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Immune cells in the placental bed

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ABSTRACT Leukocytes are an important component of the human uterine decidua in normal pregnancy. The focus of research has been on the more abundant populations such as the uterine natural killer (uNK) cells and macrophages, but more recently interest has also extended to less abundant, but functionally significant populations. Investigation of function in human pregnancy is limited by the scope of *in vitro* studies and the inability to perform *in vivo* manipulation of cell populations. Investigation of pathological pregnancy may provide clues to function, although acquisition of samples is limited until after clinical presentation. Investigation of animal models may provide clues to function in humans and this has certainly been the case for the uNK cells. However, human placentation differs substantially from the usual laboratory animal models and any extrapolation to humans from animal studies should be made with this in mind. Considerable advances have been made over the last 25 years but many questions still remain; the next 25 years may provide more answers to the role of the endometrial leukocytes in normal pregnancy, so that further advances can be made in investigation of their role, if any, in pregnancy pathology.

KEY WORDS: *uterine natural killer cell, macrophage, T cell, endometrium, decidua*

Introduction

Successful pregnancy remains to some extent an immunological enigma; the fetus inherits histocompatibility antigens from the father and yet coexists within the mother's uterus in harmony throughout pregnancy. Considerable research effort has focused on antigen expression in the placenta and in particular by trophoblast; villous trophoblast is in contact with the maternal circulating blood in the intervillous space throughout pregnancy, while extravillous trophoblast invasion into the uterine tissue and arteries is an essential feature of successful pregnancy. Apart from factors inherent within the placenta and trophoblast which enable it to survive and thrive in normal pregnancy, factors within the maternal uterine tissues are likely to play a role in the immune success of normal pregnancy; leukocytes account for a substantial population within the decidualized endometrium which lines the uterus in pregnancy. Apart from the maternal leukocytes within the decidua, there are immune cells present within the stroma of the chorionic villi; in normal pregnancy these are predominantly macrophages (Hofbauer cells) which are fetal in origin but in pathological situations such as villitis of unknown etiology, these may also include maternally derived cells, including T lymphocytes. The circulating maternal cells in the intervillous spaces have received very scant attention, although intervillositis is an important feature of some pregnancy pathology, such as placental malaria infection. This review discusses the distribution of the key immune cells in the human endometrium which are present around the time of implantation and throughout pregnancy decidua, their potential functions and relationship to pathological pregnancy.

Leukocytes in the placental bed

Leukocytes are an important component of the endometrial stroma. Although stromal leukocytes are present in proliferative and early secretory phase non-pregnant endometrium, numbers increase substantially in the midsecretory phase, around the time of expected implantation and continue to increase in early pregnancy (Bulmer *et al.*, 1991); in the first trimester of pregnancy, approximately 30-40% of endometrial (decidual) stromal cells are leukocytes. Three major populations have been identified: granulated lymphocytes termed uterine natural killer (uNK) cells, macrophages and T lymphocytes (Bulmer *et al.*, 1991). Other less abundant but functionally important endometrial leukocyte populations are dendritic cells (Gardner and Moffett, 2003), natural killer T (NKT) (Tsuda *et al.*, 2001) cells and regulatory T cells (Heikkinen *et al.*, 2004). These leukocytes are prominent at the

Abbreviations used in this paper: NKT, natural killer T cell; uNK, uterine natural killer cell.

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implantation site where they come into close contact with the implanting blastocyst and with invading extravillous trophoblast.

The large increase in endometrial leukocyte numbers in the mid secretory phase of the menstrual cycle at the expected time of implantation in a fertilized cycle and in early pregnancy (Bulmer *et al.*, 1991; Klentzeris *et al.*, 1992) is one of the major observations implicating endometrial leukocytes as playing a key role in establishment of the fetal-placental unit and the subsequent immunological maintenance of pregnancy. Whether this increase in leukocyte numbers is due to recruitment from other sites or to *in situ* proliferation in response to direct/indirect hormonal influences and/or locally produced soluble factors is not known. Despite intensive *in vitro* investigations, the *in vivo* functions of the different leukocytic cell types in human endometrium remain unknown; suggested roles include regulation of trophoblast invasion (uNK cells and macrophages), spiral artery remodelling (uNK cells) and immune tolerance (T cells, regulatory T cells and dendritic cells). There have also been many studies of the various endometrial leukocyte populations in pathological pregnancy and, in particular, macrophages and NK cells have been implicated in pregnancy pathology such as pre-eclampsia and miscarriage.

Uterine natural killer cells

Uterine natural killer (uNK) cells are the most abundant of all decidual leucocytes. They account for approximately 70% of stromal CD45-positive cells and nearly 30% of all stromal cells in early pregnancy decidua. In the original histological descriptions, uterine NK cells were most often characterised by the presence of phloxinophilic cytoplasmic granules in the phloxine tartrazine stain (Hamperl and Hellweg, 1958; Kazzaz, 1972; Dallenbach-Hellweg, 1987) and these early studies documented their distribution in non-pregnant and pregnant endometrium. After confirmation that they are leukocytes (Bulmer and Sunderland, 1983), rather than stromal derived cells as originally suggested (Dallenbach-Hellweg, 1987), they were confirmed as an NK-type cell (suggested by the large granular lymphocyte morphology) by expression of CD56 (Ritson and Bulmer, 1987), which was noted in immunohistochemical studies to be particularly intense. Subsequent flow cytometry and immunohistochemical studies have provided detailed phenotypic characterization of uterine NK cells and revealed expression of various NK receptors, both inhibitory and activating (Voss *et al.*, 1998; King *et al.*, 2000; Eriksson *et al.*, 2004; Kusumi *et al.*, 2006).

The phenotype of uterine granulated lymphocytes is known to differ from 'usual' peripheral blood NK cells, being CD56^{bright} CD16- CD57-. This phenotype is similar to a small subgroup of peripheral blood NK cells, although in contrast to uterine NK cells, in peripheral blood CD56^{bright} CD16- NK cells are generally agranular. Expression of NK receptors also differs between peripheral blood and uterine NK cells within any one individual (Trundley and Moffett, 2004).

Distribution of uterine NK cells

In early studies uterine NK cells were identified by the presence of cytoplasmic granules. The cells were reported to be absent in proliferative endometrium, increasing in numbers premenstrually and in pregnancy until the third month of gestation, thereafter declining to be virtually absent at term (Hamperl and Hellweg, 1958; Dallenbach-Hellweg and Nette, 1964). This distribution was confirmed for pregnancy in phloxine tartrazine staining of pregnancy hysterectomies from 8 weeks to term (Bulmer and Lash, 2005); granulated leukocytes were rare after 20 weeks gestation. Immunostaining for CD56 has largely confirmed this distribution, although CD56+ cells are present in the proliferative and early secretory phase, albeit in small numbers (Bulmer *et al.*, 1991; King *et al.*, 1989a). Numbers increase in the late secretory phase of the menstrual cycle and even higher numbers are observed in early pregnancy. Several studies have recorded a reduction in CD56+ cells in third trimester decidua. However, we have noted that substantial numbers of CD56+ cells remain in both decidua basalis and decidua parietalis in third trimester placental bed biopsies (Scaife *et al.*, 2003). Reports from others of lower numbers of CD56+ cells in term decidua (Haller *et al.*, 1993; Vargas *et al.*, 1993) may be due to the use of different tissues, some studies being based on decidua attached to the placental membranes and delivered placenta, while others have studied decidua in placental bed biopsies. The possibility that there is a population of agranular CD56+ cells in proliferative and early secretory phase endometrium and in decidua in the second half of pregnancy has not been analysed in detail.

