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The distribution of uterine macrophages in virgin and early pregnant mice

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INTRODUCTION

Macrophages are bone marrow derived cells which differentiate in tissue spaces from blood borne monocytes and which have a capacity for endocytosis, have cytoplasmic inclusions containing hydrolytic enzymes and express a number of specific membrane antigens including Fc_γ receptors (Van Furth, 1986). Some of the antigens present on the surface of macrophages can be detected using commercially available antibodies. Macrophages are present in the rodent uterus during pregnancy and their suggested functions in this location can be grouped into 2 broad areas: (1) immunological functions including antigen presentation (Elcock & Searle, 1985), immunosuppression (Hunt, Manning & Wood, 1984; Scodras, Parhar, Kennedy & Lala, 1990) and cytokine production (Hunt, 1989, 1990), and (2) endocytic functions associated with the removal of cell debris and repair of the uterine wall in the late stages of pregnancy and the postpartum period (Deno, 1937; Lobel & Deane, 1962; Larkin, 1972).

Previous studies of the immunological functions of uterine macrophages have largely been carried out using cells isolated from the uterine wall but with little or no attempt to determine how closely such macrophages are related to the fetal allograft in vivo. Although a small number of studies have been carried out which have included a description of the location of macrophages the picture was unclear and we therefore initiated our own study of the distribution of macrophages in the mouse uterus.

In the present study we examined the spatiotemporal distribution of macrophages in the uterine wall of virgin mice (Fig. 1*a*) and at implantation sites (Fig. 1*b*) during the first half of pregnancy. Because many of the features used to characterise macrophages (e.g. Fc_γ receptors) are also shared with other cell types which are present in the uterus during pregnancy (Daki, Stewart & Wild, 1989), a number of carefully selected characteristics were used to study the distribution of uterine macrophages. Macrophages were therefore identified using a combination of morphological criteria, evidence of endocytic activity and the expression of Mac-1 antigen. The results are discussed in relation to the purported immunological and endocytic functions of uterine macrophages. An attempt is also made to account for the apparent disparity in numbers of macrophages found in the decidua and the numbers reported in cell suspensions isolated from decidual tissue.

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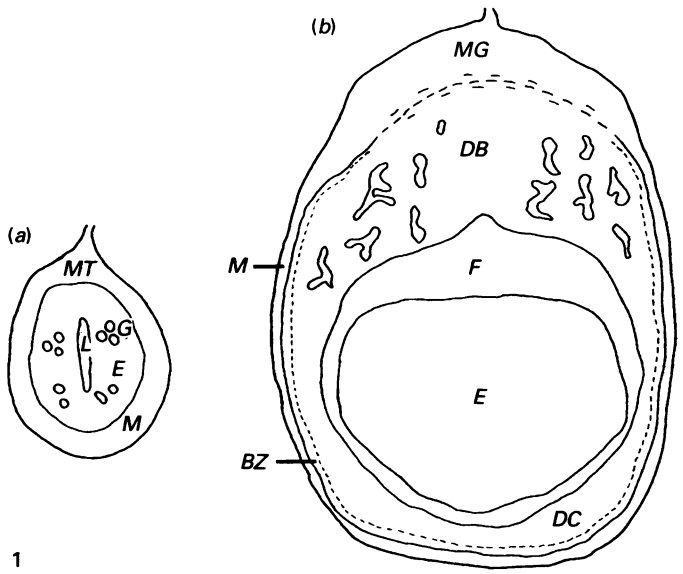


Fig. 1. (*a*) Diagram of a transverse section of virgin mouse uterus. *L*, uterine lumen; *E*, endometrium; *G*, uterine glands; *M*, myometrium; *MT*, mesometrial triangle. × 20. (*b*) Diagram of a midtransverse section through an implantation site at day 10 of pregnancy. *E*, embryonic sac; *F*, fetal placenta; *DB*, decidua basalis; *DC*, decidua capsularis; *BZ*, basal zone; *M*, myometrium; *MG*, metrial gland. × 14.

MATERIALS AND METHODS

Animals

Virgin female Porton mice aged 10–16 weeks were used in this study. Vaginal smears were prepared to identify mice at oestrus or dioestrus. To obtain timed pregnancies, mice were mated overnight with males of their own strain and the presence of a vaginal plug the following morning was taken as day 0 of pregnancy. Mice were killed by cervical dislocation under ether anaesthesia at oestrus and dioestrus and at days 4, 5, 8 and 10 of pregnancy. Implantation sites (or pieces of uterus) from each animal were randomly selected for plastic histology or for immunohistochemistry.

Injection of horseradish peroxidase

A single intravenous (i.v.) injection of horseradish peroxidase (HRP) (type II, Sigma, 5 mg/100 g body weight) in 0·9% saline was given to some mice 2 h before death. Other control mice received a single intravenous injection of 0·9% saline (0·3 ml) 2 h before death. At least 3 mice at each stage of the oestrous cycle and pregnancy were given HRP and at least 3 mice at each stage were given saline alone.

Location of implantation sites at day 4 of pregnancy

Mice killed at day 4 of pregnancy were given a single i.v. injection of 0·25 ml of a 1% solution of pontamine sky blue 6BX in 0·9% saline 15 min before death to locate implantation sites (Finn & McLaren, 1967). In 3 control mice given saline without HRP 2 h before death, implantation sites were clearly defined at laparotomy as blue patches distributed along each uterine horn. However, of 6 mice given an i.v. injection of HRP 2 h before death none had blue patches denoting the implantation sites. It was not possible, therefore, to dissect implantation sites from the day 4 pregnant mice

given HRP. This finding suggests that HRP interferes with the normal physiological changes in uterine vasculature at this time and necessitates cautious use of this tracer in studies of the uterus during the peri-implantation period.

