration in future studies regarding immune populations in eutopic endometrium as multiple factors can confound experimental findings and WERF-EpHect protocols are applicable here (Becker *et al.*, 2014; Rahmioglu *et al.*, 2014; Vitonis *et al.*, 2014): developing standard operating procedures (SOPs) for biospecimen collection, processing and storage prior to analysis; identifying where and how samples are obtained (e.g. endometrial biopsy, curettage, hysterectomy, full-thickness); extensive clinical phenotyping and history (age, menstrual cycle phase, fertility/pregnancy history, pain symptoms, medications, co-morbidities, personal habits, exposures and family history); extensive phenotyping of controls and lesions (peritoneal, ovarian and deep infiltrating); surgical staging (Johnson *et al.*, 2017); and validation of reagents, SOPs and reproducibility of results.

Immunohistochemistry, one of the most commonly used analytical approaches to study immune populations in endometrium, reveals the location of specific cells *in situ*. However, the results are based on fixed tissue, which could alter marker composition and are limited to a small area, which may not be representative of the total population in the whole tissue, and may miss detection of especially less abundant immune cells. Different groups have used different markers to detect the same immune cell population or, sometimes, the same marker is tested using a distinct clone, which may give different results. Thus, uniformity of markers is essential for reproducibility of results, although it is essential to acknowledge that specific markers may display lack of fidelity for distinct populations. New techniques are emerging, such as single-cell RNA sequencing and multidimensional flow cytometry, which offer deep phenotyping of specific populations, including transcriptomics and cell-specific activation marker phenotyping. Regarding flow cytometry studies, as in immunohistochemistry, results are often difficult to compare across studies because different groups use different markers or antibodies. The advantage, however, is that abundance of immune cell subtypes is assessed in whole-tissue specimens, giving more confidence to greater accuracy of numbers of detected cells being representative of the whole-tissue sample. However, an issue that has received little attention is blood contamination in endometrium and endometriosis lesion samples. Without accounting for this, data about tissue immune cell abundance will be confounded unless peripheral blood levels and percent contamination are included in the analyses. Furthermore, as explained above, in some immune populations, there are specific activation markers or different expression levels of specific markers in endometrial immune cell populations compared with their counterparts in blood. As most studies have not considered this, we believe that the described number of immune cells and their activation states could be over- or underestimated, leading to inconsistent results reported in the literature by various groups.

Although it is challenging to compare results among different studies, and considering the issues described above, we conclude that some immune populations are more abundant in eutopic endometrium of women with endometriosis compared with women without disease and some behave differently throughout the menstrual cycle in both groups. A summary of their behaviour throughout the cycle is shown in Fig. 3. An important issue is whether immune cells express ER and PR, as the literature is conflicting in this regard. Endometriosis is an oestrogen-dependent disorder with a blunted response to progesterone in selected cell populations, thus underscoring the plausibility of an endocrine-immune network that participates in the development and progression of the disease. We anticipate this important issue will be resolved in the near future, as immune cell phenotypes and secretion of cytokines and chemokines in response to steroid hormones in eutopic endometrium, a steroid hormone–responsive tissue, and in endometriosis, a hormone-driven disorder, are further analysed. Functions of each immune population and their fluctuations across the menstrual cycle in endometrium from women without and with disease and in ectopic endometriosis lesions and comparisons among the different tissues are summarized in Table II.

Striking in endometriosis is the network of immune cell populations in the pelvis derived from the innate and adaptive immune systems that together confer an optimal environment in ectopic endometrium to develop into lesions. In eutopic endometrium, immune cells aberrantly expressed in women with the disease are more related to an inhospitable environment for embryo implantation, relevant to infertility and poor pregnancy outcomes in women with disease. In addition, altered immune cells in eutopic endometrium of women with disease can affect other endometrial cell populations, predisposing to lesion establishment, survival and growth. Immune cell populations that are aberrant in this tissue throughout the menstrual cycle in women with endometriosis are showed in Fig. 3.

Although there are discrepancies between studies, we tried to link possible interactions of all immune populations in eutopic

Table II Immune cell populations in endometrium and endometriosis.