There is little recent information regarding the distribution of uNK cells in the second trimester of pregnancy. Normal pregnancy samples are difficult to obtain after the first trimester and most studies have focused on this period. Numbers of uterine NK cells are thought to reduce in the second half of pregnancy but the mechanism for this reduction is unclear. Using immunohistochemistry, we have analysed leukocyte numbers in the first half of pregnancy in a series of placental bed biopsies obtained after termination of normal pregnancy at 8-20 weeks of gestation. There were no significant differences in CD56+ cell numbers between first (8-12 weeks' gestation) and early second (13-20

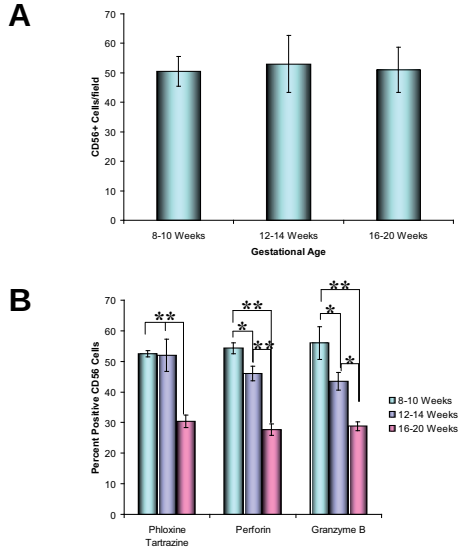

Fig. 1. Distribution of CD56+ uNK cells in early pregnancy decidua. **(A)** *Graphical representation of decidual CD56 numbers at 8-10, 12-14 and 16-20 weeks gestation (mean ± sem; n=4 each group).* **(B)** *Graphical representation of proportion of CD56 cells that were also positive for perforin, granzyme B and phloxine tartrazine at 8-10, 12-14 and 16-20 weeks gestation (mean ± sem; n=4 each group)* * P<0.02; ** P<0.0002.

weeks' gestation) trimester decidua, with uNK cells still forming the majority leukocyte population in the early second trimester (Scaife *et al.*, 2003).

Since the reduced number of uterine NK cells in previous studies has been based on a reduction in number of cells with cytoplasmic granules in standard histochemical stains, we analysed expression of perforin and granzyme B in placental bed biopsies from 8 – 20 weeks of gestation; numbers of CD56+ uNK cells did not alter but numbers of cells expressing cytoplasmic perforin or granzyme B were reduced at 16-20 weeks gestation compared to 8-10 and 12-14 weeks gestational age (Figure 1). It is therefore possible that the detection of unaltered numbers of CD56+ cells, but a decrease in perforin, granzyme B and cytoplasmic granules, in early second trimester decidua compared with first trimester decidua may reflect loss of granules. Degranulation after the first trimester has been earlier suggested based on electron microscope studies (Spornitz, 1992). Loss of cytoplasmic granules containing perforin and granzyme may reflect altered cellular function as gestation progresses.

In early pregnancy several studies have compared the distribution of uNK cells between decidua basalis, which underlies the placenta and is infiltrated by extravillous trophoblast, and decidua parietalis which lines the remainder of the uterine cavity and which does not show trophoblast infiltration. Results between different groups have been inconsistent. Sindram-Trujillo *et al.* (2003) reported increased numbers of CD56-positive CD16-negative uNK cells in decidua parietalis compared with decidua basalis at term. In contrast, others have suggested that the close association between uNK cells and extravillous trophoblast supports a role in the control of trophoblast invasion (Kusumi *et al.*, 2006). Others, including ourselves using immunohistochemistry, have failed to detect any difference in the different decidual areas (Khong, 1987; Haller *et al.*, 1995) but do find a close association between uNK cells and extravillous trophoblast cells in the placental bed (Figure 2). The discrepancy between these studies is difficult to explain. Flow cytometric studies rely on accurate identification of decidual types which is easier at term when decidua attached to the placental membranes can be sampled as decidua parietalis. Immunohistochemical studies allow localization of trophoblast within the same sections but still do not allow precise localization within the placental bed and it is possible that peripheral areas which contain trophoblast may differ from central zones. Furthermore, analysis of placental bed biopsies which include deeper decidua may also differ from studies based on decidua obtained at termination of pregnancy.

Trafficking from blood or local differentiation of uterine NK cells?

The origin of uNK cells in endometrium and decidua has been widely debated. There is evidence for both trafficking of cells from the peripheral circulation and *in situ* differentiation and proliferation (reviewed in Bulmer and Lash, 2005; Manaster and Mandelboim *et al.*, 2008).

In late secretory phase endometrium and early pregnancy decidua uNK cells are detected predominantly in the stratum functionalis, often forming aggregates around spiral arteries and glands (Bulmer *et al.*, 1991). This perivascular distribution was considered by early workers to reflect diffusion of progesterone from blood into perivascular tissues. More recently their perivascular distribution has been considered to reflect trafficking of uNK cells or their precursors from the circulation (Trundley and Moffett, 2004). Several studies have focused on interactions between uNK cells and receptors expressed by trophoblast cells as potential mechanisms of trafficking. Monocyte inflammatory protein (MIP) 1α (Drake *et al.,* 2001), MIP-1β (Kitaya *et al.,* 2003) and CXCL12 (Hanna *et al.,* 2003) have all been implicated.

However, there are several arguments against trophoblast-induced trafficking of uNK cells. For example uNK cells are prominent in endometrium in the absence of trophoblast in premenstrual phase endometrium and in intrauterine decidua in ectopic pregnancy, but not at the site of implantation in tubal pregnancy (Von Rango *et al.*, 2001). In addition, they are most notable in endometrium showing effects of exogenous progesterone, either from oral medication or a progesterone secreting