Plastic histology

Pieces of uterus were immersion fixed for 24 h in a mixture of 2% glutaraldehyde and 4% formaldehyde in 0·1 M phosphate buffer pH 7·2–7·4 (modified from Karnovsky, 1965) and then washed in the phosphate buffer. Thin (< 0·25 mm) transverse slices of uterus (from nonpregnant and day 4 pregnant mice) or of midimplantation sites were cut and placed in 0·1 M-Tris-HCl buffer pH 7·6 for 1 h. Slices of uterus were then incubated in a mixture of 5 mg diaminobenzidine tetrahydrochloride in 10 ml 0·1 M-Tris-HCl buffer pH 7·6 with 0·1 ml 1% hydrogen peroxide (DAB) for 1 h to locate peroxidase activity (Graham & Karnovsky, 1966). Control slices of tissue were incubated in the Tris-HCl buffer alone. The slices of tissue were washed in buffer, postfixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Araldite. Sections (1 μm) were cut and examined by bright field or phase contrast microscopy, with or without prior staining with toluidine blue. In view of the poor penetration properties of DAB all sections were carefully screened to ensure that all red blood cells showed a strong reaction for peroxidase activity. Sections showing only a moderate or weak reaction for red blood cell peroxidase activity were discarded.

Immunohistochemistry

Implantation sites, and pieces of uterus from nonpregnant mice and day 4 pregnant mice were frozen in 2-methylbutane cooled with liquid nitrogen. Cryostat sections (8 μm) cut transversely through the middle of implantation sites or through pieces of uterus were mounted on clean glass slides, air dried and fixed in absolute acetone for 10 min. Some sections were incubated for 10 min in DAB to locate peroxidase activity. Sections were then reacted in an indirect immunofluorescence method using a rat antimouse macrophage (Mac-1) antibody (Seralab MAS 034, culture supernatant) as the primary antibody at a dilution of 1 in 10 in phosphate buffered saline (PBS). A fluorescein isothiocyanate (FITC) conjugated rabbit or sheep antirat IgG antibody (Nordic) was used as the 2nd layer at a dilution of 1 in 100 in PBS. Control sections in which the primary antibody was omitted or replaced with nonimmune rat serum were prepared. The sections were mounted in glycerol-PBS (AF1, Citifluor) and examined in a combined fluorescence and phase contrast microscope.

Nonspecific binding of FITC conjugated antibodies by granules of eosinophilic leucocytes has been described (Valnes & Brandtzaeg, 1981) and was observed in the present study. The morphology of eosinophilic leucocytes and their distinctive binding to the FITC conjugated antibodies allowed easy identification of these cells. Eosinophils are common in uterine tissues taken at oestrous but not common at other stages of the oestrous cycle or during early pregnancy (Wharton, 1988). Thus the nonspecific antibody binding did not seriously interfere with our analysis of the distribution of Mac-1 positive cells.

RESULTS

General

In sections of tissue from mice given HRP and which had been reacted with DAB, numerous cells were identified which contained dark brown peroxidase positive

inclusions of various sizes. However, many of the cells contained only 2 or 3 positive inclusions which were very small (being near to the limit of resolution of the light microscope). For the purpose of the present study these latter cells were not regarded as endocytic cells.

In sections of tissue from mice given HRP or saline alone and not treated with DAB, no dark brown inclusions were identified in any cells. In sections of tissue from control mice given saline alone and subsequently treated with DAB, peroxidase-positive red blood cells and cells which contained peroxidase-positive inclusions were identified. From their size, nuclear morphology and location in both blood vessels and tissue spaces, some of these latter cells were identified as polymorphonuclear leucocytes. The other cells each contained only a small number of peroxidase-positive inclusions of various sizes.

Oestrous, dioestrous and day 4 *pregnant mice*

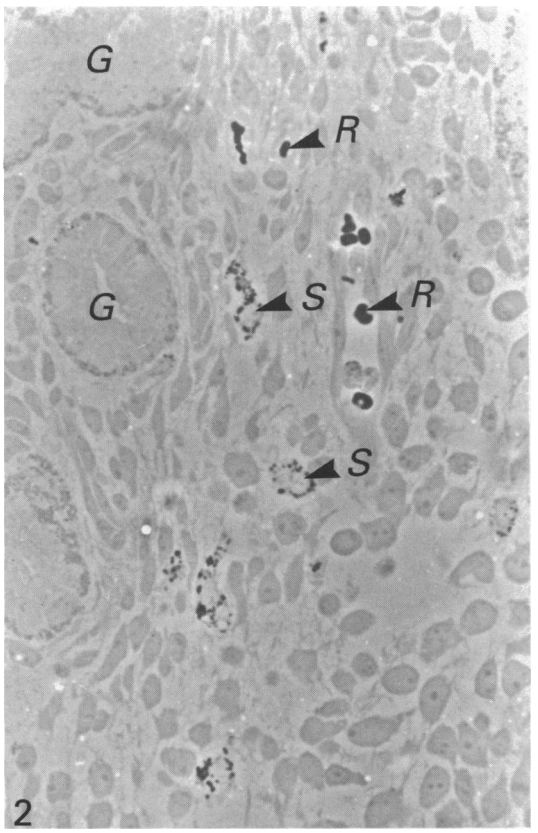
Cells which had endocytosed HRP were found throughout the endometrium in this group (Fig. 2), although they appeared to be more sparsely distributed adjacent to the luminal epithelium than in the rest of the endometrium. This tendency was most evident in the endometrium at day 4 of pregnancy. In mice killed at oestrus numerous peroxidase-positive cells were found in tissue spaces which from their size and nuclear morphology were identified as polymorphonuclear leucocytes. These cells were only sparsely distributed in the endometrium of mice at dioestrus and at day 4 of pregnancy. Polymorphonuclear leucocytes contain endogenous peroxidase and were therefore stained dark brown in sections from control mice reacted with DAB. Other cells were identified which had extensive cytoplasm containing numerous inclusions, some peroxidase positive and some peroxidase negative. Similar cells were identified in the mesometrial triangle. They had a small euchromatic nucleus and in stained sections the cytoplasm was more palely stained than that of the general stromal cell population. In control mice given saline alone and treated with DAB, some cells with this morphology contained a few peroxidase-positive inclusions but always less than these cells in mice given HRP. (Other cells in the endometrium were mainly the fibroblast-like stromal cells with an ovoid nucleus and moderately stained cytoplasm. In animals at day 4 of pregnancy these fibroblast-like stromal cells generally appeared to have a larger more euchromatic nucleus and more cytoplasm than in virgin mice.) Other minor cell populations were identified, including at day 4 of pregnancy an occasional small granulated metrial gland (GMG) cell and some small lymphoid cells, but none of these minor cell populations or the stromal cells appeared to have endocytosed HRP or contained endogenous peroxidase activity.