Population	Normal eutopic endometrium	Endometriosis eutopic endometrium	Ectopic endometriosis
Mϕ	**Fluctuation menstrual cycle:** Increase in secretory and menstrual phases (Salamonsen *et al.*, 2002; Berbic *et al.*, 2009; Bonatz *et al.*, 1992; Deloia *et al.*, 2002; King, 2000; De and Wood, 1990) **Function:** Clearance of cell debris during menstruation (Khan *et al.*, 2005)	**Fluctuation menstrual cycle:** No fluctuation throughout cycle (Berbic *et al.*, 2009) **Function:** Immunotolerance of cell debris, defect in apoptosis, migration of endometrial cells to peritoneal cavity **Comparison with normal eutopic endometrium:** Increased Mϕ2 in normal (Berbic *et al.*, 2009; Khan *et al.*, 2010; Takebayashi *et al.*, 2015) and increased Mϕ1 in endometriosis (Takebayashi *et al.*, 2015). Increased Mϕ in global (Mϕ1 and Mϕ2) in endometriosis (Berbic *et al.*, 2009; Takebayashi *et al.*, 2015; Khan *et al.*, 2010)	**Fluctuation menstrual cycle:** No fluctuation throughout cycle (Berbic *et al.*, 2009) **Function:** Immunotolerance of cell debris, lesion formation, increase of angiogenesis in lesion (Mϕ2). Increase of nerve fibres growth (Morotti *et al.*, 2015) **Comparison with normal:** Increased Mϕ2 in peritoneal fluid and ectopic lesion (Bacci *et al.*, 2009; Khan *et al.*, 2004)
DC	**Fluctuation menstrual cycle:** Increase of iDC in secretory and menstrual phases (Marbaix, 2005), increase (King *et al.*, 1996) and no fluctuation of mDC (Marbaix, 2005) **Function:** Tissue breakdown in menstruation	**Fluctuation menstrual cycle:** No fluctuation throughout cycle (Schulke *et al.*, 2009) **Function:** DC dysfunction, inefficient targeting of cell debris, migration of endometrial debris to peritoneal cavity **Comparison with normal eutopic endometrium:** Increased iDC and decrease of mDC in endometriosis (Schulke *et al.*, 2009; Maridas *et al.*, 2014)	**Fluctuation menstrual cycle:** Not described **Function:** DC dysfunction (mDC), no clearance of cell debris, lesion establishment. Increase of angiogenesis and lesion growth and increase of nerve fibres growth (iDC) **Comparison with normal:** Increase of iDC and decrease of mDC in blood (Maridas *et al.*, 2014). Increase of iDCs in adjacent peritoneum (Schulke *et al.*, 2009)
uNK	**Fluctuation menstrual cycle:** Increase in secretory and menstrual phases (Lee *et al.*, 2011; King and Critchley, 2010; Flynn *et al.*, 2000; Wira *et al.*, 2005; King, 2000; Mselle *et al.*, 2007; Salamonsen and Woolley, 1999; Bulmer *et al.*, 1991; Drury *et al.*, 2018; Manaster *et al.*, 2008; Kodama *et al.*, 1998) **Function:** Fight against microorganisms, establishment of a suitable environment for embryo implantation (low cytotoxicity)	**Fluctuation menstrual cycle:** Increase in secretory and menstrual phases (Jones *et al.*, 1996; Herington *et al.*, 2011) **Function:** Decreased cytotoxicity, decreased apoptosis, survival endometrial debris and migration to peritoneal cavity **Comparison with normal eutopic endometrium:** Decreased uNK percentage and activity (Yang *et al.*, 2011) and no differences (Giuliani *et al.*, 2014) (Controversies)	**Fluctuation menstrual cycle:** Not described **Function:** Decreased cytotoxicity, decreased apoptosis, survival endometrial debris implantation of lesions **Comparison with normal:** No differences in number in blood (Oosterlynck *et al.*, 1994; Thiruchelvam *et al.*, 2015)/Increase in number in blood (Dias *et al.*, 2012)/Decreased NK activity in peritoneal fluid (Kikuchi *et al.*, 1993; Thiruchelvam *et al.*, 2016)
MC	**Fluctuation menstrual cycle:** Increase activation in menstruation (Drudy *et al.*, 1991) or no fluctuation (Jeziorska *et al.*, 1995; Sivridis *et al.*, 2001) **Function:** Shedding of endometrium in menstruation, regeneration and angiogenesis	**Fluctuation menstrual cycle:** No fluctuation (Matsuzaki *et al.*, 1998) **Function:** Increase of EN, NT and Mϕ, pro-inflammatory responses **Comparison with normal eutopic endometrium:** Increased MC infiltration (Sugamata *et al.*, 2005)	**Fluctuation menstrual cycle:** Not described **Function:** Higher activation, formation of fibrous adhesions in lesion **Comparison with normal:** Increased MC activation in ectopic endometriotic lesion compared to eutopic endometrium (Matsuzaki *et al.*, 1998)
EN	**Fluctuation menstrual cycle:** Increase in secretory and menstrual phases (Salamonsen and Woolley, 1999; Jeziorska *et al.*, 1995) **Function:** Initiation of inflammatory responses, vasoactivity, role in menstruation by degrading endometrial tissue, wound healing and regeneration	**Fluctuation menstrual cycle:** Not described **Function:** Not described **Comparison with normal eutopic endometrium:** Increased eotaxin concentration (Hornung *et al.*, 2000), chemoattractant for EN	**Fluctuation menstrual cycle:** Not described **Function:** Increase of eotaxin in peritoneal fluid, formation of fibrous adhesions **Comparison with normal:** Increased eotaxin concentration in peritoneal fluid of severe endometriosis (Hornung *et al.*, 2000)
NT	**Fluctuation menstrual cycle:** Increase in menstruation (Lee *et al.*, 2015; Salamonsen and Lathbury, 2000; Salamonsen and Woolley, 1999) **Function:** Fight against foreign microorganisms	**Fluctuation menstrual cycle:** Increase in menstruation (Arici, 2002; Takehara et al., 2004; Donnez et al., 1998) **Function:** Angiogenesis and possible migration to ectopic sites **Comparison with normal eutopic endometrium:** Increased NT activation (Arici, 2002; Takehara *et al.*, 2004; Donnez *et al.*, 1998)	**Fluctuation menstrual cycle:** Not described **Function:** Angiogenesis in lesions **Comparison with normal:** Increased NT in ectopic endometriotic lesion (Osuga *et al.*, 2016; Takamura *et al.*, 2016)