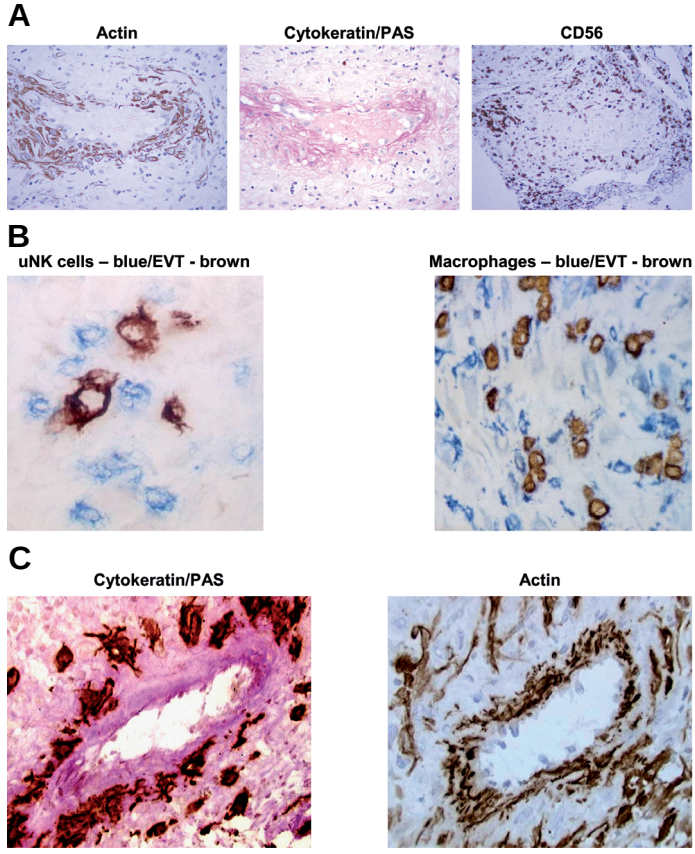

Fig. 2. Immunohistochemical demonstration of cell populations in human decidua. (A) *Representative photomicrographs of serial sections of a decidual spiral artery in the placental bed immunostained for vascular smooth muscle cells (actin), fibrinoid and extravillous trophoblast cells (EVT) (PAS/Cytokeratin) and uNK cells (CD56). Note the separation of actin staining vascular smooth muscle cells, slight deposition of fibrinoid material, high number of uNK cells surrounding the vessel and the complete absence of EVT.* **(B)** *Representative photomicrographs of association between uNK cells and macrophages with EVT. Left: uNK cells (blue), EVT (brown). Right: macrophages (blue), EVT (brown).* **(C)** *Representative photographs of serial sections of a decidual spiral artery in placental bed of woman undergoing second trimester miscarriage. Left: PAS and cytokeratin showing vessel wall surrounded by interstitial EVT but no intramural or endovascular EVT. Right: actin showing untransformed vessel wall.*

intrauterine system. Furthermore, if trophoblast had an effect on recruitment it would be expected that uNK cell numbers would differ consistently between decidua basalis in the presence of extravillous trophoblast and decidua parietalis which lacks trophoblast. Thus, if the increase in uNK cells is due to recruitment of this specialised cell from peripheral blood, this process is more likely to be regulated by factors within the endometrium. There have been several studies of chemokines and cytokines which may play a role in the recruitment of leukocytes into endometrium with several potential candidates identified, including amongst others, macrophage derived chemokine (MDC), monocyte chemoattractant protein (MCP) 3, fractalkine (FKN), 6Ckine, MIP-1β (Jones *et al.*, 2004), CXCL10, CXCL11 (Sentman *et al.*, 2004) and interleukin (IL) 15 (Kitaya *et al.*, 2005). Several of these show cyclical changes and may be regulated partly by steroid hormones. However, endometrial uNK cells do not express many of the chemokine receptors (e.g. CXCR1, CXCR2, CXCR3, CXCR4, CCR1, CCR3, CCR5 and CCR7) (Manaster *et al.*, 2008).

A striking feature of uNK cells is their close association with endometrial stromal cell decidualization which is entirely independent of trophoblast and the endometrial stromal cells first undergo predecidual change in perivascular areas in the late secretory phase of the cycle (Robertson, 1981; Dallenbach-Hellweg, 1987). Areas of ectopic decidualization such as may be seen on the ovarian surface or in the cervix in pregnancy also invariably contain uNK cells. Furthermore, comparable uNK cell numbers are seen in intrauterine decidua associated with ectopic pregnancy (Vassiliadou & Bulmer, 1998a; Von Rango *et al.*, 2001) compared with normal intrauterine pregnancy. Recently, Carlino *et al.* (2008) reported that peripheral blood NK cells from pregnant women had a greater migratory capacity through decidual endothelial cells and stromal cells than peripheral blood NK cells from males or non-pregnant women. When decidual stromal cells were pre-treated with progesterone, a greater level of NK migration was also observed. On contact with decidual stromal cells peripheral blood NK cells acquired a chemokine receptor pattern similar to that of decidual uNK cells (Carlino *et al.*, 2008).

Recruitment of cells from peripheral blood does not preclude the possibility of local differentiation into uNK cells of immature precursor cells derived from the circulation. There is evidence of local differentiation of uterine NK cells in mouse (reviewed in Bilinski *et al.*, 2008) and Lynch *et al.* (2007) have reported CD34-positive CD56-positive leukocyte stem cells in secretory phase endometrium. CD56-positive uNK cells proliferate locally in secretory phase endometrium and early pregnancy decidua (Pace *et al.,* 1989). It is possible that recruitment of cells into endometrium is of immature precursor cells rather than the phenotypically specialised subset and that cells differentiate and proliferate in response to local stimuli.

Uterine NK cells are a phenotypically distinct subset of NK cells and to date, similar cells have not been described in substantial numbers in other organs. Similarly, there are no reports of substantial numbers of 'usual' NK cells in endometrium at any stage of the menstrual cycle or in early pregnancy. Another possibility is that local cytokine products may lead to differentiation of uterine NK cells from—'usual' NK cells; incubation of peripheral blood NK cells in TGBβ1 has been shown to lead to conversion into a uterine NK cell phenotype (Keskin *et al.*, 2007). If this occurred *in vivo* it would be anticipated that at some stage of the menstrual cycle or pregnancy 'usual' NK cells would be readily detected but this is not the case.

Although the focus of the perivascular distribution of uterine NK cells has been based on their recruitment and differentiation, an alternative explanation is that this distribution reflects a role in the remodelling of uterine spiral arteries which is an essential feature of pregnancy (see below).

Functional investigations

Since the identification of uNK cells as the major endometrial leukocyte population around the time of implantation and in early pregnancy, functional studies of endometrial leukocyte function have focused particularly on these cells (reviewed in Bulmer and Lash, 2005). Their identification as a type of natural killer cell inevitably focused attention on cytotoxicity. CD56+ cells isolated from non-pregnant endometrium at various menstrual cycle stages (Jones *et al.*, 1997) and early pregnancy decidua (King *et al.*, 1989b; Ritson and Bulmer, 1989; Ferry *et al.*, 1990) consistently exhibited cytotoxic activity against the NK cell target K562. It is recognized, however, that their cytotoxic activity is lower than that of peripheral blood NK cells, despite their content of perforin (Kopcow *et al.*, 2005). Demonstration of cytotoxic potential and the close association with extravillous trophoblast led to speculation of an *in vivo* role in control of trophoblast invasion into decidua. However, extravillous trophoblast cells appear to be protected from uNK cytotoxicity due to their expression of HLA-G (Chumbley *et al.*, 1994; Rouas-Freiss *et al.*, 1997). Although uNK cells express several activating receptors including NKp46, NKp44, NKp30, NKG2d and 2B4, it is believed that the lack of cytotoxicity towards trophoblast cells may be due to interactions between inhibitory receptors and either the classical class 1a MHC-molecule (HLA-C) or non-classical class Ib MHC-molecules (HLA-E, HLA-F and HLA-G). These inhibitory receptors include LIR-1, KIR2DL4 and CD94/NKG2A (reviewed in Manaster and Mandelboim, 2008). Indeed, a mismatch of HLA-C and KIR2DL4 haplotypes has been associated with pre-eclampsia and recurrent miscarriage (Hiby *et al.*, 2004; Hiby *et al.*, 2008).

Uterine NK cells are a rich source of many different cytokines and growth factors including TNF-α, IL-10, GM-CSF, IL-1β, TGF-β1, CSF-1, LIF and IFN-γ (Saito *et al.*, 1993; Jokhi *et al.*, 1994; Jokhi *et al.*, 1997; Rieger *et al.*, 2001). In general investigation of cytokine production has been on uNK cells purified from early pregnancy decidua, with no separation of decidua basalis and decidua parietalis, although Von Rango *et al.* (2003) compared cytokine production by uNK cells from decidua basalis and decidua parietalis and noted differences in cytokine profile.