Cells which had endocytosed HRP were identified in the connective tissue between the bundles of smooth muscle fibres in the myometrium (Fig. 3). Generally these cells had similar characteristics to the HRP-positive cells in the mesometrial triangle and endometrium except that they generally seemed to be smaller and of a more diverse form, often appearing flattened or stellate. As in the endometrium and mesometrial triangle there were a few cells of this type which contained a small number of peroxidase-positive inclusions in saline injected control mice treated with DAB.

Days 5, 8 *and* 10 *of pregnancy*

Endocytic cells in the decidua

At day 5 of pregnancy many cells which had endocytosed HRP were found in the endometrium. These were generally located in the periphery of the endometrium where the stromal cells showed relatively little evidence of decidualisation. In the



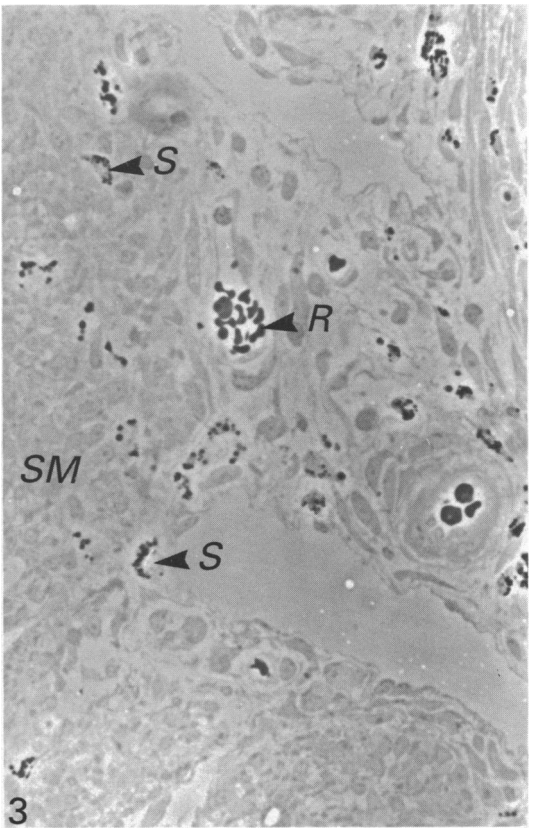


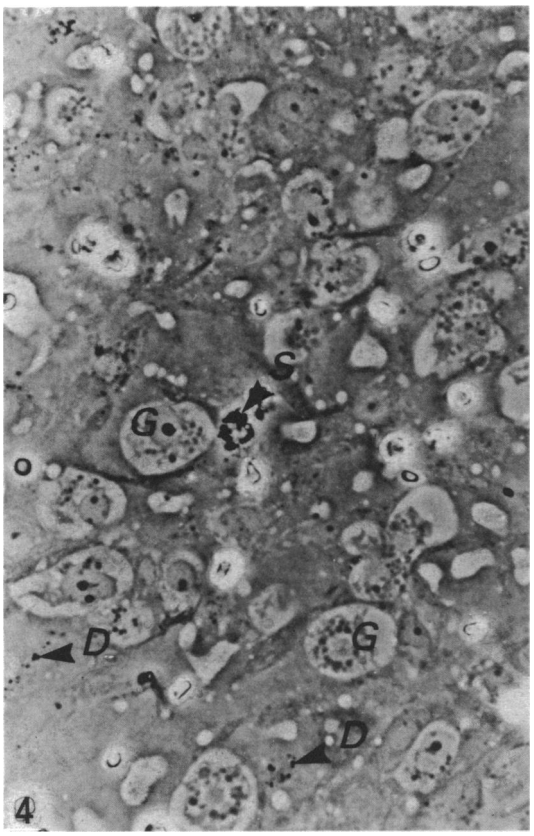
Fig. 2. Cells (arrowed *S*) in the endometrium which have endocytosed HRP. Red blood cells (arrowed *R*). *G*, uterine glands. HRP 2 h. DAB reaction. Unstained 1 μm section. Phase-contrast microscopy. Day 4 of pregnancy. × 525.

