

Control of the immunological environment of the uterus

Sarah A. Robertson

Department of Obstetrics and Gynaecology and The Reproductive Medicine Unit, University of Adelaide, Adelaide SA 5005, Australia

The uterine immune axis holds the key to solving major problems in female reproductive health, including infertility, many pathologies of pregnancy, and sexually transmitted disease. The molecular determinants of tolerance and immunity in the reproductive tract are now being identified, and the governing principles are similar to those in other mucosal tissues. Cytokines are implicated as pivotal regulators at important 'decision-making' points in each phase of the induction and elicitation of a response. Indeed, the flexibility to deal appropriately with antigens as disparate as infectious micro-organisms, spermatozoa and the conceptus is likely to be attributable to the sophistication of the cytokine network in driving immune deviation. A better understanding of the factors controlling the development of immune activity in the uterus, particularly the significance of the inductive cytokine environment in determining the destiny of T-lymphocyte responses, will assist the rational design of new therapeutic strategies to treat immune-based reproductive disorders.

The uterus is uniquely adapted to support pregnancy while retaining the barrier function that is characteristic of all mucosal tissues. The capacity to distinguish between, and appropriately respond to, the array of foreign entities to which it is exposed is achieved through a highly specialized local immune system. Specific mechanisms acting to elicit immunological tolerance in the case of semen and the conceptus co-exist with active immunity to defend the uterus from pathogenic bacteria and viruses. Since populations of regulatory and effector T lymphocytes are likely to be the key orchestrators of any endometrial or decidual immune activity or tolerance, it is essential that the molecular events involved in their selective activation and expansion are deciphered. Studies in other mucosal tissues show that the character and strength of a T-lymphocyte response is the net consequence of pivotal 'decision-making' points in the inductive (afferent) and effector (efferent) pathways. This overview will highlight the significance of T lymphocytes in uterine immune responses and the nature of the major determinants of their generation, with a focus on the integral role of cytokines (Box 1) in these processes. Finally, the prospects for exploiting this knowledge to intervene therapeutically in immune responses to uterine pathogens or the conceptus will be discussed.

Immunological competence of the uterus

The uterus is part of the common mucosal immune system, sharing structural and functional similarities, and common lymphocyte trafficking networks, with the intestinal, bronchial, nasal-associated and ocular tissues, and salivary and mammary glands. The uterus is supplied with ample lymphatic drainage and contains the full range of lymphohaemopoietic cells and molecular regulators required to generate and elicit 'adaptive' (antigen-specific) immunity. The most striking difference between the uterus and other mucosal surfaces is the lack of highly organized secondary lymphoid nodules analogous to Peyer's patches and bronchus-associated lymphoid tissue (BALT). This

deficiency may be related to the smaller antigenic load encountered in the uterus. In the absence of infectious disease, antigen exposure is confined to the intermittent introduction of semen or conceptus tissue, and micro-organisms ascending from the vagina or introduced at insemination.

Antibody-mediated (humoral) and cell-mediated immunity can be induced in the uterus after infection, or immunization by antigen delivery to the reproductive tract or other mucosal surfaces (for reviews, see Parr and Parr, 1994; Wira *et al.*, 1999). Although there have been few systematic efforts to define the variables that determine the magnitude or quality of the uterine response to exogenous antigens, it is clear that the nature of the antigen, the route of sensitization and the use of adjuvants all have a major influence. The uterus is exceptional among mucosal tissues in that ovarian steroid hormones also have considerable effects on both afferent and efferent immune events (for review, see Wira *et al.*, 1999). Thus, the outcome of an immune response and the elicitation of protective immunity to pathogens can be markedly influenced by the stage of the oestrous cycle at which priming or infection takes place (Kaushic *et al.*, 1998; Parr and Parr, 1999).

'Immune deviation' and the significance of T lymphocytes

The quality and effectiveness of an adaptive immune response is contingent on the relative abundance and activities of alternative phenotypes of T lymphocytes (Box 2) recruited into the site of the antigenic insult. Different subsets of T lymphocytes elicit the full spectrum of immune responses, ranging from antibody-mediated or cell-mediated types of immunity, to various forms of anergy or active suppression. This capacity for 'deviation' in the immune response is now recognized to underlie most forms of immunological tolerance, as well as protective immunity, in mucosal tissues and is, therefore, implicated as the underlying mechanism for flexibility in uterine immune activity.

Box 1. Cytokine regulation of the uterine immune response

The cells of the immune system are often loosely distributed and nomadic, in continual flux through the blood and peripheral tissues. The immune system relies on the presence of a highly sophisticated communication network mediated by cytokines and other soluble messenger molecules to raise a rapid, integrated response to an antigenic stimulus. Cytokines are small, usually glycosylated proteins expressed and secreted in a highly regulated fashion, with the flexibility to act locally or at a distance, to limit their sphere of influence or act at a systemic level, and to exert transient or sustained effects. The special characteristics of cytokines and cytokine receptors, including the molecular basis of their complex patterns of diverse and overlapping actions, have been reviewed by Kelso (1998).

The cytokine network operating in the female reproductive tract is governed predominantly by ovarian steroid hormones, and it is through the agency of cytokines that reproductive hormones exert their powerful influence on local immune activity (Robertson *et al.*, 1994). During the oestrous cycle and pregnancy, fluctuating concentrations of ovarian steroid hormones have a marked effect on the pattern of cytokines emanating from endometrial somatic cells, particularly luminal and glandular epithelial cells. Further modulation of the cytokine axis occurs in response to insemination (Robertson *et al.*, 1992; Choudhuri and Wood, 1993; Tremellen *et al.*, 1998), the presence of a conceptus (McMaster *et al.*, 1993; Cocchiara *et al.*, 1996) or the introduction of foreign agents or infection (Rasmussen *et al.*, 1997). Additional factors, including genetic polymorphisms in cytokine genes, developmental events, nutrition and stress, contribute to quantitative differences in the magnitude and profile of the cytokine response among and within individuals (Table 1).

T-lymphocyte populations in the endometrium and cervix

T lymphocytes, defined on the basis of their expression of surface CD3 and T-cell receptor (TCR) molecules, comprise a significant lymphocyte population in the cyclic and pregnant endometrium of all species so far investigated. They are generally fewer in number than the distinctive, antigen non-specific population of CD56⁺CD16⁻CD3⁻ cells classified as 'uterine natural killer (NK) cells' (Whitelaw and Croy, 1996). B lymphocytes and plasma cells are also present in uterine tissues, demonstrating active humoral immunity (for review, see Parr and Parr, 1994). The abundance and distribution of T lymphocytes in uterine tissues is influenced by the species, sexual maturity and parity of the animal, and particularly by the stage of the menstrual cycle or pregnancy. Compartmentalization is evident in the patterns of T-lymphocyte localization within tissues, particularly in women, in whom, for example, the cervical tissues are more richly populated than the endometrium (Edwards and Morris, 1985; Morris *et al.*, 1985).

In humans, it is generally agreed that most endometrial T cells express the $\alpha\beta$ form and fewer cells express the $\gamma\delta$ form of the TCR (Vassiliadou and Bulmer, 1996). Most endometrial T cells also express CD8 and are located in intra-epithelial locations, or scattered singly or in aggregates adjacent to glands in the endometrial stromal tissue (Morris *et al.*, 1985; Vassiliadou

Table 1. Factors regulating cytokine synthesis in the uterus

Ovarian steroid hormones
Cytokines, soluble cytokine receptors and antagonists
Infection and bacterial or viral products
Prostaglandins and other soluble mediators
Extracellular matrix molecules
Stress hormones
Nutrition
Genetics (cytokine gene polymorphisms)
Developmental events

Box 2. T-lymphocyte subsets

T lymphocytes can be categorized into subsets on the basis of their cytokine secretion patterns and functional capabilities (Table 2). The best characterized T lymphocytes were originally labelled Th1 (for T helper type 1) and Th2, but the preferred terminology is now type 1 and type 2, since both CD4⁺ (helper) and CD8⁺ (suppressor-cytotoxic) T cells can display these patterns. Type 1 T cells participate in cell-mediated immune responses, such as delayed type hypersensitivity and macrophage activation, whereas type 2 subsets stimulate a humoral response by releasing cytokines that induce B lymphocytes to proliferate, differentiate and secrete antigen-reactive antibodies (Mosmann and Sad, 1996).