Regulation of trophoblast invasion by uNK cells could be due to their cytokine products. uNK cells produce a range of cytokines that have been shown to regulate trophoblast invasion; these include TNF-α, TGF-β1 and IFN-γ, all of which inhibit trophoblast invasion from placental explants (Otun *et al.*, 2003; Lash *et al.*, 2005; Lash *et al.*, 2006a). Recently, Hanna *et al.* (2006) demonstrated that uNK cell supernatants could stimulate isolated cytotrophoblast invasion through matrigel in a mechanism that was partly dependent on IL-8 and IP-10. Results from our own laboratory using a placental villous explant invasion assay support this finding but only when uNK cell supernatants and placental explants were taken from 12-14 weeks gestation (Lash *et al.*, 2007a). In our hands, this effect is partially abrogated by blockage

of IL-8 (Oliveira *et al.*, 2007). In contrast, Hu *et al.* (2006) demonstrated that uNK cell supernatants inhibited migration of extravillous trophoblast cells in a two dimension migration assay via a mechanism dependent on IFN-γ.

Various other chemokines and cytokines produced by uNK cells have also been shown to potentially affect trophoblast invasion in a range of assays; it is likely that the *in vivo* role of uNK cells reflects complex mixtures of cytokines and chemokine products, with subtle gestational age differences having the potential to alter functional effects. Most studies have focused on decidua from first trimester samples. Rapid alterations occur within this period, notably the altered levels of oxygen in the intervillous space around 8-10 weeks of gestation, with consequent effects on trophoblast function. It is possible therefore that uNK cell function may differ between 8 weeks and 12 weeks gestation, although these would both fall within the 'first trimester' group. We have demonstrated gestational age differences in the uNK cell secretion of cytokines and growth factors from 8-10 to 12-14 weeks gestation (Lash *et al.*, 2006b; Naruse *et al.*, 2007a); future studies should focus on subtle gestational age differences which may have a profound effect on function.

Uterine NK cells are also a major source of angiogenic growth factors in early pregnancy (Lash *et al.*, 2006b; Hanna *et al.*, 2006). Recently a role for uNK cells in trophoblast independent spiral artery remodelling has been proposed (Pijnenborg *et al.*, 2006). Although complete remodelling ('transformation') of uterine spiral arteries requires a contribution from trophoblast (Kam *et al.*, 1999), the initial stages of spiral artery remodelling, including dilatation, some fibrinoid deposition and vascular smooth muscle cell separation appear to occur in the absence of extravillous trophoblast cells (Figure 2) (Craven *et al.*, 1998; Kam *et al.*, 1999). Uterine NK cells are frequently aggregated around the spiral arteries in early pregnancy; rather than reflecting their trafficking from blood, this distribution may reflect a role in mediating vascular changes in pregnancy. There is convincing evidence from mouse pregnancy that uNK cells are important for vascular remodelling in pregnancy, although changes comparable with those in human pregnancy are not seen in mouse (Croy *et al.*, 1997). The ubiquity of uNK cells, or at least granulated lymphocytes, in a wide range of species, including those with placentation types other than haemochorial, may reflect a role in mediating uterine vascular changes rather than in control of trophoblast invasion. Using a chorionic plate artery model we have recently demonstrated that uNK cell supernatants not only stimulate the separation of vascular smooth muscle cells from each other, but also initiate their de-differentiation (Bulmer *et al.*, 2007; Naruse *et al.*, 2007b). Further work is required to determine the role of uNK cells on uterine vascular changes in pregnancy.

Uterine NK cells in pathological pregnancy

The presence of a large population of NK cells in endometrium in early pregnancy has led to speculation that these cells may play a role in pregnancy loss. There have been several studies of uNK cells and 'usual' NK cells in decidua and peripheral blood in both recurrent and sporadic miscarriage but results between different studies are inconsistent (reviewed in Laird *et al.*, 2003). For example, increased numbers of CD56+ NK cells have been reported in decidua of women with sporadic miscarriage compared with controls (Zenclussen *et al.*, 2001), although we were not able to confirm this finding in a study of placental bed biopsies from aneuploid and euploid miscarriages from 8 – 20 weeks gestation, compared with normal pregnancy (Scaife *et al.*, 2004). Despite the inconsistency of data for decidual and peripheral blood leukocytes, there are several independent reports from different groups of increased numbers of uNK cells, detected using immunohistochemistry, in luteal phase endometrium of both women with unexplained recurrent miscarriage and recurrent implantation failure (Quenby *et al.*, 1999; Clifford *et al.*, 1999; Laird *et al.*, 2005; Tuckerman *et al.*, 2007). Furthermore, it has been suggested that high uNK cell numbers in mid-secretory phase endometrium from women with recurrent miscarriage predicted miscarriage in subsequent pregnancy (Quenby *et al.*, 1999). A recent larger study did not confirm prediction of pregnancy outcome from uNK cell numbers but treatment details were not provided, interval between uNK cell assessment and subsequent pregnancy was not clear and pregnancy data were only available for a subset of the group (Tuckerman *et al.*, 2007).

Increased numbers of CD56+ uNK cells have been reported in decidua from women with pre-eclampsia compared to age matched controls (Stallmach *et al.*, 1999; Wilczynski *et al.*, 2003; Bachmayer *et al.*, 2006). In contrast, in our laboratory we have demonstrated a reduction in CD56+ uNK cells in pre-eclampsia in placental bed biopsies (Scaife, Innes, Searle, Robson, Bulmer; unpublished data). Eide *et al.* (2006) also reported a decrease in decidual CD56+ uNK cell numbers in women with severe fetal growth restriction in the presence or absence of pre-eclampsia, although there were no differences in numbers of decidual CD56+ uNK cells in women with pre-eclampsia alone.

Conclusion

Uterine NK cells are an important component of the endometrium in early pregnancy but numerous questions remain unanswered after more than 20 years of research. The increase in uNK cell numbers in the secretory phase of the cycle and in early pregnancy remains largely unexplained. The influence of the hormonal milieu on recruitment and/or differentiation and proliferation of uNK cells is provided by their presence in pseudodecidua associated with progesterone treatment, in decidua associated with ectopic pregnancy and in focal decidualization in ectopic locations such as cervix and ovarian surface in pregnancy.

Their temporal and spatial distribution has led to speculation that they play a role in control of trophoblast invasion. Detection of receptors for HLA antigens expressed by invasive extravillous trophoblast also suggests a role in recognition of trophoblast. However, the presence of similar cells in species which do not exhibit trophoblast invasion as is seen in human pregnancy, including species with a non-haemochorial placentation, suggests an alternative function. The demonstration of a uNK cell-mediated effect on uterine arteries provides evidence for such a role. As yet there are few studies which have focused on the possibility that uNK cell function differs according to the location within the endometrium, such as in relation to trophoblast in decidua basalis or around vessels in early pregnancy. Intrauterine decidua in ectopic pregnancy would provide a good model for such investigations. The effect of progesterone on uNK cell function could be investigated in pseudodecidualized endometrium associated with progesterone treatment. Furthermore, subtle

gestational age differences may alter function; trophoblast invasion differs between 8 weeks and 12 weeks gestation yet most studies of uNK cells combine samples from these gestational ages within one group. Despite the incomplete understanding of the role of uNK cells in normal pregnancy, there is intensive interest in the possibility that uNK cells or 'usual' NK cells play a role in pregnancy loss.