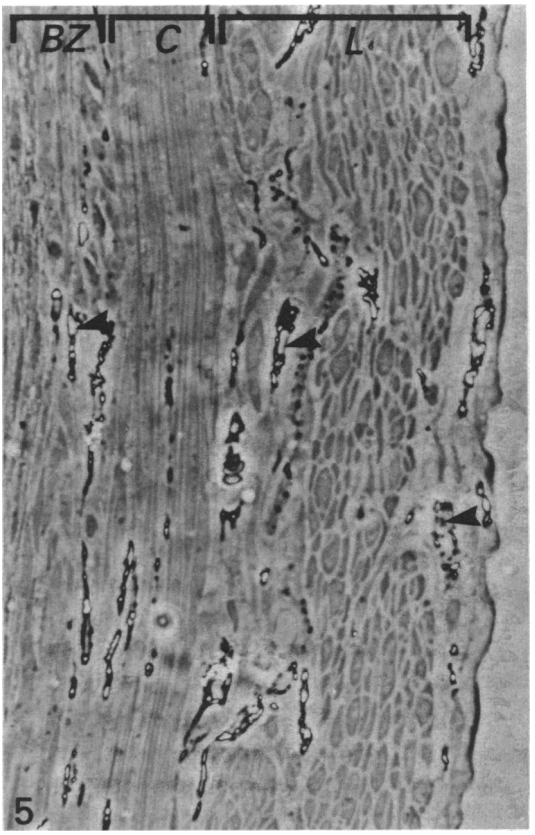
Fig. 3. Cells (arrowed *S*) in the connective tissue space of the myometrium which have endocytosed HRP. Red blood cells (arrowed *R*). *SM*, smooth muscle. HRP 2 h. DAB reaction. Unstained 1 μm section. Phase-contrast microscopy. Day 4 of pregnancy. × 525.

decidualising endometrium around the developing embryo and more mesometrially around the remains of the luminal epithelium, the hypertrophied stromal cells were closely packed. Cells which had endocytosed HRP were only occasionally found amongst these hypertrophied stromal cells and were not present in all sections.

By day 8 of pregnancy the main zones of the decidua are clearly defined although not fully developed. In the decidua basalis only a few small cells were found which contained abundant peroxidase-positive inclusions. Fewer still were found amongst the decidual cells of the decidua capsularis and lateral decidual zone. However, in the basal zone of the decidua (adjacent to the myometrium) there were numerous HRP-positive cells which appeared fusiform in shape and lying parallel to the circular smooth muscle cells.

At day 10 of pregnancy, as at day 8, only a few endocytic cells were seen in the decidua (Fig. 4) and mainly in the decidua basalis. Generally there were up to about a dozen peroxidase-positive cells in each section of the decidua basalis at days 8 and 10 of pregnancy and only about 2 or 3 in the decidua capsularis. The basal zone was clearly defined by day 10 of pregnancy, both antimesometrially and mesometrially.





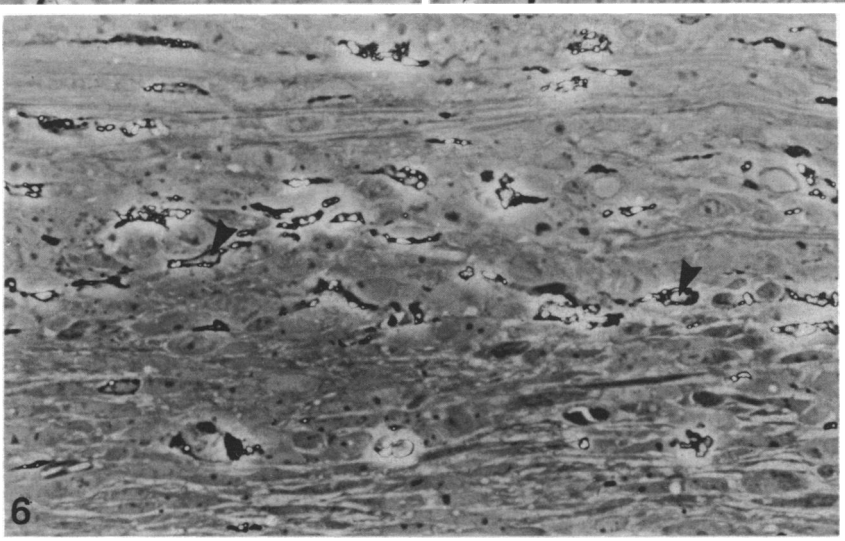


Fig. 4. An endocytic cell (arrowed *S*) in the decidua basalis. Many of the decidual cells also contain some small HRP-positive inclusions (arrowed *D*). Granulated metrial gland cells (*G*) contain no HRP-positive inclusions. HRP 2 h. DAB reaction. Unstained 1 μm section. Phase-contrast microscopy. Day 10 of pregnancy. × 525.

Fig. 5. Cells (arrows) in the myometrium and basal zone of the decidua which have endocytosed HRP. *L*, longitudinal layer of smooth muscle; *C*, circular layer of smooth muscle; *BZ*, basal zone. HRP 2 h. DAB reaction. Unstained 1 μm section. Phase-contrast microscopy. Day 10 of pregnancy. × 525.

Fig. 6. Cells (arrows) in the metrial gland which have endocytosed HRP. HRP 2 h. DAB reaction. Unstained 1 μm section. Phase-contrast microscopy. Day 10 of pregnancy. × 525.

