Immunology 1988 **65** 129–134

Cell populations in human early pregnancy decidua: characterization and isolation of large granular lymphocytes by flow cytometry

P. M. STARKEY, I. L. SARGENT & C. W. G. REDMAN *Harris-Birthright Centre for Perinatal Medicine, Nuffield Department of Obstetrics & Gynaecology, John Radcliffe Hospital, Oxford*

Accepted for publication 23 May 1988

SUMMARY

Cell populations of human pregnancy decidua, obtained by enzymic digestion from first trimester samples, were analysed by flow cytometry after labelling with monoclonal antibodies. The majority of these decidual cells (75%) were of bone marrow origin. The most abundant cell type expressed antigens characteristic of large granular lymphocytes (LGL), although macrophages and small numbers of classical T cells were also present. Three subsets of decidual LGL can be defined by single- and double-antibody labelling. Most decidual LGL are positive for NKH1, a marker of peripheral blood LGL, but negative for CD16, the Fc receptor of NK cells, and for the T-cell markers CD3 and CD5. About half the NKH1-positive cells also express CD2, associated with the E-rosette receptor, and are identical to the CD3-negative/CD2-positive cells reported previously in early pregnancy decidua. The NKH1-positive cells apparently correspond to a minor subset of peripheral blood LGL. The remaining decidual LGL are positive for CD16 and negative or only dimly positive for NKH1, and are similar to the major type of peripheral blood LGL. After purification by flow cytometry, the NKH1-positive cells were demonstrated to be of similar size to, but slightly higher granularity than, lymphocytes, whereas the CD16-positive cells were larger and more granular. The possible role of decidual LGL in modulating placental development is discussed.

INTRODUCTION

Immunohistological studies on human decidua in the first trimester of pregnancy have identified numerous cells of bone marrow origin, including macrophages, classical T cells and an unusual lymphocyte population (Bulmer & Johnson, 1984; Bulmer & Sunderland, 1984), subsequently identified as expressing antigens characteristic of large granular lymphocytes (LGL) (Ritson & Bulmer, 1987).

The function of these bone marrow-derived decidual cells and their role in early pregnancy are unknown, though they may regulate the maternal immune response to the fetal allograft. Unfractionated preparations of human first trimester decidual cells have been reported to have immunosuppressive activity *in vitro* (Daya *et al.*, 1985a,b; Nakayama *et al.*, 1985); and in mouse decidua a similar activity has been associated with a population of lymphocytes lacking both T- and B-cell markers (Clark *et al.*, 1984). Mouse decidua also contains cells with natural killer (NK) activity (Croy *et al.*, 1985).

Investigation of the functional activity of the various cell populations in human early decidua requires their isolation and purification. In this paper we describe the use of flow cytometry to define and quantify the cell populations, and to isolate decidual LGL to allow *in vitro* studies of their immune function.

Correspondence: Dr P. M. Starkey, Harris-Birthright Centre for Perinatal Medicine, Nuffield Dept. of Obstetrics & Gynaecology, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K.

MATERIALS AND METHODS

Chemicals were obtained as follows: Dutch modification of RPMI-1640 (DRPMI) from Flow Laboratories, Rickmansworth, Herts; protease (type XIV), hyaluronidase (type 1-S) and collagenase (type IV) from Sigma Chemical Co. Ltd, Poole, Dorset; Percoll from Pharmacia, Milton Keynes, Bucks; fetal calf serum (FCS) from Gibco-Europe, Uxbridge, Middlesex; normal human serum, a pool of male AB sera shown to be free from bacterial contamination, from the Blood Transfusion Service; all sera were heat-inactivated; fluorescein (FITC)-conjugated $F(ab')_2$ fragments of rabbit (anti-mouse IgG) were prepared in our laboratory; details of all other antibodies are given in Table 1.

Tissue samples were from first trimester therapeutic abortions. Gestational age was calculated from the date of the last menstrual cycle.

Preparation of cell suspensions

All stages were carried out under sterile conditions, with DRPMI containing 1 mM L-glutamine, 50 μg/ml gentamycin, 100 μg/ml streptamycin and 100 U/ml benzylpenicillin. Deci-