Macrophages

Human macrophages are mononuclear phagocytic cells that are involved in both innate and adaptive immunity. Tissue macrophages derive from pluripotent myeloid stem cells in the bone marrow which differentiate into several types of progenitor cells that differentiate into promonocytes which enter the circulation, differentiate into mature monocytes and subsequently enter various organs and tissues. Tissue-specific factors induce final maturation into tissue macrophages, a phenotypically and functionally heterogeneous population. Uterine macrophages are generally identified by immunostaining for either CD14 or CD68 with varying results (Table 1). CD14 recognises bacterial lipopolysaccharide, while CD68 detects lysosomal-associated proteins and is a marker of phagocytic cells. In a recent study of third trimester placental bed biopsies using double immunostaining for CD14 and CD68 Kim *et al*. (2007) demonstrated not only a CD14+CD68+ macrophage population but also a small CD14-CD68+ macrophage population. It is possible that these were a non-macrophage population; reactivity for CD68 has been reported to differ between different antibodies and according to fixation and in the same study reactivity of CD68 antibodies with purified fibroblasts was also reported (Kunisch *et al*., 2004). Therefore, CD14 may be a better marker of uterine macrophages. CD14-negative macrophages have been reported to exhibit reduced phagocytic activity and also do not produce key cytokines such as TGF-β and TNF-α (Smythies *et al*., 2005), which are known to potentially regulate trophoblast invasion (Otun *et al*., 2003; Lash *et al*., 2005). Interestingly, CD14-negative CD68-positive macrophages predominated in decidua while the reverse was found in superficial myometrium. The biological significance of these two macrophage populations is unclear.

Distribution of macrophages

Macrophages are the second most abundant leukocyte population in decidua comprising approximately 20% of all decidual leucocytes (Bulmer *et al*., 1988). Macrophages (CD14- or CD68-positive cells) are present in the non-pregnant endometrium; some studies have not detected any alteration in numbers throughout the menstrual cycle (Rieger *et al*., 2004) but others have noted increased macrophage numbers in the secretory phase, using both CD14 (Bulmer *et al*., 1991) and CD68 (Klentzeris *et al*., 1992). In contrast with uNK cells, macrophage numbers do not alter substantially with increasing gestational age, although the proportion of endometrial leukocytes accounted for by macrophages increases as the number of uNK cells is reduced in the second half of pregnancy. Few studies have reported in detail on the distribution of macrophages in uterine tissues. Unlike uNK cells, however, macrophages are present in both endometrium/decidua and myometrium. In early pregnancy decidua they have been described to be associated with spiral arteries and glands (Bulmer and Johnson, 1984) and extravillous trophoblast (Figure 2) (Bulmer *et al*., 1988). Using flow cytometry Repnik *et al*. (2008) did not show any differences in the number of CD14+ cells in decidua basalis and decidua parietalis from second trimester or term pregnancies. However, they did find increased levels of DC-SIGN and MMR (macrophage mannose receptor) expressing cells in decidua basalis compared to decidua parietalis from second trimester and term. In addition, increased numbers of CD80 and HLA-DR expressing cells were also observed in decidua basalis compared to decidua parietalis in term pregnancies (Repnik *et al*., 2008). CD80, CD86 and HLA-DR are generally considered to be markers of macrophage activation. Decidual macrophages also express MHC class II and receptors for GM-CSF, M-CSF and IFN-γ. In addition they express the inhibitory MHC class Ia and class Ib receptors CD85d and CD85j (Cupurdija *et al*., 2004).

Functional studies

Several functions for decidual macrophages have been proposed, although there have been few *in vitro* studies as separation of macrophage populations from decidua has been problematic and there are no reports of successful separation of pure populations. Based on their lysosomal enzyme content (Bulmer and Johnson, 1984) and proximity to apoptotic cells *in situ* it has been proposed that they play a role in phagocytosis of cell debris produced during implantation (reviewed in Mor and Abrahams, 2003; Abrahams *et al*., 2004). A role in immunosuppression has also been proposed due to their suppression of one-way mixed lymphocyte reaction (Mizuno *et al*., 1994). In addition, Heikkinen *et al*. (2003) reported that decidual macrophages from term pregnancy have low level expression of the T lymphocyte co-stimula-

TABLE 1

SUMMARY OF MACROPHAGE DISTRIBUTION STUDIES AS DETERMINED BY IMMUNOLABELLING FOR CD14 AND/OR CD68

Tissue	CD14+	CD68+	Reference
P, ES, LS, Dec. Frozen sections	No change	More variable than CD14+ with trend to higher numbers in LS, but no change	Rieger et al., 2004
P, ES, LS, Dec. Frozen sections	Increased in LS and further increased in Dec	Not tested	Bulmer et al., 1991
P, ES, LS. Frozen sections	Not tested	Increased in LS	Klentzeris et al., 1992
Basal plate, decidua and myometrium at term. Double immunolabeling of paraffin sections	CD14+CD68+ - higher in myometrium than basal plate or decidua	Total CD68+ - higher in decidua than basal plate or myometrium CD68+CD14- - higher in decidua than basal plate or myometrium	Kim et al., 2007
Decidua basalis (db) and decidua parietalis (dp) from second trimester and term. Flow cytometry	No difference between db and dp in second trimester or at term. Decrease in both db and dp at term compared to second trimester	Not tested	Repnik et al., 2008

P, proliferative stage endometrium; ES, early secretory stage endometrium; LS, late secretory stage endometrium; Dec, early pregnancy decidua.

tory molecules CD80 and CD86, and also express indoleamine-2,3-dioxygenase (IDO), suggesting a role in prevention of maternal T lymphocyte activation. Additionally one of the major cytokine products of decidual macrophages is the TH2 type cytokine interleukin (IL)-10, along with TNF-α and minimal levels of IL-1β (Heikkinen *et al*., 2003).

Macrophages have also been proposed to play a role in regulating trophoblast invasion. Renaud *et al*. (2005) demonstrated that peripheral blood macrophages activated with lipopolysacharride inhibit the invasion of the trophoblast cell line HTR-8/SVneo, via TNF-α. However, non-activated macrophages had no effect on trophoblast invasion. In addition, Huang *et al*. (2008) also demonstrated that macrophages derived from the THP-1 cell line inhibited HTR-8/SVneo invasion although a mechanism was not investigated.

Macrophages in pathological pregnancy

Several studies have investigated macrophage numbers in decidua from women with pre-eclampsia but results have varied between groups. Attention was focused on macrophages by Reister *et al*. (1999) who reported increased macrophages surrounding myometrial spiral arteries in the absence of extravillous trophoblast cells (EVT) in placental bed biopsies from women with pre-eclampsia. Furthermore, in control samples where myometrial spiral arteries were surrounded by EVT, macrophage density was sparse. In a flow cytometric and immunohistochemical study Burk *et al*. (2001) reported reduced numbers of CD14- and HLA-DR-positive macrophages in decidua from pre-eclampsia compared with controls. We have obtained similar results in placental bed biopsies from pre-eclampsia and normal pregnancies, noting a reduction in number of CD14-positive decidual macrophages in placental bed biopsies (Scaife, Innes, Searle, Robson, Bulmer; unpublished data). Kim *et al*. (2007) did not detect any alteration in numbers of decidual macrophages in pre-eclampsia compared to age matched controls, using a double labelling approach with antibodies against both CD14 and CD68. In contrast, Lockwood *et al*. (2006) reported increased numbers of decidual macrophages in pre-eclampsia; CD68-positive macrophages were detected in decidua basalis attached to delivered placentas. The distribution of macrophages in controls varied widely, ranging from absent to sparse to numerous. The increased macrophage numbers in pre-eclampsia was considered to reflect altered regulation of decidual MCP-1 by TNFα and IL1β, leading to enhanced recruitment of macrophages into the tissue. Apart from the differences between CD14 and CD68, the variation in results between these different studies is likely to reflect the different tissues studied; these range from myometrium (Reister *et al*., 1999) to superficial decidua attached to the delivered placenta (Lockwood *et al*., 2006), whereas in our study we investigated decidua in placental bed biopsies obtained at the time of caesarean section.