Flattened peroxidase-positive cells were numerous in this zone (Fig. 5). At days 8 and 10 of pregnancy the endocytic cells in the decidua and basal zone appeared smaller than seen in the endometrium at earlier stages of pregnancy and in the endometrium of nonpregnant mice but similar in size to the endocytic cells seen in the myometrium at all stages.

Mesometrial triangle and metrial gland

In the mesometrial triangle at day 5 of pregnancy there were many cells which had endocytosed HRP, many more per unit area of section than in the developed decidua. At days 8 and 10 of pregnancy there were still many HRP-positive cells in the metrial gland (Fig. 6).

Myometrium

The distribution of endocytic cells in the myometrium (Fig. 5) was similar to that found in virgin mice but as pregnancy progressed the myometrium appeared thinner. However, the proportion of cells in the connective tissue which were endocytic did not appear to change.

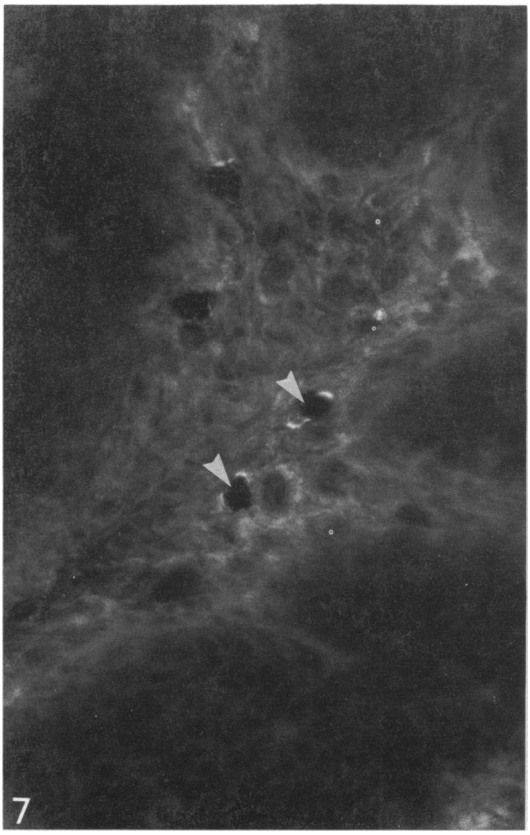
Uptake of HRP by nonendocytic cells

The cells described above as HRP positive each contained many HRP-positive inclusions of various sizes. These cells could easily be seen in sections with or without prior staining with toluidine blue. However, it was evident that in unstained preparations viewed at high magnification there were cells in the decidua basalis and developing metrial gland which contained small HRP-positive inclusions in addition to the background levels described earlier. These inclusions were clearly evident by day 10 of pregnancy (Fig. 4) when they were numerous in many of the decidual cells of the decidua basalis although they were not a feature of the cells in the decidua capsularis. They were also present, although less numerous, in the stromal cells of the developing metrial gland. These small inclusions were masked by staining the sections with toluidine blue and on this basis the cells are described as nonendocytic. Granulated metrial gland cells in the decidua basalis and developing metrial gland showed no evidence of having endocytosed HRP.

Immunohistochemistry

Mac-1 positive cells were found in the myometrium at all stages of the oestrous cycle and pregnancy examined. In mice given HRP these Mac-1 positive cells had also endocytosed HRP. In postimplantation pregnant mice many Mac-1 positive, HRP-positive cells were also located in the basal zone of the decidua. Mac-1 positive, HRP-positive cells were found randomly distributed in the endometrium of nonpregnant mice at day 4 of pregnancy. Some HRP-positive cells in the endometrium did not react with the Mac-1 antibody. In pregnant mice few Mac-1 positive cells were found in the main part of the developing decidua (Figs 8–10) and most of these had endocytosed HRP. Such cells were generally small and only about a dozen were found in each section of decidua basalis at days 8 and 10 of pregnancy with only 2 or 3 in the antimesometrial decidua. At day 10 of pregnancy a small number of Mac-1 positive cells, which had not endocytosed HRP, were located at the decidua-trophoblast interface (Fig. 11), but only in a small proportion of the sections examined.

In the mesometrial triangle of virgin mice and mice at days 4 and 5 of pregnancy, many Mac-1 positive cells (Fig. 9) were identified and these had endocytosed HRP. However, HRP-positive cells were also found which did not react with the Mac-1



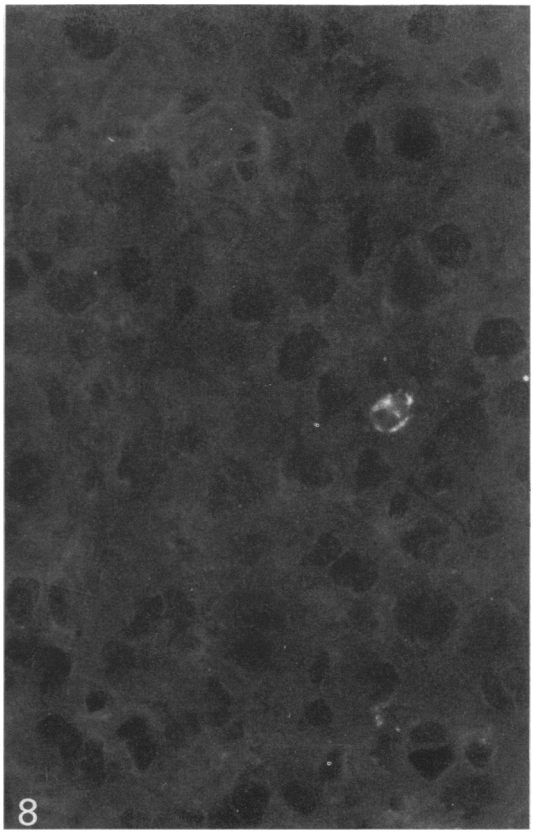


Fig. 7. Cells (arrows) in the connective tissue spaces of the myometrium which have endocytosed HRP and show binding of the Mac-1 antibody. The dark core to the arrowed cells is due to the presence of endocytosed HRP. HRP 2 h. DAB reaction. 8 μm cryostat section. Day 10 of pregnancy. × 550.