Table 1. Details of monoclonal antibodies used in this study

Antibody	Dilution	Specificity	Reference	Obtained from
W6/32	1/100	HLA class I monomorphic	Barnstable *et al.* (1978)	Gift of Professor W.F. Bodmer, ICRF, London
T3, T3*	1/40	CD3, T-cell receptor-associated	—	Coulter Electronics Ltd, Luton, Beds
Leu 1†	1/15	CD5	—	Becton-Dickinson, Oxford
T11*	1/60	CD2, E-rosette receptor-associated	—	Coulter Electronics Ltd
NKH1, NKH1*	1/50	200,000 MW protein on most LGL	Griffin *et al.* (1983)	Coulter Electronics Ltd
Leu 11c*	1/3	CD16, Fc receptor of NK cells and neutrophils	Perussia *et al.* (1983)	Becton-Dickinson
OKT10	1/5	CD38, B cells, LGL, activated T cells and macrophages	—	Ortho Diagnostic Systems, High Wycombe, Bucks
CR3/43	1/8	HLA-DR, -DP, -DQ	Sunderland *et al.* (1981)	Gift of Dr D.Y. Mason, John Radcliffe Hospital, Oxford
F10/89/4	1/100	CD45, leucocyte common antigen	Dalchau, Kirkley & Fabre (1980)	Gift of Professor J.W. Fabre, Blond McIndoe Centre, Queen Victoria Hospital, East Grinstead, Sussex

* † Indicates antibodies that were obtained directly conjugated with phycoerythrin or FITC, respectively.

dual tissue samples, identified morphologically, were picked clean of blood clots and chorionic villous tissue, teased apart, and incubated for 15 min at 37° in DRPMI (3 ml per g of tissue) containing protease (0·2 mg per ml). After repeated washing in phosphate-buffered saline containing 0·5% bovine serum albumin (PBS/BSA), the tissue fragments were resuspended in the same volume of DRPMI as above, containing 10% (v/v) normal human serum (NHS), 2 mg/ml of hyaluronidase and 1·5 mg/ml of collagenase, and digested at 37° for up to 2 hr with occasional agitation. The cell digest was filtered through a 100-gauge nylon gauze, the cells washed (three times) by centrifugation with PBS/BSA, and left to recover at 4° overnight in 10 ml of DRPMI containing 10% (v/v) fetal calf serum. The cells were resuspended in 20 ml of Percoll, 36% in DRPMI, and layered over 18 ml of 62·5% Percoll and under 2 ml of PBS. After centrifugation at 670 ***g*** for 30 min, cells were recovered from the 36%/62·5% interface, washed in a large volume of PBS and resuspended in PBS containing 20 mM glucose and 0·5% BSA (PGB).

Antibody labelling and flow cytometry

All antibodies were diluted in PBS containing 20 mM glucose and 5% NHS (PGN), and all incubations were done, and reagents kept, on ice. For analysis, 10^6 cells were incubated with 50 μl of first antibody for 30 min, then centrifuged at 9000 ***g*** for 3 seconds, washed twice with 500 μl of PGB, incubated with 50 μl of FITC-conjugated rabbit anti-(mouse IgG) $F(ab')_2$ second antibody, and washed again. For double labelling, the cells were incubated further with normal mouse serum, 10% in PGB, to block non-specific binding to the rabbit anti-(mouse IgG), washed, incubated with phycoerythrin (PE)-conjugated monoclonal antibody, and washed.

Controls for single labelling were either FITC-conjugated second antibody alone or PE-conjugated mouse IgG1 (Becton-Dickinson, Mountain View, CA). For double labelling the control was FITC-conjugated rabbit anti-(mouse IgG) $F(ab')_2$, then normal mouse serum, followed by PE-conjugated mouse IgG1.

Antibody-labelled cells suspended in 125 μl of PGB were fixed by mixing with an equal volume of PBS containing 2% formaldehyde and 0·5% BSA. Cells were stored in the dark at 4° if necessary, and analysed by flow cytometry in a Coulter EPICS 541. The percentage of cells that were antibody positive was calculated by comparison with the appropriate control using the 'Immuno' subtraction programme.

The same procedure was used for sorting except that solutions were kept sterile and protected from fluorescent light; 4×10^7 cells were incubated with 2 ml of each antibody, washed with 15 ml of PGB and centrifuged at 300 ***g*** for 10 min. The antibody-labelled cells, unfixed, were refiltered through 40-μ gauze, resuspended at 5×10^6/ml in PGB and sorted in the flow cytometer. The sorted cells were collected into 10-ml tubes containing 2 ml of FCS (50% in DRPMI).

RESULTS

Characterization of cell populations from early decidua

Maternal decidual tissue was identified in termination samples by its morphology, using a dissecting microscope, and verified later by immunohistology. Samples were a mixture of decidua basalis and decidua vera. Tissue yields varied from 2 to 12 g, from which up to 5 g were subjected to enzymic digestion. In initial experiments, the total cell dispersion without centrifugation over Percoll was labelled with W6/32 and F10/89/4, an antibody to the leucocyte common antigen (CD45), followed by analysis by flow cytometry; forward angle light scatter (FALS), 90°light scatter (90°LS), green and red fluorescence were measured. W6/32, an antibody to the non-polymorphic part of the major histocompatibility complex (MHC) class I antigen, was used to estimate the total number of cells in the digest, and to set gates on FALS and 90°LS to exclude most non-cellular debris. MHC class I, as detected by W6/32, is expressed on virtually all maternal cells, and on most fetal cytotrophoblast. The results with F10/89/4, and subsequently with all other antibodies, were expressed as a percentage of W6/32-positive cells.