Other prominent cytokine patterns have been identified in CD4⁺ cells, including cells designated T helper type 3 (Th3) cells, which are characterized by copious secretion of TGF β (Weiner, 1997) and T-regulatory 1 (Tr1) cells which exhibit high interleukin 10 secretion (Groux and Powrie, 1999). Both Th3 and Tr1 cell types have potent suppressive activity and are likely to be key mediators of mucosal tolerance, acting principally by inhibiting type 1 responses. The regulatory influences of Th3 and Tr1 cells are not confined to a single antigen but instead inhibit inflammation in a general manner within the microenvironment of the sensitizing antigen, a phenomenon called 'bystander suppression' (Groux and Powrie, 1999). In mice, special subsets of T cells expressing natural killer cell markers (NK1⁺ T cells) also suppress type 1 immunity through their unique ability to secrete interleukin 4 (Bendelac *et al.*, 1997).

and Bulmer, 1996). During early pregnancy, the abundance of T lymphocytes decreases but CD3⁺ cells still account for 10–20% of the lymphocytes in the decidua, and there remains some debate over the relative proportions of cells expressing $\alpha\beta$ and $\gamma\delta$ TCRs (Mincheva Nilsson *et al.*, 1994; Vassiliadou and Bulmer, 1996). The precise phenotypes and specific cytokine expression profiles of decidual T cells have begun to be explored, and considerable heterogeneity is evident (Christmas and Meager, 1990; Jokhi *et al.*, 1994). Their antigenic specificities also remain unknown, but the majority of those expressing $\gamma\delta$ TCRs incorporate the V δ 1 gene segment and potentially recognize a wide array of self and/or trophoblast antigens (Christmas *et al.*, 1993; Mincheva Nilsson *et al.*, 1997).

Table 2. Alternative CD4⁺ T-lymphocyte phenotypes and their distinguishing characteristics

	Th1	Th2	Th3	Tr1	NK1 + T
Inducing cytokines	IL-12, IFN γ	IL-4, IL-10	IL-4, IL-10	IL-10	
Inducing factors	dsDNA, dsRNA, LPS	TGF β , PGE	TGF β		
Eliciting APC	type 1 DC	type 2 DC	DC	DC	DC/epithelial cell
Eliciting MHC	MHC class II	MHC class II	MHC class II	MHC class II	CD1
Major cytokine secreted	IFN γ , IL-2	IL-4, IL-5	TGF β	IL-10, TGF β	IL-4
Regulatory phenotype					
Help	Cell-mediated IR	IgG1/IgE	IgA		IgG1/IgE
Suppress	Th2	Th1	Th1/Th2	Th1/Th2	Th1
Antigen specificity					
Activation	Yes	Yes	Yes	Yes	No
Function	Yes	Yes	No	No	No
Bystander suppression	No	No	Yes	Yes	Yes

Th: T-helper; NK: natural killer; IL: interleukin; IFN: interferon; TGF: transforming growth factor; PGE: prostaglandin E; LPS: lipopolysaccharide; APC: antigen presenting cell; DC: dendritic cell; MHC: major histocompatibility complex; Ig: immunoglobulin; IR: immune response.

T lymphocytes are sparse in the uteri of non-pregnant mice (Croy *et al.*, 1993), although repeated exposure to semen and multiple parity may increase the number of resident T cells as exposure to antigen increases. Pregnancy is associated with a substantial increase in the numbers of $\alpha\beta$ and $\gamma\delta$ TCR⁺ T cells, which comprise up to 20% of the total lymphocytes in mid-gestation decidua (Heyborne *et al.*, 1992; Arck *et al.*, 1997). V δ 1⁺ cells, which as in humans predominate among murine decidual $\gamma\delta$ T cells, appear to recognize and respond to mouse and human trophoblast antigens in a non-major histocompatibility antigen (MHC) restricted manner, potentially through specificity for heat shock protein (HSP)-60 (Heyborne *et al.*, 1994). Specificity for paternal MHC class I antigens is detected among CD8⁺ T cells in lymph nodes draining the uterus, and evidence of downregulated TCR expression indicates functional unresponsiveness in these cells (Tafari *et al.*, 1995; Zhou and Mellor 1998). Although some individual $\gamma\delta$ decidual clones display a 'type 1' phenotype (see Box 2), on the basis of their expression of cytokines including interleukin 2 (IL-2) and tumour necrosis factor α (TNF α) (Heyborne *et al.*, 1992), other T cells can be categorized as 'type 2' or 'Th3' on the basis of their expression of transforming growth factor β (TGF β) of either the β 1 or β 2 isoform. These TGF β cells can suppress alloantigen-induced lymphocyte proliferation *in vitro* in a non-antigen-specific manner (Suzuki *et al.*, 1995; Clark *et al.*, 1997).

Infection, as well as pregnancy, can affect the populations of T cells found in the uterus. The absolute numbers and relative proportions of lymphocytes, particularly CD4⁺ T cells, are increased in the reproductive tracts of pathogen-infected mice or women who have sexually transmitted disease (Kaushic *et al.*, 1998; Levine *et al.*, 1998): the precise numbers and phenotypes are probably dependent on the nature of the pathogen and the stage of progression of the disease.

A more comprehensive picture of the exact nature of the T cells residing in the endometrium and decidua will only

emerge after a detailed characterization of their surface antigen- and cytokine-secreting profiles is achieved, preferably using multi-parameter analysis of individual cells by flow cytometry or reverse transcriptase (RT)-PCR. However, it is reasonable to conclude on the basis of current knowledge that decidual T cells bear similarities to subsets of regulatory and effector T lymphocytes found in other mucosal tissues, in terms of both their surface antigen expression and their cytokine secretion profile (Table 2). In particular, the identification of populations of cells secreting TGF β supports the proposition that a form of 'bystander suppression' mediated by Th3-like cells operates in the decidua during pregnancy. The presence in the uterus of populations analogous to the Tr1 or NK-T subsets, which are implicated in tolerance in other mucosae (Box 2), remains to be explored.

T-lymphocyte perturbations in pathologies of pregnancy

The functional significance of T lymphocytes in the uterine immune response is most clearly demonstrated by their associations with pathologies of pregnancy. An excess of type 1 activity in the implantation site is emerging as a key feature of disorders of pregnancy believed to have an immunological aetiology, including spontaneous abortion and pre-eclampsia (Raghupathy, 1997). The detrimental effect of type 1 immunity is exerted on the growth and development of the placenta, through eliciting inflammatory and pro-thrombotic cascades that interfere with blood supply (Clark *et al.*, 1998). In mice, studies in the abortion-prone CBA \times DBA strain combination have established causal relationships between specific subsets of decidual T cells and pregnancy outcome. Cell depletion experiments *in vivo* indicate that CD8⁺ $\alpha\beta$ T cells are absolutely necessary for immunological tolerance of the conceptus, and identify $\gamma\delta$ T cells with a type 1 cytokine secretion profile as predisposing to pregnancy loss (Arck *et al.*, 1997). The fact