In a study of placental bed biopsies from early and late sporadic miscarriage, we detected increased CD14-positive macrophage numbers in decidua associated with early aneuploid sporadic miscarriage, and a trend towards increased macrophage numbers in both early and late chromosomally normal miscarriage (Scaife *et al*., 2004). This is in keeping with Vassiliadou and Bulmer (1996) who noted a trend towards increased numbers of CD68 in decidua in sporadic miscarriage, although Quack *et al*. (2001) did not detect differences in macrophages between miscarriage and controls. All studies of decidua in miscarriage suffer from the criticism of 'cause or result' since samples are collected after clinical presentation and any alteration in leukocyte populations may simply reflect an inflammatory response to pregnancy demise. Nevertheless, macrophage numbers were also increased in luteal phase endometrium from women with a history of recurrent miscarriage, supporting a causative rather than a secondary effect (Quenby *et al*., 1999).

T Lymphocytes

CD3-positive T lymphocytes comprise approximately 10% of the endometrial stromal leukocyte population in early pregnancy and have been relatively neglected compared with uNK cells and macrophages. In contrast with peripheral blood there are consistent reports that CD3+CD8+ cells predominate and CD3+CD4+ T cells are less abundant (CD8+:CD4+ ratio 3:1) (Bulmer *et al*., 1991). Immunohistochemical detection of CD4+ T cells in endometrium is, however, difficult, since there is also expression of CD4 by macrophages in decidua.

Induction and activation of cytotoxic T lymphocytes is initiated when the T cell receptor recognizes a specific antigen presented by antigen presenting cells and CD3+CD8+ cytotoxic T lymphocytes use two pathways for their cytotoxic activity, perforin/granzyme B and Fas/Fas ligand (Lowin *et al*., 1994; Kagi *et al*., 1995). CD3+CD4+ T lymphocytes are involved in both cell mediated and humoral immune responses and can be divided into Th0, 1 and 2 cells based on their cytokine production profile. Th0 cells are considered to be progenitor cells that differentiate into Th1 (cell mediated immunity) or Th2 (humoral immunity) cells in response to the cytokines produced at the time of antigen presentation.

During early pregnancy the fetus must be protected from attack by maternal non-tolerized naïve T lymphocytes. The apparent lack of a rejection reaction may be explained either by fetal alloantigens being concealed from maternal immune cells, or alternatively, T lymphocyte tolerance being induced upon exposure to these antigens. There is some evidence that maternal T lymphocytes are aware of fetal alloantigens and have been tolerized, thereby rendering them unresponsive (Tafuri *et al*., 1995; Jiang and Vacchio, 1998). Extravillous trophoblast which invades decidua and spiral arteries expresses HLA-C as well as HLA-E and HLA-G. There are also potential breaks in the villous syncytiotrophoblast covering the villous stroma which expresses fetal and therefore semiallogeneic class I MHC antigens. Maternal T lymphocytes may therefore contact fetal alloantigens within decidua, or fetal alloantigens may be transported by antigen presenting cells to local draining lymph nodes where antigen presentation may occur.

Distribution of T lymphocytes

The T cell population within the endometrial stroma does not show dramatic alteration with menstrual cycle phase or gestational age in pregnancy, although the proportion of leukocytes accounted for by T lymphocytes varies widely as other cells types, notably the uNK cells, alter in number. Klentzeris *et al*. (1992) noted an increase in numbers of CD8+ T cells in timed endometrial biopsies between 4 and 7 days after the luteinising hormone surge but others have not noted any difference between proliferative and secretory phases of the cycle (Bulmer *et al.,* 1991). Since numbers of T lymphocytes vary little if any during the menstrual cycle and pregnancy it has been suggested that these cells do not have a key

role in the establishment and maintenance of pregnancy (Vassiliadou and Bulmer, 1996), and their role is simply to provide immune protection against external antigens. However, the finding that there is a reversal in the CD8:CD4 T lymphocyte ratio in endometrium, compared with peripheral blood does suggest a specific role for decidual T lymphocytes in pregnancy maintenance (Bulmer *et al.*, 1991; Klentzeris *et al.*, 1992). There is also considerable interest in the balance between Th1 and Th2 type cytokines in pregnancy. It is thought that successful pregnancy is a Th2 phenomenon, with Th1 cytokines being implicated in pathological pregnancy such as miscarriage. CD4+ T cells are a relatively minor component of the decidual leukocyte population and several other cells types within the decidua are able to produce both Th1 and Th2-type cytokines. Although data from murine pregnancy have been compelling, the Th1/Th2 paradigm has been questioned in human pregnancy; amongst several examples, notably IFN-γ which was proposed as a Th1 cytokine and detrimental to pregnancy has been shown in murine pregnancy to play a crucial role in mediating uterine vascular changes (Ashkar *et al.*, 2000).

Functional studies

Decidual T lymphocytes have been reported to exhibit increased expression of the activation markers HLA-DR, CD38 and CD49a in comparison to peripheral blood T lymphocytes (Geiselhart *et al.*, 1995), leading to the suggestion that they play a role in control of trophoblast growth and placental development, either directly via cytotoxic mechanisms or indirectly via cytokine production (Geiselhart *et al.*, 1995). Decidual CD8+ T lymphocytes have also been reported to have immunosuppressive activity (Nagarkatti and Clark, 1983; Thomas and Erickson, 1986).

CD8+ T lymphocytes isolated from non-pregnant endometrium are capable of cytotoxic activity, with a substantial reduction in cytolytic ability in the secretory phase of the menstrual cycle (White *et al.*, 1997). This result suggests that the loss of cytolytic potential of uterine CD8+ T lymphocytes could prevent rejection of the implanting conceptus. However, studies from our laboratory on CD8+ T lymphocytes isolated from early pregnancy decidua demonstrated cytolytic activity of this cell type in both redirected and standard chromium release assays (Scaife *et al.*, 2006). Although extravillous trophoblast cells express HLA-C, there is no evidence that CD8+ T lymphocytes lyse extravillous trophoblast cells *in vivo*; rather it is the uNK cells and macrophages which have been reported to be particularly closely associated with trophoblast within the placental bed.

An alternative or additional function of T lymphocytes within decidua may be in regulating trophoblast invasion. CD8+ T lymphocytes isolated from early pregnancy decidua secrete a wide range of cytokines (particularly IL-8) and when stimulated with phytohemagglutinin-P supernatants produced by purified decidual CD8+ cells were able to stimulate extravillous trophoblast invasion (Scaife *et al.*, 2006). The mechanism for this stimulatory effect remains to be determined.