Fig. 8. A Mac-1 positive cell in the developing decidua capsularis. 8 μm cryostat section. Day 5 of pregnancy. × 550.

antibody. In the developing metrial gland at days 8 and 10 of pregnancy some Mac-1 positive cells were found (Fig. 12) but fewer per unit area of section than was apparent in the mesometrial triangle at day 5 of pregnancy. These Mac-1 positive cells had endocytosed HRP. However, there were many more HRP-positive cells than Mac-1 positive cells.

The GMG cells in the decidua basalis and metrial gland did not react with the Mac-1 antibody.

DISCUSSION

Cells with a rounded nucleus and cytoplasm containing abundant inclusions, which had endocytosed HRP and which expressed the Mac-1 antigen were identified in each compartment of the uterine wall at each stage of the oestrous cycle and pregnancy examined. These cells are considered to be macrophages. Other cells with a similar morphology were found which had either endocytosed HRP or expressed the Mac-1 antigen, but not both. The Mac-1 antigen is not unique to the monocyte-macrophage

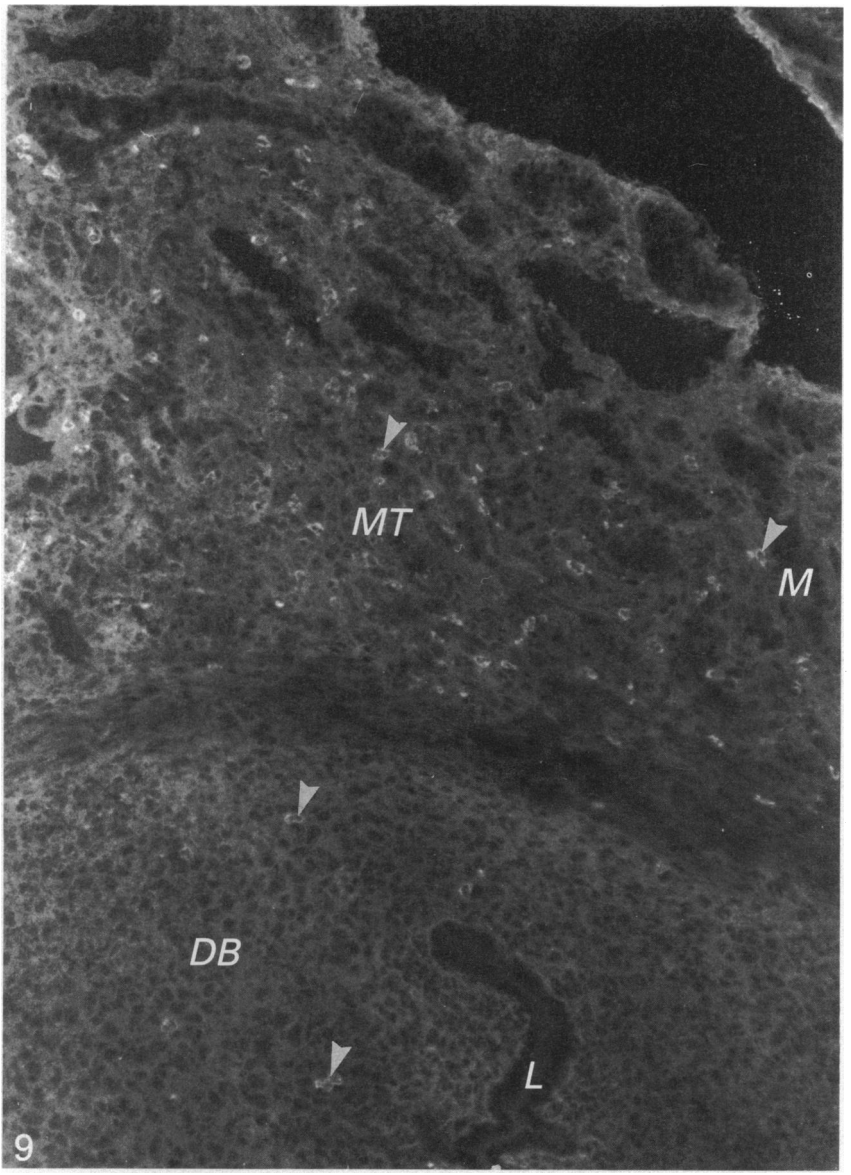
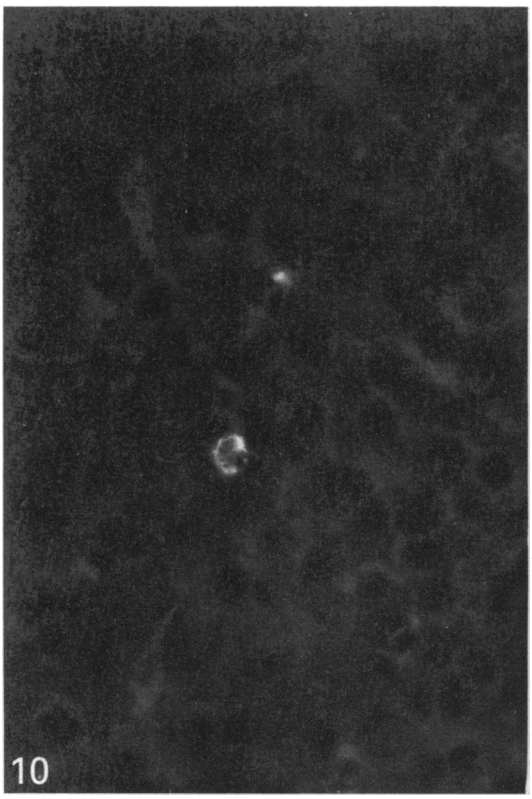