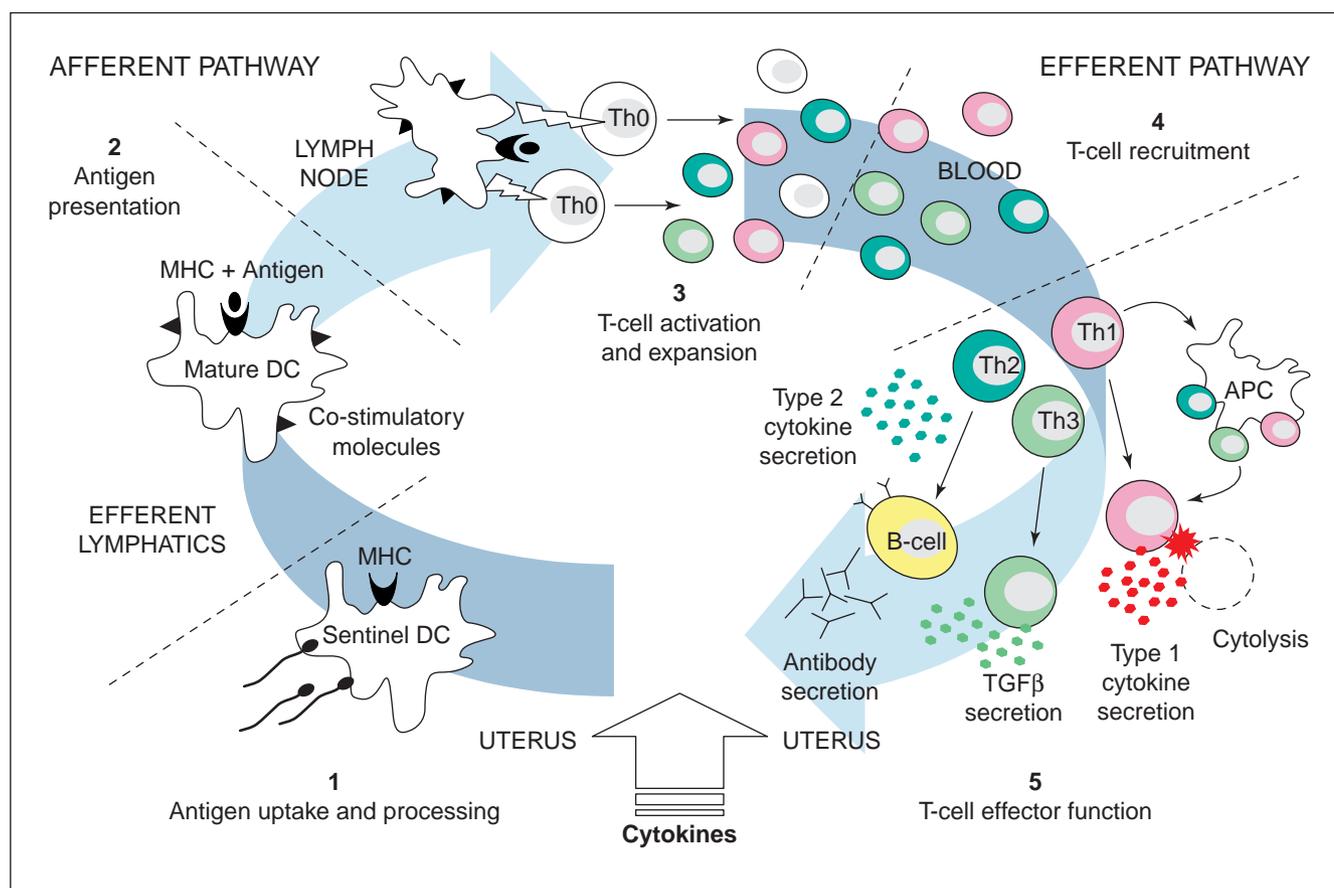


Fig. 1. The major events in the induction (afferent) and elicitation (efferent) pathways of an immune response include: (1) antigen (Ag) uptake and processing within antigen presenting cells (APCs); (2) trafficking of APCs to draining lymph nodes, and presentation of antigen fragments on the surface of the APC in association with major histocompatibility (MHC) molecules; (3) interaction between the APC and cognate lymphocytes to elicit the activation, proliferation and differentiation of specific populations of effector cells; (4) recruitment of specific effector cell populations from the circulation back into the inductive site, and (5) further differentiation of lymphocytes and exertion of specific effector functions, including synthesis of antibodies or cytokines, or ligation and cytolysis of specific target cells. Th0, Th1, Th2, Th3: T-helper type 0, type 1, type 2 and type 3, respectively; IL: interleukin; TGF: transforming growth factor; DC: dendritic cell.

that pregnancy can proceed in T-cell-deficient *scid/scid* mice indicates that the relative balance between these apparently beneficial and detrimental cell subsets is likely to be of paramount importance, rather than any trophic effect of T cells on placental development.

Although a similar cause and effect relationship is difficult to demonstrate in humans, there is now convincing evidence that an aberration in decidual T-lymphocyte phenotypes is associated with abortion in women. Expression of type 2 cytokines leukaemia inhibitory factor (LIF), IL-4 and IL-10 is diminished in both CD4⁺ and CD8⁺ decidual T-cell clones recovered from women who have experienced recurrent pregnancy loss (Piccinni *et al.*, 1998). This type 1 cytokine bias is reflected systemically in trophoblast antigen-reactive peripheral blood lymphocytes (Hill *et al.*, 1995).

Kinetics of T-lymphocyte generation

The relative proportions of alternative phenotypes of T lymphocytes comprising an antigen-specific pool is the net result

of a stepwise process that begins with activation of naïve lymphocytes by antigen presenting cells, continues with their differentiation and clonal expansion, and concludes with their recruitment back into the site of antigen entry (Fig. 1). There are many opportunities for cytokines to influence the development of different subsets at each stage of the processes of recognition and response to antigen.

The inductive phase – priming for tolerance versus immunity

Antigen uptake and processing

The first event in the induction of a new immune response is the uptake of antigen by endocytosis or phagocytosis into antigen-presenting cells (APCs) at the site of antigen encounter. Internalized antigen is degraded within the endolysosomal pathway into small immunogenic fragments, which are then presented on the cell surface in association with MHC molecules, in readiness for interaction with cognate T lymphocytes.

In the endometrium and cervix, the predominant cells implicated in antigen uptake and processing are MHC class II⁺ dendritic cells and macrophages. These cells form a contiguous network immediately subjacent to the epithelial surface in rodents (Head and Gaede, 1986) and in women, in whom they are especially concentrated at the transformation zone in the cervix (Edwards and Morris, 1985; Morris *et al.*, 1985; Tabibzadeh *et al.*, 1986).

There are substantial differences in the abundance and location of APCs within tissues, particularly macrophages, during both the oestrous cycle and pregnancy, which are the consequence of fluctuations in the expression of the cytokines and chemokines driving their recruitment into various tissue compartments (Jones *et al.*, 1997; Wood *et al.*, 1997). At some stages of the reproductive process, for example after insemination in mice, macrophages with an activated phenotype co-localize with dendritic cells in the endometrial and cervical tissues (McMaster *et al.*, 1992; Robertson *et al.*, 1996a). However, because of the paucity of markers for distinguishing accurately between these cells, their precise abundance, locations and ontogenic relationship have yet to be fully characterized. In addition, the roles of non-professional uterine APCs, such as luminal and glandular epithelial cells, which can express MHC class II constitutively and in response to infection (Kaushic *et al.*, 1998), as well as the non-classical MHC class I-b molecule CD1, remain to be explored (Canchis *et al.*, 1993).

Although soluble proteins instilled into the uterine lumen can be endocytosed into epithelial cells, passage across the epithelium into stromal APCs does not occur readily (Parr and Parr, 1994), and immune responses are difficult to elicit with soluble proteins administered into the uterine lumen in the absence of adjuvants (for review, see Parr and Parr, 1994). The access of APCs to luminal antigens may be facilitated by trauma to the epithelial surface, for example, in the event of infection or endometrial sloughing, and this explains why invasive micro-organisms such as *Chlamydia* elicit stronger responses than protein antigens (Stagg *et al.*, 1998). Constitutive dendritic cell phagocytosis of apoptotic epithelial cells has been documented in intestinal tissues (MacPherson and Liu, 1999), demonstrating that the phenomenon of 'cross-priming', where by dendritic cells can prime CD8⁺ cells after acquisition of antigen from apoptotic cells, may be a common mechanism facilitating transport of luminal antigens into stromal APCs in mucosal tissues. In addition, there is clear evidence of uptake of sperm antigens into phagocytes in the uterine stroma after insemination in mice (Watson *et al.*, 1983), indicating that factors in semen elicit increased epithelial permeability to luminal antigens, or that spermatozoa have special adhesive properties facilitating their uptake into endometrial tissue.

During human pregnancy, cells expressing MHC class II molecules are abundant in decidual tissues recovered during the first trimester and also at term (Bulmer *et al.*, 1988) but, in mice, they are sequestered away from the decidual-placental interface from early after implantation, and are relegated to the metrial gland and mesometrial tissue (Redline and Lu, 1989). Even in this more remote location, APCs may acquire antigens released in soluble form or as the consequence of the apoptosis of trophoblast cells. It is possible that trophoblast cells present antigens to decidual lymphocytes, in the context of their non-polymorphic class I-b molecules, a notion supported by the

finding that human leukocyte antigen G (HLA-G) presents peptide (Diehl *et al.*, 1996).

Dendritic cell activation and emigration

Effective T-cell priming to antigens encountered in the uterus by conventional APCs would require that, once loaded with antigen, the APCs emigrate from the endometrial tissues to draining lymph nodes via the afferent lymphatics. By analogy with events described in other mucosae, this emigration would be accompanied by a phenotypic and functional maturation characterized by increased expression of molecules required for effective antigen presentation, including MHC class II and co-stimulatory molecules (Banchereau and Steinman, 1998; Sallusto and Lanzavecchia, 1999).