T lymphoctyes in pathological pregnancy

There have been several reports of perturbation of the normal CD8:CD4 ratio (reduced CD8-positive cell numbers) in the non-pregnant endometrium of women who suffer unexplained infertility (Klentzeris *et al.*, 1994) and recurrent miscarriage (Lachapelle *et al.*, 1996; Quenby *et al.*, 1999). In decidua associated with pregnancy loss, several studies have reported no difference in decidual CD3-positive numbers in women with recurrent (Quack *et al.*, 2001) or sporadic (Vassiliadou and Bulmer, 1998b; Scaife *et al.*, 2004) miscarriage compared to normal early pregnancy. Furthermore, in a study of decidua in placental bed biopsies we observed no alteration in numbers of CD8-positive T lymphocytes in decidua from women with sporadic miscarriage (Scaife *et al.*, 2004). In contrast, Zenclussen *et al.* (2001) reported increased numbers of CD3-positive and CD8-positive T lymphocytes in decidua of women with sporadic miscarriage. Investigation of decidua in miscarriage is difficult and relies on speedy clinical presentation to avoid secondary changes. Piccinni *et al.* (1998) produced CD4-positive and CD8-positive clones from decidua undergoing recurrent miscarriage; production of LIF, IL-4 and IL-10 was reduced compared with controls. Since these results were on clones, the extent to which these T cell responses reflect the *in vivo* situation are not clear. Despite the interest in Th1/Th2 cytokines, there have been very few studies of freshly isolated decidual CD3-positive T cells in either recurrent or sporadic miscarriage, the focus being on uNK cells and other regulatory cell types.

Increased numbers of CD4+ T lymphocytes have been reported in decidua from women with pre-eclampsia compared to age matched controls (Stallmach *et al.*, 1999; Wilczynski *et al.*, 2003). However, work in our laboratory demonstrated a reduction in both CD3+ and CD8+ cells in pre-eclampsia (Scaife, Innes, Searle, Robson, Bulmer; unpublished data). This contrasts with Eide *et al.* (2006) who reported no difference in numbers of decidual CD3+ T lymphocytes in women with fetal growth restriction, pre-eclampsia or pre-eclampsia with fetal growth restriction compared to control samples.

Natural Killer T Cells

Natural Killer T (NKT) cells, characterised by expression of either an invariant (iNKT) or a non-invariant (non-iNKT) T cell receptor, CD161 (C-type lectin) and CD56, have also been identified in early pregnancy decidua (Tsuda *et al.*, 2001; Uemura *et al.*, 2008). The relative proportion of both iNKT and non-iNKT is greatly enriched in decidua compared to peripheral blood of the same patients (Tsuda *et al.*, 2001; Boyson *et al.*, 2002; Uemura *et al.*, 2008).

Distribution and function

Numbers of CD3+CD56+ cells are significantly lower in decidua at term compared to first trimester (Scaife *et al.*, 2003). A role for NKT cells in immune responses has been recognized. NKT cells contain cytoplasmic perforin and granzyme B, suggesting that this cell type may have cytotoxic activity. In addition, a role for NKT cells in decidua and in the maintenance of pregnancy has been suggested by their ability to rapidly produce large amounts of cytokines. Several studies have demonstrated that NKT cells produce large amounts of either IL-4 or IFN-γ (Bendelac *et al.*, 1997; Tsuda *et al.*, 2001). Both cytokines have been reported in decidua and IFN-γ may play a role in vascular remodelling in early pregnancy (Croy *et al.*, 1997; Lash *et al.*, 2006b; Lash *et al.*, 2006c).

CD1d is expressed by trophoblast cells and is able to present a limited set of glycolipids for recognition by NKT cells (Bendelac *et al.*, 2007). The placenta is enriched for glycosphingolipids

(Mikami *et al*., 1993) which may be presented to NKT cells by trophoblast expressing CD1d. Uemura *et al*. (2008) demonstrated that decidual non-iNKT cells did not recognize fetal alloantigens and demonstrated that non-iNKT cells were able to suppress anti-fetal alloresponses as observed by inhibition in a mixed lymphocyte reaction that was mediated by CD1d recognition and subsequent IL-10 production.

NKT cells in pathological pregnancy

Decreased numbers of CD3+CD56+ cells in decidua of women with recurrent miscarriage compared to controls have been reported (Yahata *et al*., 1998; Yamamoto *et al*., 1999). To date the prevalence of this cell type in decidua of women with pre-eclampsia has not been investigated in detail, although in a small study of decidua in placental bed biopsies we did not demonstrate any difference in numbers of CD3+CD56+ cells between pre-eclampsia and normal pregnancy (Scaife, Innes, Searle, Robson, Bulmer; unpublished data).

Regulatory T Lymphocytes

T regulatory cells have been found to play an important role in the suppression of peripheral T cells. This T cell population is highly heterogeneous and includes several subpopulations such as the naturally occurring CD4+CD25+ T regulatory cells, antigen-induced Tr1 and Th3 cells, CD8+CD28+ T regulatory cells and NKT regulatory cells (Wang and Wang, 2007). The CD4+CD25+ T regulatory cells are the most widely studied, although it is important to distinguish between CD4+CD25dim (activated T lymphocytes) and CD4+CD25bright (T regulatory) cells. They derive from the neonatal thymus and express specific, although not unique, surface markers. In addition to CD4 and CD25, naturally occurring T regulatory cells express high levels of glucocorticoid-induced TNFR family related gene (GITR) and Foxp3 molecules. They also express several members of the Toll-like receptors (TLR) family and transforming-growth factor-β (TGF-β).

Distribution and function

Recent publications suggest a potential role of naturally occurring CD4+CD25+ T regulatory cells in maternal-conceptus cross talk and in induction of maternal tolerance to the fetal alloantigens. Heikkinen *et al*. (2004) reported that 10-15% of decidual CD4+ T lymphocytes are CD4+CD25+ T regulatory cells. Both Sasaki *et al*. (2004) and Yang *et al*. (2008) reported an increased percentage of CD25bright cells in the decidual CD4+ population in early pregnancy, compared with that in the peripheral blood CD4+ population from the same subjects, although the proportions of CD25bright cells that were detected differed between the two studies. A greater proportion of CD4+CD25+ cells have been identified in decidua parietalis compared to decidua basalis in both early pregnancy and at term (Tilburgs *et al*., 2006). CD8+CD28+ T regulatory cells have also been identified in decidua from early pregnancy and term using flow cytometry (Tilburgs *et al*., 2006). We have recently identified a small population of both CD3+CD4+ and CD3+CD8+ T regulatory cells that also express HLA-G as determined by double labelling immunohistochemistry, Western Blot analysis and RT-PCR (Lash *et al*., 2007b).

The exact way in which T regulatory cells are activated and finally induce tolerance to alloantigens during pregnancy remains to be elucidated. It has been suggested that this subpopulation expands in the periphery after encountering male antigens presented by antigen presenting cells. T regulatory cells may then migrate into the maternal-fetal interface, where they induce a tolerant microenvironment characterized by high levels of protective molecules, such as TGF-β, LIF and HO-1 (Zenclussen *et al*., 2006). IDO is an enzyme that metabolises tryptophan, a molecule essential for T cell activation. Therefore, expression of IDO by T regulatory cells, which can be upregulated by IFN-γ may be a mechanism by which T regulatory cells are able to suppress T lymphocyte activity (Heikkinen *et al*., 2004).