Fig. 9. Mac-1 positive cell (arrows) in the developing decidua basalis (*DB*), in the myometrium (*M*) and in the mesometrial triangle (*MT*). There are relatively few Mac-1 positive cells in the decidua. *L*, luminal epithelium. 8 μm cryostat section. Day 5 of pregnancy. ×210.

lineage but is also expressed by neutrophils (Springer, Galfre, Secher & Milstein, 1979). In addition, neutrophils exhibit endogenous peroxidase activity (Cormack, 1987). However, the morphology of neutrophils is quite distinct and allows for a clear identification of this cell type. The Mac-1 positive, peroxidase-negative cells are probably also macrophages but further characterisation including immunohistochemical analysis directed against other macrophage specific antigens will provide a more definite answer.

Cells with peroxidase activity which were Mac-1 negative were also detected. From



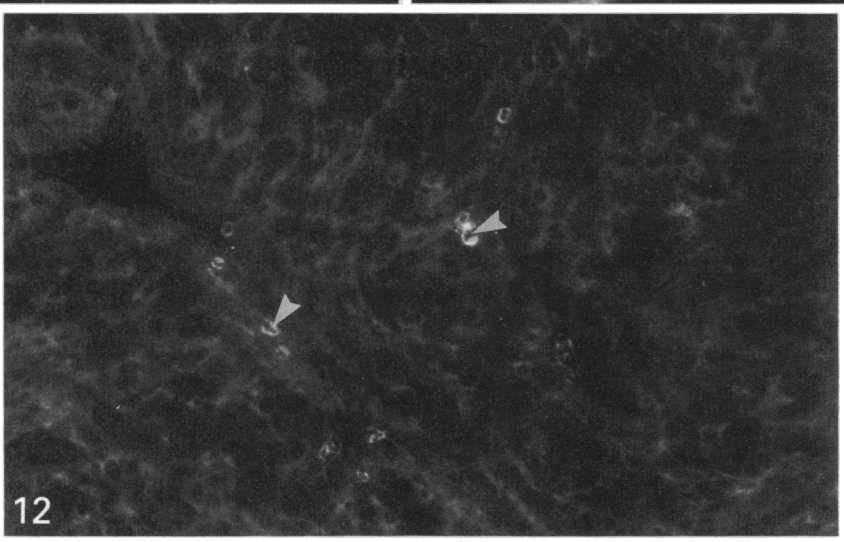


Fig. 10. A Mac-1 positive cell in the decidua basalis. 8 μm cryostat section. Day 10 of pregnancy. $\times 550$.

Fig. 11. Mac-1 positive cells (arrows) in the decidua basalis adjacent to the giant trophoblast cell layer. *N*, nucleus of a giant cell. 8 μm cryostat section. Day 10 of pregnancy. $\times 800$.

Fig. 12. Mac-1 positive cells (arrows) in the metrial gland. 8 μm cryostat section. Day 10 of pregnancy. $\times 275$.

their morphology some of these cells were polymorphonuclear leucocytes but other cells which had rounded nuclei and a morphology generally similar to the majority stromal cell population were identified. These latter cells were most common in the mesometrial triangle/metrial gland but they were also found in the myometrium and basal zone. Although the morphology of these cells and their capacity to endocytose HRP are both compatible with their being macrophages, the absence of expression of the Mac-1 antigen suggests that these cells represent a group of endocytic cells which are not part of the monocyte–macrophage lineage. The possibility that endocytic cells in the uterus may fall into 2 categories has been considered by Cornillie & Lauweryns (1984) in studies of protein removal from tissue fluid of the rat endometrium. Their suggestion that endocytic cells may be either monocyte derived or stromal cell derived is supported by our study. Care will need to be taken to ensure that, when appropriate, distinctions are made between the 2 groups of cells.

Changes were detected in the distribution of Mac-1 positive macrophages during the period of pregnancy studied. In pregnancy implantation is followed by a wave of proliferative activity and hypertrophy of the endometrial stromal cells around the blastocyst to form the decidua (Finn & Martin, 1967). However, the wave of proliferative activity and hypertrophy of stromal cells does not extend to the myometrium and a zone of flattened cells known as the basal zone becomes evident by day 8 of pregnancy. In the developing decidua basalis, immigrant bone marrow derived cells proliferate and hypertrophy as they differentiate into GMG cells (Stewart & Peel, 1977, 1982; Peel, Stewart & Bulmer, 1983). The reduction in numbers of macrophages in the decidua is probably mainly due to a failure of this population to increase in number while the other cell populations present are undergoing extensive proliferative activity (i.e. the macrophages become diluted). Studies by Redline & Lu (1988) have provided good evidence that decidual factors, as yet undefined, restrict access of monocytes (and T lymphocytes) into the decidua at least during the last week of pregnancy. If similar factors are present in the early decidua they could be responsible for preventing the maintenance of, or increase in, the macrophage population by new infiltrating monocytes. In addition, many of the macrophages which are distributed in the more peripheral parts of the endometrium in virgin and day 4 pregnant mice probably become compressed into the basal zone as a consequence of the decidualisation process. Similarly the formation of the metrial gland is characterised by the proliferation and hypertrophy of stromal cells and GMG cells and the apparent decline in numbers of cells of the monocyte–macrophage population in this region can be accounted for by a failure of this cell population to proliferate or invade at sufficient levels. The turnover of macrophages in relation to the changing hormonal environment during the oestrous cycle and pregnancy was not addressed in the present study but is a factor which could influence the overall distribution of macrophages (De & Wood, 1990). This possibility awaits detailed investigation.