Several cytokines known to influence APC maturation and function are expressed by somatic cells and leukocytes in endometrial tissues under the regulation of ovarian steroid hormones (for review, see Robertson *et al.*, 1994). Granulocyte macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor α (TNF α), the key cytokines implicated in the maturation of sentinel dendritic cells, are expressed in uterine epithelial cells and mast cells after induction by oestrogen (Hunt *et al.*, 1997a). In contrast, cytokines that inhibit these events, such as CSF-1 and IL-10, and other products of local leukocytes, including nitric oxide (Bilyk and Holt, 1995), are induced by progesterone in the secretory phase of the human cycle and during early pregnancy in mice (Daiter and Pollard, 1992; Hunt *et al.*, 1997a). Progesterone induces a decrease in GM-CSF expression (Robertson *et al.*, 1996b), an effect that might be augmented by the destabilizing effects of IL-10 on mRNA encoding GM-CSF (Brown *et al.*, 1996). Taken together, these observations indicate a major role for steroid hormones in driving the inductive phase of the immune response through differential effects on cytokine-regulated antigen uptake and processing (Fig. 2). This proposal may account for the well-recognized immunosuppressive effects of progesterone, and is supported by functional data from experiments showing that steroid hormones regulate the immunostimulatory activity of rat uterine cell preparations *in vitro* (Wira, 1999).

The predisposing effects of oestrogen on antigen uptake and processing may be augmented by exposure to semen (Fig. 2). In mice, there is a further upregulation in expression of GM-CSF, IL-1 and TNF α after insemination (McMaster *et al.*, 1992; Robertson *et al.*, 1992) and the effects of these cytokines could be boosted by seminal TGF β (Tremellen *et al.*, 1998), which counteracts the inhibitory effects of macrophages (Bilyk and Holt, 1995). Changes in the abundance and activation phenotypes of rodent uterine macrophages and dendritic cells that are consistent with an effect of insemination on antigen processing activity have been reported by De *et al.* (1991) and Robertson *et al.* (1996a). Whether a similar influence of insemination is exerted in the cervical tissues of women is not known. Furthermore, specific chemokines that direct the movements of dendritic cells by differentially targeting immature and activated cells have now been described, and exploration of their occurrence and temporal expression in the uterus would be illuminating (Sallusto and Lanzavecchia, 1999).

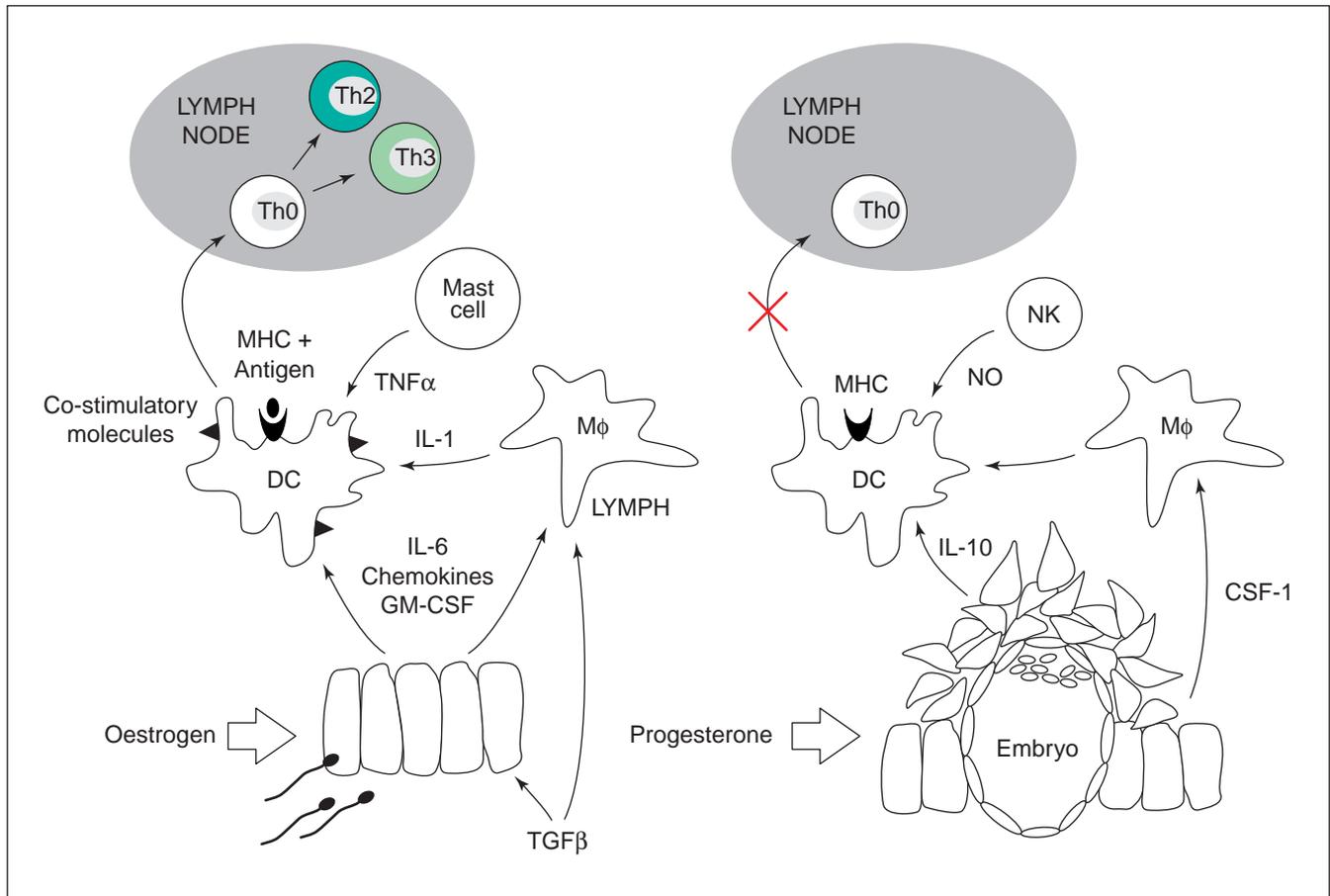


Fig. 2. Effect of ovarian steroid hormones and insemination on the expression of cytokines and the behaviour of antigen-presenting cells in the mouse endometrium. The cytokine environment at the time of insemination is conducive to induction of new immune responses. Transforming growth factor β (TGF β) contained in seminal plasma induces expression of granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin 6 (IL-6) and chemokines in uterine epithelial cells, which elicit the recruitment and activation of macrophages and dendritic cells in the endometrial stroma. Expression of IL-1 and tumour necrosis factor α (TNF α) in macrophages would augment the effects of GM-CSF in driving the maturation of sentinel dendritic cells (DC) and their emigration to draining lymph nodes. In the absence of 'danger' signals, this cytokine environment would be expected to elicit T lymphocytes with type 2 and T-helper type 3 phenotypes. Conversely, the cytokine environment at the time of implantation is not conducive to the induction of immune responses. Progesterone-induced synthesis of CSF-1, IL-10 and nitric oxide in uterine epithelial cells and leukocytes would be expected to constrain DC activity in the implantation site. In other mucosal tissues, these cytokines act directly and indirectly through the agency of macrophages to inhibit DC transition from the sentinel to the mature state. NK: natural killer cell.

Antigen presentation and lymphocyte activation – general principles

Once recruited into lymph nodes, APCs induce the activation, proliferation and differentiation of antigen-reactive T lymphocytes. The factors that drive the selective differentiation of alternative subsets include: (1) the cytokine environment, (2) the nature of the antigen, (3) the dose of antigen, and (4) the presence or absence of a repertoire of soluble and membrane-bound 'co-stimulatory' signals delivered by the APC to the T lymphocyte (Seder and Paul, 1994; Constant and Bottomly, 1997). The most clearly defined variable is the nature of the cytokine environment in which the APC-antigen encounter takes place (Table 2). Thus, local conditions within the site of antigen encounter are thought to polarize the

capacity of dendritic cells to trigger alternative subsets of effector and regulatory T cells (Stephens and Thomson, 1996; Kapsenberg *et al.*, 1999). IL-12 and, to a lesser extent, interferon γ (IFN γ), IFN α and IL-18 appear to be the most important cytokines for promoting type 1 differentiation, whereas IL-4 has the greatest influence in driving type 2 differentiation (Table 2). The induction of Th3 cells is augmented by IL-4, IL-10, bacterial lipopolysaccharide (LPS), and IFN β (Weiner, 1997), whereas Tr1 cells arise when antigen is delivered in the presence of IL-10 (Groux and Powrie, 1999). The APC lineage also has a potent influence on the character of the ensuing response, with epithelial cells and haemopoietic APCs expressing CD1 appearing to trigger the activation of NK1 $^+$ T cells (Bendelac *et al.*, 1997) and regulatory cells with suppressive functions (Mayer, 1997) preferentially.