Continued

Table II Continued

Population	Normal eutopic endometrium	Endometriosis eutopic endometrium	Ectopic endometriosis
B cells	**Fluctuation menstrual cycle:** No fluctuation (Salamonsen and Lathbury, 2000) **Function:** Production of antibodies	**Fluctuation menstrual cycle:** Not described **Function:** Not described **Comparison with normal eutopic endometrium:** Increased (Antsiferova *et al.*, 2005; Scheerer *et al.*, 2016)/No differences (Klentzeris *et al.*, 1995; Witz *et al.*, 1994)(Discrepancies)	**Fluctuation menstrual cycle:** Not described **Function:** Production of autoantibodies **Comparison with normal:** Increased (Antsiferova *et al.*, 2005; Badawy *et al.*, 1989; Berbic *et al.*, 2013; Chishima *et al.*, 2000; Gleicher *et al.*, 1987; Hever *et al.*, 2007; Lachapelle *et al.*, 1996; Odukoya *et al.*, 1996a; Odukoya *et al.*, 1996b; Riccio *et al.*, 2017; Scheerer *et al.*, 2016)/Decreased (Gagné *et al.*, 2003; Oosterlynck *et al.*, 1993)/No differences (Christofolini *et al.*, 2011; Gebel *et al.*, 1993; Klentzeris *et al.*, 1995; Nomiyama *et al.*, 1997; Witz *et al.*, 1994; Yeol *et al.*, 2015) in peritoneal fluid, blood and ectopic lesion (Discrepancies)
CD8+ T cells	**Fluctuation menstrual cycle:** Increased in proliferative phase (Klentzeris *et al.*, 1995; Marchal, 1997; Flynn *et al.*, 2000) and activity decreased in secretory phase (Mselle *et al.*, 2007; Salamonsen and Lathbury, 2000; White *et al.*, 1997; Xu *et al.*, 2007) **Function:** Destroy cells presented by APC	**Fluctuation menstrual cycle:** Decreased activity in secretory phase (Mettler *et al.*, 1996) **Function:** Not described **Comparison with normal eutopic endometrium:** Decreased CD8+ T cells activity (White *et al.*, 1997)	**Fluctuation menstrual cycle:** No fluctuation in ectopic lesion (Bulmer *et al.*, 1998) nor in blood while they do in controls (Slabe *et al.*, 2013) **Function:** Lack of CD8+ T-cells activity regulation **Comparison with normal:** Increased CD8+ T cells in ectopic endometriosis lesion compared to eutopic endometrium of endometriosis (Witz *et al.*, 1994)
CD4+ Th17	**Fluctuation menstrual cycle:** Increased in proliferative phase (Mettler *et al.*, 1996) **Function:** Fighting against infections	**Fluctuation menstrual cycle:** Not described **Function:** Not described **Comparison with normal eutopic endometrium:** Not described	**Fluctuation menstrual cycle:** Not described **Function:** Involved in growth lesion **Comparison with normal:** Increased CD4+ Th17 cells in peritoneal fluid and blood compared to controls (Gogacz *et al.*, 2016)/No variation in serum or blood (Andreoli *et al.*, 2011)
CD4+ Th1	**Fluctuation menstrual cycle:** Increased in proliferative phase (Mettler *et al.*, 1996) **Function:** Pro-inflammatory responses	**Fluctuation menstrual cycle:** Not described **Function:** Pro-inflammatory responses **Comparison with normal eutopic endometrium:** Not described	**Fluctuation menstrual cycle:** Not described **Function:** Probably associated with severity **Comparison with normal:** Increased CD4+ Th1 in blood of endometriosis (de Barros *et al.*, 2017) and decreased CD4+ Th1 in endometriotic lesion compared to eutopic endometriosis endometrium (Takamura *et al.*, 2015)
CD4+ Th2	**Fluctuation menstrual cycle:** Increased in secretory phase (Krasnow *et al.*, 1996) **Function:** Anti-inflammatory responses	**Fluctuation menstrual cycle:** Not described **Function:** anti-inflammatory responses **Comparison with normal eutopic endometrium:** No differences (Antsiferova *et al.*, 2005)	**Fluctuation menstrual cycle:** Not described **Function:** Anti-inflammatory environment, lesion growth, chemotaxis of NT, increase of angiogenesis **Comparison with normal:** Increased CD4+ Th2 cells in peritoneal fluid and blood (Antsiferova *et al.*, 2005; Podgaec *et al.*, 2007)
Treg cells	**Fluctuation menstrual cycle:** Increased in proliferative phase (Arruvito *et al.*, 2007) **Function:** Suppression of immune responses	**Fluctuation menstrual cycle:** Increased in secretory phase (Berbic *et al.*, 2010)/No fluctuation (Basta *et al.*, 2010; Podgaec *et al.*, 2014) **Function:** Suppression of immune responses **Comparison with normal eutopic endometrium:** Increased Treg in proliferative phase (Chen *et al.*, 2012a)	**Fluctuation menstrual cycle:** No fluctuation (Tanaka *et al.*, 2017) **Function:** Anti-inflammatory environment against lesion implantation, lesion growth **Comparison with normal:** Increased Treg in endometriotic lesion compared to eutopic endometrium (Basta *et al.*, 2010; Chen *et al.*, 2012a; Olkowska-Truchanowicz *et al.*, 2013; Podgaec *et al.*, 2014), increased in peritoneal fluid (Li *et al.*, 2014) and decrease in endometriotic lesions (Tanaka *et al.*, 2017)
$\gamma\delta$T cells	**Fluctuation menstrual cycle:** Not described **Function:** Recognition of super-antigens or heat shock proteins	**Fluctuation menstrual cycle:** Not described **Function:** Not described **Comparison with normal eutopic endometrium:** Increased $\gamma\delta$T cells (Ota *et al.*, 1996)	**Fluctuation menstrual cycle:** Not described **Function:** Not described **Comparison with normal:** Not described