Regulatory T lymphocytes in pathological pregnancy

A role for peripheral regulatory CD4+CD25+ T lymphocytes in successful pregnancy has also been suggested for murine (Aluvihare *et al*., 2004) as well as human pregnancy (Somerset *et al*., 2004). Increased numbers of CD4+CD25+ regulatory T lymphocytes have been identified in peripheral blood from pregnant women compared to non-pregnant controls, peaking in the second trimester with numbers falling after delivery (Somerset *et al*., 2004). Expansion of T regulatory lymphocytes in normal pregnancy may be associated with immune protection for the fetus. In women with sporadic miscarriage reduced numbers of decidual CD4+CD25+ T regulatory lymphocytes were observed compared to normal age matched controls (Sasaki *et al*., 2004). Yang *et al*. (2008) have reported reduced proportions of CD4+ CD25bright cells in decidua in unexplained recurrent miscarriage compared with controls; whereas there was a significant difference between the percentage of CD25bright cells in the decidual and peripheral blood CD4+ populations, no such difference was noted in recurrent miscarriage subjects. Reduced numbers of CD25bright CD4+ regulatory T lymphocytes have also been reported in both peripheral blood and decidua in pre-eclampsia compared with normal pregnancy controls (Sasaki *et al*., 2007).

Dendritic Cells

Dendritic cells comprise approximately 1-2% of decidual leucocytes during early pregnancy, with numbers not appearing to alter through gestation. Dendritic cells are closely related to macrophages but are more potent antigen presenters. There are three origins of dendritic cells in humans; lymphoid (plasmacytoid), non-hematopoietic (follicular cells of the lymph nodes) and myeloid origin (myeloid) (reviewed in Banchereau and Steinman, 1998; Steinman *et al*., 2003). Immature dendritic cells capture antigens from dying, infected or allogeneic cells and present them to T cells inducing antigen-specific T cell tolerance. However, mature dendritic cells function as highly potent antigen presenting cells capable of activating naïve and memory helper T cells, cytotoxic T cells and B cells. Therefore the maturation status of dendritic cells determines whether they exert immunogenic or tolerogenic effects (Lutz and Schuler, 2002).

Distribution of dendritic cells

Uterine dendritic cells are of myeloid origin and are generated from several potential progenitors including bone marrow precursors, blood monocytes, blood CD34+ stem cells and possibly

tissue macrophages (Palucka *et al*., 1998; Steinman and Inaba, 1999). Both mature (CD83+) and immature (CD83-) dendritic cells have been identified in human uterine tissues (Kammerer *et al*., 2000). There appears to be several related immature dendritic cell types some of which are DC-SIGN+CD14+ and others DC-SIGN-CD14-DEC205+ (Gardner and Moffett, 2003; Kammerer et a., 2003). It is proposed that DC-SIGN+ cells are precursors of both macrophages and dendritic cells (Soilleux *et al*., 2002).

In an immunohistochemical study of CD83+ cells in early pregnancy decidua approximately 50% were associated with lymphoid clusters, 40% were closely associated with endometrial glands, 10% with lymphatic vessels, with only a few scattered CD83+ cells through the decidual stroma. Anatomically, in both decidua and non-pregnant endometrium CD83+ cells were predominantly identified in the basal layer. No differences were observed between decidua basalis and decidua parietalis (Kammerer *et al*., 2000). Kammerer *et al*. (2003) demonstrated significantly higher numbers of CD83+ dendritic cells in non-pregnant endometrium and early pregnancy decidua compared with myometrium. In the same study DC-SIGN+ cells were virtually absent in non-pregnant endometrium but were dramatically increased in early pregnancy through to the end of gestation (Kammerer *et al*., 2003). In contrast, Reiger *et al*. (2004) reported the presence of DC-SIGN+ cells in non-pregnant endometrium with a significantly higher density in early pregnancy. Reiger *et al*. (2004) also reported low numbers of CD83+ dendritic cells in non-pregnant endometrium and in early pregnancy decidua, with highest numbers observed in the late secretory phase of the menstrual cycle. No co-expression of CD83+ was observed with DC-SIGN in non-pregnant endometrium (Reiger *et al*., 2004) or early pregnancy (Kammerer *et al*., 2003). In addition, in non-pregnant endometrium less than 1% of the DC-SIGN cells were proliferative (as determined by double-labelling with Ki67) while this figure rose to approximately 10% in early pregnancy (Reiger *et al*., 2004). Schulke *et al*. (2008) studied CD83+ and CD1a+ dendritic cells in non-pregnant endometrium; numbers of CD1a+ cells were higher than CD83+ cells and both populations were more numerous in the basal endometrium compared with the stratum functionalis, with some cycle variations. Numbers of CD1a+ but not CD83+ cells increased between proliferative and secretory phases.

Gardner and Moffett (2003) primarily used flow cytometry analysis to demonstrate and characterise the dendritic cell population in early pregnancy decidua. Immunohistochemical analysis using DEC-205 and CD83 demonstrated that the majority of these cells were associated with lymphoid aggregates, lymphatic vessels and venules (Gardner and Moffett, 2003).

Functional studies

In vitro functional experiments performed with dendritic like cells derived from the THP-1 cell line suggest that dendritic cells have no effect of trophoblast invasion (Huang *et al*., 2008). In addition, while isolated decidual immature CD14+DC-SIGN+ dendritic cells were able to mature into CD25+CD83+ mature dendritic cells and take up antigen, they were not able to stimulate naïve allogeneic T cells (Kammerer *et al*., 2003). However the *in vitro* matured cells demonstrated strong immunostimulatory activity in this assay (Kammerer *et al*., 2003).

Dendritic cells in pathological pregnancy

Investigation of dendritic cells in pathological pregnancy has been limited. Increased numbers of both CD209+ immature dendritic cells and mature CD83+ activated dendritic cells have been reported but whether this is a cause or effect of this condition is unclear (Huang *et al*., 2008). In contrast, Scholz *et al*. (2008) reported no difference in numbers of DC-SIGN+ (CD209+) or DEC-205 in term decidual tissue (attached to the delivered placenta) from women with pre-eclampsia and fetal growth restriction compared to age matched controls. In contrast, both of these DC markers were found to be increased in decidua from women with HELPP syndrome compared to age matched controls (Scholz *et al*., 2008). The density and localisation of this cell type has not been investigated in other pregnancy pathologies. Higher numbers of CD83+ dendritic cells have been observed in decidua of women with recurrent miscarriage at 8 weeks gestation compared to age matched controls (although the total number of samples do not match) (Askelund *et al*., 2004). However, when all gestational ages were taken together (7-11 weeks gestational age) there was no difference in the number of CD83+ cells between women with recurrent miscarriage and controls (Askelund *et al*., 2004). To date the distribution of this cell type has not been investigated in sporadic miscarriage.

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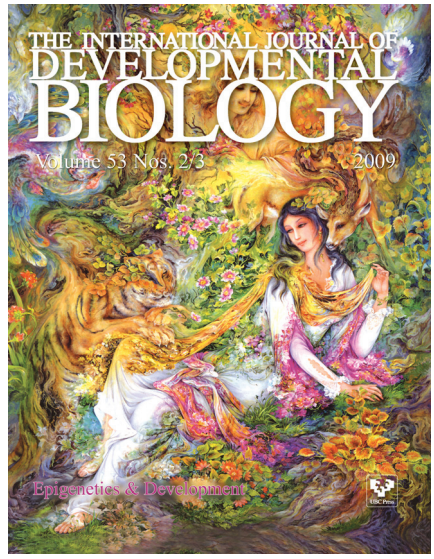

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