Antigen presentation and lymphocyte activation in the endometrium and cervix

In the uterus, the influence of the oestrous cycle and the intermittent presence of semen and conceptus tissue cause considerable fluctuations in the concentrations of cytokines that affect APC–T cell interactions. Probably the greatest and most frequent antigenic challenge to the female reproductive tract occurs at insemination when, at least in mice, there is clear evidence for resultant T-cell priming (Beer and Billingham, 1974). The character of the ensuing response is likely to be profoundly influenced by the considerable immune-deviating activity of seminal plasma. TGF β , present in seminal plasma at concentrations of 100–200 ng ml⁻¹ (Nocera and Chu, 1993; Tremellen *et al.*, 1998), is a highly potent immune-deviating agent and key tolerance-inducing molecule in the ocular, intestinal and respiratory mucosae (Letterio and Roberts, 1998). In humans, the activity of seminal TGF β is augmented by prostaglandin E₂ (PGE₂) (Kelly *et al.*, 1997), which acts to programme a type 2-inducing phenotype in dendritic cells through its capacity to inhibit IL-12 synthesis (Kapsenberg *et al.*, 1999). A ‘tolerance-inducing’ effect of seminal plasma was indicated by the finding that seminal plasma abrogates the priming for delayed type hypersensitivity achieved by intrauterine instillation of spermatozoa in rats (Beer and Billingham, 1974). In addition, human seminal plasma has been shown to skew the phenotype of lymphocytes induced to proliferate *in vitro* (Kelly *et al.*, 1997). Functional evidence for a type 1 inhibiting effect is provided by the finding that peripheral tolerance to paternal transplantation antigens can be induced in mice by mating them with intact but not seminal vesicle-deficient males (S. A. Robertson, K. Branson and R. F. Seamark, unpublished). Diminished type 1 immunity when antigen is delivered in the context of seminal plasma may also account for the marked attenuation by semen of simian immunodeficiency virus infectivity in macaques (Neildez *et al.*, 1998).

The effects of seminal cytokines are augmented by TGF β and PGE₂ secreted from endocervical and endometrial cells in women (Fichorova and Anderson, 1999) and endometrial epithelial cells in rodents (Tamada *et al.*, 1990). Additional type 2- and Th3-deviating cytokines, including IL-4, IL-6 and IL-10, are secreted in the endometrium in women (Lim *et al.*, 1998) and are likely to ensure that tolerance is the default outcome to challenge with luminal antigens. Although type 1-deviating cytokines, including IL-12, IL-2 and IFN γ , are rarely detected in non-pregnant endometrial tissue (Lim *et al.*, 1998), the uterus and endocervical tissues have the capacity to override this default situation and mount a type 1 immune response when confronted with ‘danger signals’ identifying the threat of infection (Stagg *et al.*, 1998; Rasmussen *et al.*, 1997). One such signal is IFN γ , which can evoke macrophage expression of the type 1-skewing cytokine IL-12. In immortalized endometrial and cervical epithelial cells, IFN γ is a potent inducer of cytokine and chemokine expression, and also of MHC class II antigens (Tabibzadeh *et al.*, 1986; Fichorova and Anderson, 1999). Intraepithelial lymphocytes and, unexpectedly, neutrophils are capable of producing IFN γ in endometrial tissue (Yeaman *et al.*, 1998), but a correlation between expression of these cytokines and local expansion of type 1 T lymphocytes has yet to be demonstrated.

Cytokines with the capacity to skew immune responses through influencing the differentiation and expansion of specific lymphocyte populations are abundant in the implantation site. Copious quantities of IL-4, IL-10 and PGE₂ secreted by placental trophoblast and amnion epithelial cells (Jones *et al.*, 1995; Kelly *et al.*, 1995; Roth *et al.*, 1996), as well as IL-4 and TGF β secreted by uterine NK cells and decidual T lymphocytes (Jokhi *et al.*, 1994; Clark *et al.*, 1997; Whitelaw and Croy 1996), ensure that any local APCs are continuously bathed in type 2- and Th3-deviating cytokines. The effects of these cytokines are bolstered by the type 2-deviating effects of progesterone-induced blocking factor (PIBF), a 34 kDa protein released by lymphocytes in response to progesterone (Szekeres-Bartho and Wegmann, 1996). In contrast, TNF α and IFN γ would be expected to counteract the type 2-polarizing cytokines and, indeed, aberrant secretion of TNF α and IFN γ by decidual macrophages and activated NK cells is associated with fetal resorption in mice (Clark *et al.*, 1998). Human decidual neutrophils and macrophages can also express IFN γ and IL-12, for example after stimulation with LPS (Haddad *et al.*, 1997; Yeaman *et al.*, 1998).

Thus, there is a molecular framework for achieving immune deviation by influencing the inductive phase of the immune response during pregnancy. However, considerable experimental work is now required to demonstrate causal links between expression of immune-deviating cytokines, the existence of specific phenotypes in decidual APCs, and the activation and expansion of T-lymphocyte populations with regulatory or effector functions that can influence the outcome of pregnancy.

The effector phase – achieving acceptance versus rejection

The effector phase of the immune response involves recruitment of T lymphocytes from the circulation back into the site of antigen encounter, further rounds of proliferation and differentiation within that site, and the exertion of effector functions. Each of these processes is susceptible to molecular regulation that affects the final outcome.

T-lymphocyte recruitment into the reproductive tract

Specific patterns of recruitment or homing are likely to contribute to the specificity in T-lymphocyte phenotypes present in the cyclic and pregnant uterus. All lymphocytes are programmed during their development to follow specific migration pathways through the body that enable antigen-specific immune responses to be concentrated at certain sites. Directed migration, or ‘homing’, into mucosal tissues is controlled by expression of distinct patterns of adhesion molecules on the lymphocyte cell surface, which mediate differential recognition and adherence to endothelium in mucosal sites.

Cell-tracking experiments show that cells primed in the nasal-associated lymphoid tissue (NALT) home preferentially to the reproductive tract, indicating that similar molecules might control lymphocyte homing to both sites. However, any regional specificity is not absolute, since priming of rectal tissue and, to a lesser extent, the upper intestinal tract, can also elicit cells that migrate into the urogenital tissues (Parr and Parr, 1994). Little is known about the molecular regulation

of T-lymphocyte homing into endometrial tissues, but there is evidence of temporal patterns of adhesion molecule expression in endometrial endothelial cells that could regulate access of lymphocytes both spatially and temporally (Rees *et al.*, 1993). Local microdomains are also evident within the implantation site, with differential expression of adhesion molecules among local vessels (Burrows *et al.*, 1994). One example of this is mucosal vascular addressin (MAdCAM-1), which unexpectedly has been found in a proportion of vessels at the leading edge of trophoblast invasion in women, despite being otherwise absent from the reproductive tract (Kruse *et al.*, 1999). It would be of great interest to investigate whether differential expression in the endometrium and decidua of specific chemokines relates to the preferential recruitment of type 1 and type 2 T-lymphocyte populations in different situations (Sallusto *et al.*, 1998).

Lymphocyte differentiation, survival and effector function in situ

Once recruited into local tissues, the retention and further proliferation of antigen-specific lymphocytes is driven by the presence of local antigen, leading to further expansion of reactive T-cell clones. Like naïve T-cell priming, this expansion is dependent on the interaction between T cells and resident APCs and is equally susceptible to the prevailing cytokine environment. Although dendritic cells are essential for stimulating naïve T cells during the first encounter with antigen, activated macrophages and B cells can stimulate experienced ('activated' or 'memory') T lymphocytes.

Despite the fact that highly structured secondary lymphoid nodules are not observed in the uterus, there is evidence of local expansion of T-lymphocyte clones. Small aggregates of lymphocytes, comprised mainly of CD8⁺ T lymphocytes, but also B lymphocytes and MHC⁺ class II APCs, have been described in the endometrium of women (Yeaman *et al.*, 1997; Vassiliadou and Bulmer, 1998). The increase in size of these aggregates at ovulation and during the secretory phase, and their absence in post-menopausal women, indicates regulation by steroid hormone-driven cytokines.