Abbreviations: Mø, macrophages; DC, dendritic cells; uNK, uterine natural killer cells; MC, mast cells; EN, eosinophils; NT, neutrophils; B cells, B lymphocytes; T cells, T lymphocytes; $CD8^+$ T cells, cytotoxic T cells; $CD4^+$ T cells, T helper cells (Th1, Th2 and Th17); Treg, regulatory T cells; NK T cells, natural killer T cells; $\gamma\delta$T cells, gamma-delta-T cells.

endometrium of women with and without endometriosis and the repercussions in ectopic sites. A summary of their interactions is shown in Fig. 2. In general terms, it is possible that eutopic endometrium of women with endometriosis is more pro-inflammatory than normal endometrium. Overall, the available evidence supports that aberrant functions of immune populations participate in different processes leading to an inhospitable environment for embryo implantation, disease establishment outside the uterus, fibrosis and pain. These include roles in promoting the survival of endometrial cells, attachment to the peritoneum and neuroangiogenesis.

In conclusion, understanding mucosal immune populations in eutopic and ectopic endometrium of women with endometriosis is anticipated to lead to better understanding of the pathophysiology of this multifactorial polygenic disorder and the development of new and less invasive diagnostics and novel therapeutics. The development of non-invasive diagnostic tools based on cytokines and autoantibodies would be a huge advance in the management of the disease. Moreover, there is promise for development of novel therapeutics for endometriosis symptoms, as well as for treating reproductive dysfunction by focusing on immunomodulators and functionally aberrant immune populations. There is a need to develop new approaches for the medical treatment of endometriosis. The use of non-hormonal therapies would be a great advance, since most of the women are treated with contraceptive steroids, estradiol synthesis inhibitors and GnRH analogues, which are all contraindicated in pursuing pregnancy. Nonsteroidal anti-inflammatory drugs are helpful in managing pain caused by endometriosis, although usually in combination with other therapies. Thus, an increased understanding of the immune and inflammatory aspects of endometriosis would be beneficial in the search for novel treatment strategies.

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Authors' roles

J.V.-J. conceived the initiative for this manuscript and played a major role in writing, assembling the figures and tables and doing literature search for the references. S.H., co-senior author, extensively contributed to conceiving, writing and editing the manuscript and participated extensively in discussions about content, format, inclusion, references and process. L.C.G., senior author, extensively contributed to conceiving, writing and editing the manuscript and participated extensively in discussions about content, format, inclusion, references and process.

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Conflict of interest

The authors have no conflicts of interest.

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