The majority of monocyte-derived uterine macrophages are located in the myometrium and mesometrial triangle and, in pregnancy, also in the basal zone of the decidua. This distribution appears to remain fairly constant at all stages of the oestrous cycle and through pregnancy. The myometrium, the mesometrial triangle and the peripheral part of the endometrium (basal zone in pregnancy) are the sites where lymphatic capillaries begin (Malaney, Reid & Scothorne, 1980). The bulk of the endometrium is believed to be lacking in lymphatic capillaries (Head & Lande, 1983; Head & Seelig, 1984). Thus although the majority of uterine macrophages are located

in the periphery of the uterine wall they are still well placed to monitor tissue fluid entering lymphatic vessels and to remove particulate matter and bind antigen.

It has been suggested that macrophages are involved in the protection of the fetal allograft by releasing factors (e.g. prostaglandin E2) which suppress cytolytic cells such as NK cells or cytotoxic T cells (Hunt, Manning & Wood, 1984; Tawfik, Hunt & Wood, 1986; Matthews & Searle, 1987; Scodras *et al.* 1990). Uterine macrophages have also been implicated in antigen presentation (Searle, Bell & Billington, 1983; Elcock & Searle, 1985) and in cytokine production (Hunt, 1989). These functions could best be performed at or near the decidua-trophoblast interface, but our results support and extend the findings of Redline & Lu (1988, 1989) and Parr, Young, Parr & Young, (1990) who, in their studies of the decidua in late pregnancy, indicated that there are few macrophages in the decidua and these appear to be randomly distributed. Alternatively, macrophage-derived soluble suppressor factors and cytokines which are needed at the materno-trophoblast interface could be produced by blood borne cells or by cells resident in the spleen. We identified a small number of nonendocytic, Mac-1 positive cells at the decidua-trophoblast interface at day 10 of pregnancy but only in some implantation sites. A study by Krcek, Dickson & Biddle (1983) identified small mononuclear cells (macrophages) at the decidua-trophoblast interface in some implantation sites for a short period during day 10 of pregnancy in the mouse. That such macrophages were not usually seen in our material may be due to their transitory appearance as described by Krcek *et al.* (1983). It is at day 10 of pregnancy that the formation of the definitive placenta becomes apparent and new trophoblast is exposed to maternal blood (Stewart, 1984). The transitory appearance of macrophages at this time and in this location could represent a local monitoring of new trophoblastic alloantigen.

Our results clearly indicate that few macrophages are present in the decidua, and therefore single cell suspensions of decidua that contain a representative cellular composition should only contain a small macrophage population. In a previous study in our laboratory, single cell suspensions of decidua were prepared and maintained in culture (Stewart & Mukhtar, 1988). Five groups of cells were identified on morphological criteria, a minor one of which was suggested to be macrophages. Another cell type, the GMG cell, was only rarely identified even in cell isolates prepared from day 8 decidua when such cells form about 15% of cells in the decidua basalis (Stewart & Peel, 1982). The latter result shows that cell isolation procedures may not yield cell suspensions which reflect the proportions of cells in heterogenous populations found in vivo. There seems to be little general concern by other workers about the composition of single cell suspensions of decidua used in functional assays or for cell culture and whether they form a representative sample of the cellular constituents of the decidua in vivo. Indeed, it is often unclear in such preparations whether decidual tissue had been dissected from the rest of the uterine wall prior to mechanical and enzymatic disruption of the tissue. It is likely that there are 2 main reasons for the artificially high proportion of decidual macrophages in cell samples used in many studies. First, the tissue from which the cell suspensions were prepared contains not just decidua but also some basal zone and myometrium, which contains a higher proportion of macrophages. Second, the preparation methods used may preferentially preserve small leucocyte populations including macrophages. Greater concern needs to be directed towards ensuring that the interpretation of data from assays using cell isolates gives consideration to the relationship between the cellular constitution of the isolates and of the tissue in vivo. The paucity of decidual

macrophages revealed in the present study suggests that many conclusions drawn from previous studies using macrophage rich cell isolates need to be reassessed.

SUMMARY

The spatiotemporal distribution of macrophages in the uterine wall of virgin mice and mice in the first half of pregnancy has been studied. Macrophages were identified using a combination of morphological criteria, the capacity to endocytose horseradish peroxidase and the expression of the Mac-1 antigen. In virgin mice and mice at preimplantation stages of pregnancy, macrophages were found throughout the endometrium, myometrium and mesometrial triangle. Following implantation, and in parallel with decidualisation, the density of macrophages appeared to decline in the decidua with advancing gestation. It is suggested that this change in density is due to a dilution of the macrophage population rather than a loss of individual cells. The numbers and distribution of decidual macrophages indicate that this group of cells does not play a major regulatory role in the success of pregnancy.

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