Discovery of expression of the recombinase activating enzymes (RAG-1 and RAG-2) in the implantation site in mice has led to speculation that the decidua comprise a site for extrathymic T-cell differentiation during early pregnancy (Kimura *et al.*, 1995). RAG-1 and RAG-2 expression has also been reported within decidual aggregates of CD3⁺ T lymphocytes during the first trimester of human pregnancy (Hayakawa *et al.*, 1994; Mincheva Nilsson *et al.*, 1997). Hayakawa *et al.* (1994) and Mincheva Nilsson *et al.* (1997) have claimed that RAG-1 and RAG-2 expression is also evident in decidual CD56⁺ cells, but this claim has been disputed by King *et al.* (1998). The ontogenic relationship between cells classified as uterine NK cells and mucosal T lymphocytes remains unclear, especially now that components of the CD3 complex, a defining marker of T lymphocytes, have been found in the cytoplasm of decidual CD56⁺ cells (Hayakawa *et al.*, 1994; King *et al.*, 1998).

Even after terminal differentiation, the behaviour of T lymphocytes can be influenced *in situ* depending on local cytokine activity. For example, it has been shown that IFN γ release and anti-trophoblast cytotoxic activity in uterine NK cells can both be augmented by culture in IL-2 and IL-12 (Marzusch *et al.*, 1997; Hayakawa *et al.*, 1999). The differential survival of

T lymphocytes in the uterus is likely to be constrained by specific apoptotic lysis of certain subsets. This mechanism appears to be particularly important in pregnancy, during which fas ligand-expressing decidual and trophoblast cells are ideally positioned to delete fas⁺ lymphocytes (Hunt *et al.*, 1997b).

Exploiting cytokines to manipulate the uterine immune response

Each 'decision-making' step in the induction and elicitation of the uterine immune response is open to exogenous intervention. The cytokines that drive the generation of immune responses are obvious tools and targets for strategies designed to stimulate or inhibit aspects of a new immune response, or even to redirect existing responses (Kelso, 1998).

Immunization against conception and sexually transmitted pathogens

The host defence mechanisms operating to control sexually transmitted infections, including human immunodeficiency virus, human papilloma virus, HSV-2 and *Chlamydia*, are being elucidated, and key antigens and specific types of protective immunity have been identified (Parr and Parr, 1999). The challenge now is to construct vaccines that effectively elicit and sustain the desired type of immunity, in the appropriate tissue. Similarly, contraceptive vaccines involving immunization against reproductive antigens to confer protection against conception or embryo implantation have enormous potential for the humane control of pest animal species, and perhaps also in human fertility regulation. Prophylactic and contraceptive vaccines of the future will almost certainly use cytokines as adjuvants to deviate the immune response in the desired manner (Ramsay and Ramshaw, 1997). Various strategies for the incorporation of cytokines in vaccines, either as soluble proteins, naked DNA or in replicating vectors, are currently being explored. A further consideration will be the route of administration, with the established link between the nasal and genital tissues indicating that aerosol administration to the airways might prove an efficacious and practical alternative to reproductive tract delivery.

Immunotherapy for pathologies of pregnancy?

A second, and potentially major, application of emerging knowledge will be in the development of therapeutic treatments to treat infertility and other immunological disorders including recurrent abortion and pre-eclampsia. The underlying lesion in many of these disorders is likely to be imbalance in the phenotypes of regulatory lymphocytes present in decidual tissues, specifically a deficiency in those subsets that suppress the formation of type 1 activity (Raghupathy, 1997). This imbalance may reflect disturbances in some or all of the stages of the generation of the immune response to conceptus antigens, but current paradigms identify the cytokine environment during the initial stages of the response, particularly at antigen presentation, are most critical.

Exogenous manipulation of the activities of cytokines has been used to considerable effect in improving pregnancy outcome in mouse models (Chaouat *et al.*, 1990, 1995) and, at face

value, seems an attractive proposition in women. Theoretically, type 1 cytokines such as TNF α could be neutralized by systemic administration of specific antibodies, soluble cytokine receptors, or natural or synthetic cytokine antagonists. Administration of a cytokine that counteracts the effect of TNF α (for example, IL-10) might achieve a comparable effect. The problem with these approaches is the potential for toxic side-effects in tissues unrelated to the target site. Innovative strategies for overcoming this impediment include targeted delivery systems, or virally encoded or naked cytokine DNA to achieve sustained cytokine exposure at selected sites (Kelso, 1998). An alternative approach is cell therapy to exploit the phenomenon of by-stander suppression, whereby suppressive T cells generated against antigens known to be present in the implantation site might be used to inhibit a pathogenic response without the necessity for identical antigenic specificity.

Conclusions and perspectives

The picture emerging from the studies highlighted in this review is of a uterine immune environment featuring a series of molecular mechanisms to ensure that tolerance is the default response to foreign entities that breach the epithelial barrier. This tolerance appears to be achieved principally through the agency of cytokine environments that either drive the induction of type 2 or Th3 T-lymphocyte responses (for example, at insemination), or interfere with the induction of new responses by inhibiting the activation and emigration of antigen-presenting cells (for example, during pregnancy). The elicitation of type 1 immunity seems to require antigen exposure in the context of specific cytokines or other signalling agents of bacterial or viral origin that denote 'danger'. Dysregulation in the mechanisms governing alternative immune outcomes is likely to manifest as infertility, or inadequate protection from infectious disease. The prospects for therapeutic intervention to manipulate the uterine immune environment will become clearer as a deeper knowledge is acquired of the interactions between different effector and regulatory T-lymphocyte populations in the uterus, and the key roles of cytokines in regulating each phase of their induction and function.

S. A. Robertson is supported by a Research Fellowship and Project Grants from the NH and MRC of Australia.

References

Key references are indicated by asterisks.

- Arck PC, Ferrick DA, Steele Norwood D, Croitoru K and Clark DA (1997) Murine T cell determination of pregnancy outcome I. Effects of strain, alphabeta T cell receptor, gammadelta T cell receptor, and gammadelta T cell subsets *American Journal of Reproductive Immunology* **37** 492–502
- *Banchereau J and Steinman RM (1998) Dendritic cells and the control of immunity *Nature* **392** 245–252
- Beer AE and Billingham RE (1974) Host responses to intra-uterine tissue, cellular and fetal allografts *Journal of Reproduction and Fertility Supplement* **21** 59–88
- Bendelac A, Rivera MN, Park SH and Roark JH (1997) Mouse CD1-specific NK1 T cells: development, specificity, and function *Annual Review of Immunology* **15** 535–562
- Bilyk N and Holt PG (1995) Cytokine modulation of the immunosuppressive phenotype of pulmonary alveolar macrophage populations *Immunology* **86** 231–237
- Brown CY, Lagnado CA, Vadas MA and Goodall GJ (1996) Differential regulation of the stability of cytokine mRNAs in lipopolysaccharide-activated blood monocytes in response to interleukin-10 *Journal of Biological Chemistry* **271** 20 108–20 112
- Bulmer JN, Morrison L and Smith JC (1988) Expression of class II MHC gene products by macrophages in human uteroplacental tissue *Immunology* **63** 707–714
- Burrows TD, King A, Loke YW (1994) Expression of adhesion molecules by endovascular trophoblast and decidual endothelial cells: implications for vascular invasion during implantation *Placenta* **15** 21–33
- Canchis PW, Bhan AK, Landau SB, Yang L, Balk SP and Blumberg RS (1993) Tissue distribution of the non-polymorphic major histocompatibility complex class I-like molecule, CD1d *Immunology* **80** 561–565
- Chaouat G, Menu E, Clark DA, Dy M, Minkowski M and Wegmann TG (1990) Control of fetal survival in CBA \times DBA/2 mice by lymphokine therapy *Journal of Reproduction and Fertility* **89** 447–458
- Chaouat G, Assal Meliani A, Martal J, Raghupathy R, Elliot J, Mosmann T and Wegmann TG (1995) IL-10 prevents naturally occurring fetal loss in the CBA \times DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by *in vivo* injection of IFN-tau *Journal of Immunology* **154** 4261–4268
- Choudhuri R and Wood GW (1993) Production of interleukin-1, interleukin-6, and tumor necrosis factor alpha in the uterus of pseudopregnant mice *Biology of Reproduction* **49** 596–603
- Christmas SE and Meager A (1990) Production of interferon-gamma and tumour necrosis factor-alpha by human T-cell clones expressing different forms of the gamma delta receptor *Immunology* **71** 486–492
- Christmas SE, Brew R, Deniz G and Taylor JJ (1993) T-cell receptor heterogeneity of gamma delta T-cell clones from human female reproductive tissues *Immunology* **78** 436–443
- Clark DA, Merali FS, Hoskin DW, Steel Norwood D, Arck PC, Croitoru K, Murgita RA and Hirte H (1997) Decidua-associated suppressor cells in abortion-prone DBA/2-mated CBA/J mice that release bioactive transforming growth factor beta2-related immunosuppressive molecules express a bone marrow-derived natural suppressor cell marker and gamma delta T-cell receptor *Biology of Reproduction* **56** 1351–1360
- Clark DA, Chaouat G, Arck PC, Mitruecker HW and Levy GA (1998) Cytokine-dependent abortion in CBA \times DBA/2 mice is mediated by the procoagulant fg12 prothrombinase *Journal of Immunology* **160** 545–549
- Cocchiara R, Lampiasi N, Albegiani G, Azzolina A, Bongiovanni A, Gianaroli L, Di Blasi F and Geraci D (1996) A factor secreted by human embryo stimulates cytokine release by uterine mast cell *Molecular Human Reproduction* **2** 781–791
- *Constant SL and Bottomly K (1997) Induction of Th1 and Th2 CD4⁺ T cell responses: the alternative approaches *Annual Review of Immunology* **15** 297–322
- Croy BA, Stewart CM, McBey BA, and Kiso Y (1993) An immunohistologic analysis of murine uterine T cells between birth and puberty *Journal of Reproductive Immunology* **23** 223–233
- Daitei E and Pollard JW (1992) Colony stimulating factor-1 (CSF-1) in pregnancy *Reproductive Medicine Review* **1** 83–97
- De M, Choudhuri R and Wood GW (1991) Determination of the number and distribution of macrophages, lymphocytes, and granulocytes in the mouse uterus from mating through implantation *Journal of Leukocyte Biology* **50** 252–262
- *Diehl M, Munz C, Keilholz W, Stevanovic S, Holmes N, Loke YW and Rammensee HG (1996) Nonclassical HLA-G molecules are classical peptide presenters *Current Biology* **6** 305–314
- Edwards JN and Morris HB (1985) Langerhans' cells and lymphocyte subsets in the female genital tract *British Journal of Obstetrics and Gynaecology* **92** 974–982
- Fichorova RN and Anderson DJ (1999) Differential expression of immunobiological mediators by immortalized human cervical and vaginal epithelial cells *Biology of Reproduction* **60** 508–514
- *Groux H and Powrie F (1999) Regulatory T-cells and inflammatory bowel disease *Immunology Today* **20** 442–446
- Haddad EK, Duclos AJ, Anteckka E, Lapp WS and Baines MG (1997) Role of interferon-gamma in the priming of decidual macrophages for nitric oxide production and early pregnancy loss *Cellular Immunology* **181** 68–75
- Hayakawa S, Saito S, Nemoto N *et al.* (1994) Expression of recombinase-activating genes (RAG-1 and 2) in human decidual mononuclear cells *Journal of Immunology* **153** 4934–4939

- Hayakawa S, Nagai N, Kanaeda T, Karasaki Suzuki M, Ishii M, Chishima F and Satoh K (1999) Interleukin-12 augments cytolytic activity of peripheral and decidual lymphocytes against choriocarcinoma cell lines and primary culture human placental trophoblasts *American Journal of Reproductive Immunology* **41** 320–329
- Head JR and Gaede SD (1986) Ia antigen expression in the rat uterus *Journal of Reproductive Immunology* **9** 137–153
- Heyborne K, Cranfill RL, Carding SR, Born WK and O'Brien RL (1992) Characterization of gamma delta T lymphocytes at the maternal–fetal interface *Journal of Immunology* **149** 2872–2878
- Heyborne K, Fu YX, Nelson A, Farr A, O'Brien R and Born W (1994) Recognition of trophoblasts by gamma delta T cells *Journal of Immunology* **153** 2918–2926
- Hill JA, Polgar K and Anderson DJ (1995) T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion *Journal of the American Medical Association* **273** 1933–1936
- Hunt JS, Miller L, Roby KF, Huang J, Platt JS, DeBrot BL (1997a) Female steroid hormones regulate production of pro-inflammatory molecules in uterine leukocytes *Journal of Reproductive Immunology* **35** 87–99
- Hunt JS, Vassmer D, Ferguson TA and Miller L (1997b) Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus *Journal of Immunology* **158** 4122–4128
- Jokhi PP, King A, Sharkey AM, Smith SK and Loke YW (1994) Screening for cytokine messenger ribonucleic acids in purified human decidual lymphocyte populations by the reverse-transcriptase polymerase chain reaction *Journal of Immunology* **153** 4427–4435
- Jones CA, Williams KA, Finlay Jones JJ and Hart PH (1995) Interleukin 4 production by human amnion epithelial cells and regulation of its activity by glycosaminoglycan binding *Biology of Reproduction* **52** 839–847
- Jones RL, Kelly RW and Critchley HO (1997) Chemokine and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation *Human Reproduction* **12** 1300–1306
- *Kapsenberg ML, Hilkens CM, Wierenga EA and Kalinski P (1999) The paradigm of type 1 and type 2 antigen-presenting cells. Implications for atopic allergy *Clinical and Experimental Allergy* **29** Supplement 2 33–36
- Kaushic C, Murdin AD, Underdown BJ and Wira CR (1998) *Chlamydia trachomatis* infection in the female reproductive tract of the rat: influence of progesterone on infectivity and immune response *Infection and Immunity* **66** 893–898
- Kelly RW, Carr GG, Elliott CL, Tulppala M and Critchley HO (1995) Prostaglandin and cytokine release by trophoblastic villi *Human Reproduction* **10** 3289–3292
- *Kelly RW, Carr GG and Critchley HO (1997) A cytokine switch induced by human seminal plasma: an immune modulation with implications for sexually transmitted disease *Human Reproduction* **12** 677–681
- Kelso A (1998) Cytokines: principles and prospects *Immunology and Cell Biology* **76** 300–317
- Kimura M, Hanawa H, Watanabe H, Ogawa M and Abo T (1995) Synchronous expansion of intermediate TCR cells in the liver and uterus during pregnancy *Cellular Immunology* **162** 16–25
- King A, Gardner L, Sharkey A and Loke YW (1998) Expression of CD3 epsilon, CD3 zeta, and RAG-1/RAG-2 in decidual CD56⁺ NK cells *Cellular Immunology* **183** 99–105
- Kruse A, Merchant MJ, Hallmann R and Butcher EC (1999) Evidence of specialized leukocyte-vascular homing interactions at the maternal/fetal interface *European Journal of Immunology* **29** 1116–1126
- Letterio JJ and Roberts AB (1998) Regulation of immune responses by TGF-beta *Annual Review of Immunology* **16** 137–161
- Levine WC, Pope V, Bhoomkar A, Tambe P, Lewis JS, Zaidi AA, Farshy CE, Mitchell S and Talkington DF (1998) Increase in endocervical CD4 lymphocytes among women with nonulcerative sexually transmitted diseases *Journal of Infectious Disease* **177** 167–174
- Lim KJ, Odukoya OA, Ajjan RA, Li TC, Weetman AP and Cooke ID (1998) Profile of cytokine mRNA expression in peri-implantation human endometrium *Molecular Human Reproduction* **4** 77–81
- McMaster MT, Newton RC, Dey SK and Andrews GK (1992) Activation and distribution of inflammatory cells in the mouse uterus during the pre-implantation period *Journal of Immunology* **148** 1699–1705
- McMaster MT, Dey SK and Andrews GK (1993) Association of monocytes and neutrophils with early events of blastocyst implantation in mice *Journal of Reproduction and Fertility* **99** 561–569
- MacPherson GG and Liu LM (1999) Dendritic cells and Langerhans cells in the uptake of mucosal antigens *Current Topics in Microbiology and Immunology* **236** 33–53
- Marzusch K, Buchholz F, Ruck P, Handgretinger R, Geiselhart A, Engelmann L and Dietl J (1997) Interleukin-12- and interleukin-2-stimulated release of interferon-gamma by uterine CD56⁺ large granular lymphocytes is amplified by decidual macrophages *Human Reproduction* **12** 921–924
- Mayer L (1997) Regulation of mucosal immune responses: distinct antigens and antigen presenting cells *Journal of Clinical Immunology* **17** 349–353
- Mincheva Nilsson L, Baranov V, Yeung MM, Hammarstrom S and Hammarstrom ML (1994) Immunomorphologic studies of human decidua-associated lymphoid cells in normal early pregnancy *Journal of Immunology* **152** 2020–2032
- *Mincheva Nilsson L, Kling M, Hammarstrom S, Nagaeva O, Sundqvist KG, Hammarstrom ML and Baranov V (1997) Gamma delta T cells of human early pregnancy decidua: evidence for local proliferation, phenotypic heterogeneity, and extrathymic differentiation *Journal of Immunology* **159** 3266–3277
- Morris H, Edwards J, Tiltman A and Emms M (1985) Endometrial lymphoid tissue, an immunohistological study *Journal of Clinical Pathology* **38** 644–652
- Mosmann TR and Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more *Immunology Today* **17** 138–146
- Neildez O, Le Grand R, Cheret A, Caufour P, Vaslin B, Matheux F, Theodoro F, Roques P and Dormont D (1998) Variation in virological parameters and antibody responses in macaques after atraumatic vaginal exposure to a pathogenic primary isolate of SIVmac251 *Research in Virology* **149** 53–68
- Nocera M and Chu TM (1993) Transforming growth factor beta as an immunosuppressive protein in human seminal plasma *American Journal of Reproductive Immunology* **30** 1–8
- Parr MB and Parr EL (1994) Mucosal immunity in the female and male reproductive tracts. In *Handbook of Mucosal Immunology* pp 677–689 Eds Ogra PL et al. Academic Press, New York
- Parr MB and Parr EL (1999) Female genital tract immunity in animal models. In *Mucosal Immunology* 2nd Edn pp 1395–1409 Eds Ogra PL et al. Academic Press, San Diego
- *Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G and Romagnani S (1998) Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions *Nature Medicine* **4** 1020–1024
- *Raghupathy R (1997) Th1-type immunity is incompatible with successful pregnancy *Immunology Today* **18** 478–482
- Ramsay AJ and Ramshaw IA (1997) Cytokine enhancement of immune responses important for immunocontraception *Reproduction, Fertility and Development* **9** 91–97
- Rasmussen SJ, Eckmann L, Quayle AJ, Shen L, Zhang YX, Anderson DJ, Fierer J, Stephens RS and Kagnoff MF (1997) Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in chlamydial pathogenesis *Journal of Clinical Investigation* **99** 77–87
- Redline RW and Lu CY (1989) Localization of fetal major histocompatibility complex antigens and maternal leukocytes in murine placenta. Implications for maternal–fetal immunological relationship *Laboratory Investigation* **61** 27–36
- Rees MC, Heryet AR, Bicknell R (1993) Immunohistochemical properties of the endothelial cells in the human uterus during the menstrual cycle *Human Reproduction* **8** 1173–1178
- Robertson SA, Mayrhofer G and Seamark RF (1992) Uterine epithelial cells synthesize granulocyte-macrophage colony-stimulating factor and interleukin-6 in pregnant and nonpregnant mice *Biology of Reproduction* **46** 1069–1079
- Robertson SA, Seamark RF, Guilbert LJ and Wegmann TG (1994) The role of cytokines in gestation *Critical Reviews in Immunology* **14** 239–292
- Robertson SA, Mau VJ, Tremellen KP and Seamark RF (1996a) Role of high molecular weight seminal vesicle proteins in eliciting the uterine inflammatory response to semen in mice *Journal of Reproduction and Fertility* **107** 265–277
- Robertson SA, Mayrhofer G and Seamark RF (1996b) Ovarian steroid hormones regulate granulocyte-macrophage colony stimulating factor (GM-CSF) production by uterine epithelial cells in the mouse *Biology of Reproduction* **54** 183–196

- Roth I, Corry DB, Locksley RM, Abrams JS, Litton MJ and Fisher SJ (1996) Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10 *Journal of Experimental Medicine* **184** 539–548
- *Sallusto F and Lanzavecchia A (1999) Mobilizing dendritic cells for tolerance, priming, and chronic inflammation *Journal of Experimental Medicine* **189** 611–614
- Sallusto F, Lenig D, Mackay CR and Lanzavecchia A (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes *Journal of Experimental Medicine* **187** 875–883
- Seder RA and Paul WE (1994) Acquisition of lymphokine-producing phenotype by CD4⁺ T cells *Annual Review of Immunology* **12** 635–673
- Stagg AJ, Tuffrey M, Woods C, Wunderink E and Knight SC (1998) Protection against ascending infection of the genital tract by *Chlamydia trachomatis* is associated with recruitment of major histocompatibility complex class II antigen-presenting cells into uterine tissue *Infection and Immunity* **66** 3535–3544
- *Stephens RJ and Thomson AW (1996) Dendritic cells and tolerance induction *Clinical and Experimental Immunology* **105** 397–402
- Suzuki T, Hiromatsu K, Ando Y, Okamoto T, Tomoda Y and Yoshikai Y (1995) Regulatory role of gamma delta T cells in uterine intraepithelial lymphocytes in maternal antifetal immune response *Journal of Immunology* **154** 4476–4484
- Szekeres-Bartho J and Wegmann TG (1996) A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance *Journal of Reproductive Immunology* **31** 81–95
- Tabibzadeh SS, Gerber MA and Satyaswaroop PG (1986) Induction of HLA-DR antigen expression in human endometrial epithelial cells *in vitro* by recombinant gamma-interferon *American Journal of Pathology* **125** 90–96
- Tafari A, Alferink J, Moller P, Hammerling GJ and Arnold B (1995) T cell awareness of paternal alloantigens during pregnancy *Science* **270** 630–633
- Tamada H, McMaster MT, Flanders KC, Andrews GK and Dey SK (1990) Cell-type specific expression of transforming growth factor-B1 in the mouse uterus during the periimplantation period *Molecular Endocrinology* **4** 965–972
- Tremellen KP, Seamark RF and Robertson SA (1998) Seminal transforming growth factor β 1 stimulates granulocyte-macrophage colony-stimulating factor production and inflammatory cell recruitment in the murine uterus *Biology of Reproduction* **58** 1217–1225
- Vassiliadou N and Bulmer JN (1996) Quantitative analysis of T lymphocyte subsets in pregnant and nonpregnant human endometrium *Biology of Reproduction* **55** 1017–1022
- Vassiliadou N and Bulmer JN (1998) Characterization of endometrial T lymphocyte subpopulations in spontaneous early pregnancy loss *Human Reproduction* **13** 44–47
- Watson JG, Carroll J and Chaykin S (1983) Reproduction in mice: the fate of spermatozoa not involved in fertilization *Gamete Research* **7** 75–84
- *Weiner HL (1997) Oral tolerance: immune mechanisms and treatment of autoimmune diseases *Immunology Today* **18** 335–343
- Whitelaw PF and Croy BA (1996) Granulated lymphocytes of pregnancy *Placenta* **17** 533–543
- Wira CR, Kaushic C, Richardson J (1999) Role of sex hormones and cytokines in regulating the mucosal immune system in the female reproductive tract. In *Mucosal Immunology* 2nd Edn pp 11 449–11 461 Eds Ogra PL *et al.* Academic Press, San Diego
- Wood GW, Hausmann E and Choudhuri R (1997) Relative role of CSF-1, MCP-1/JE, and RANTES in macrophage recruitment during successful pregnancy *Molecular Reproduction and Development* **46** 62–69
- Yeaman GR, Guyre PM, Fanger MW, Collins JE, White HD, Rathbun W, Orndorff KA, Gonzalez J, Stern JE and Wira CR (1997) Unique CD8⁺ T cell-rich lymphoid aggregates in human uterine endometrium *Journal of Leukocyte Biology* **61** 427–435
- Yeaman GR, Collins JE, Currie JK, Guyre PM, Wira CR and Fanger MW (1998) IFN-gamma is produced by polymorphonuclear neutrophils in human uterine endometrium and by cultured peripheral blood polymorphonuclear neutrophils *Journal of Immunology* **160** 5145–5153
- Zhou M and Mellor AL (1998) Expanded cohorts of maternal CD8⁺ T-cells specific for paternal MHC class I accumulate during pregnancy *Journal of Reproductive Immunology* **40** 47–62