In cell digests from 14 tissue samples, taken from pregnancies of 8–12 weeks gestational age, 75% (range 37–99%) of the W6/32-positive cells were also positive for the leucocyte common antigen, and were therefore derived from bone marrow.

The autofluorescence of these cell preparations was very high and made analysis difficult, particularly with double-

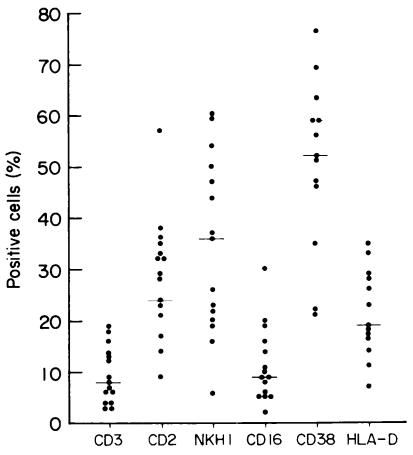

Figure 1. Antigen expression on decidual cells, as detected by single-antibody binding. Cell dispersions from early pregnancy decidua ($n=15$, gestational age 7–11 weeks) were labelled with a variety of different antibodies followed by FITC-conjugated rabbit anti-(mouse IgG) $F(ab')_2$ and then analysed by flow cytometry. For each sample the number of cells positive for a given antibody are expressed as a percentage of those cells positive with W6/32. The bars indicate median values.

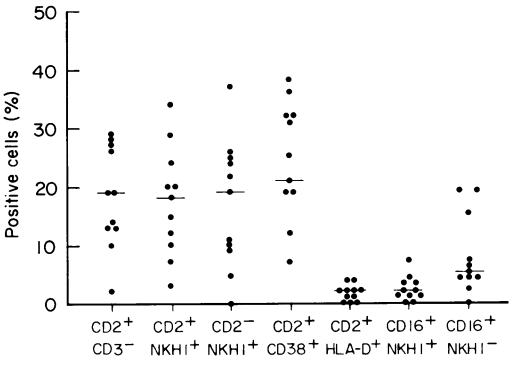

Figure 2. Antigen expression on decidual cells, as detected by double-antibody binding. Cell dispersions from early pregnancy decidua ($n=11$, gestational age 7–11 weeks) were labelled with the following combinations of antibodies; T3-T11, NKH1-T11, OKT10-T11, CR3/43-T11 and NKH1-Leu 11c. In each case the first antibody was detected with FITC-conjugated rabbit anti-(mouse IgG) $F(ab')_2$ and the second antibody was directly conjugated with phycoerythrin. The number of cells in each subset is expressed as a percentage of those cells positive with W6/32. The bars indicate median values.

labelled cell preparations. To remove more of the dead cells and debris which were highly autofluorescent, the procedure was modified to include centrifugation of the cell dispersion through a step-wise gradient of PBS/36% Percoll/62·5% Percoll. The majority of the viable cells were recovered from the interface between the two Percoll phases, with debris and non-viable cells at the medium/36% Percoll interface, and red cells sedimenting to below the 62·5% Percoll. In all subsequent experiments, antibody-labelling was done with cell populations recovered after Percoll fractionation.

Single antibody labelling

The results of the analysis of 15 first trimester decidua samples are summarized in Fig 1. There was great variability between individual samples, which showed no discernible trend with gestational age. Nevertheless, certain patterns were evident. In every sample the percentage of cells positive for CD2, associated with the receptor for sheep erythrocytes, was significantly greater than the percentage of cells positive for CD3, associated with the T-cell receptor. Classical T cells are positive for both CD3 and CD2, but a cell population has been detected previously by immunohistology of early human pregnancy decidua that is negative for most classical T-cell markers, including CD3, but positive for CD2 (Bulmer & Sunderland, 1984).

A large proportion of the decidual cells were positive for NKH1, an antibody that reacts with the LGL of peripheral blood including NK cells (Griffin *et al.*, 1983). Most NKH1-positive cells in peripheral blood are also positive for CD16, the Fc receptor of NK cells and neutrophils (Lanier *et al.*, 1986), but the number of decidual cells positive for NKH1 exceeded the number of CD16-positive cells by up to 10-fold. Leu 7, an antibody that binds to cytotoxic T cells and some NK subsets (Lanier *et al.*, 1983), did not bind to decidual cells.

Of the other two antibodies used, one to CD38, a marker of B cells, LGL and activated macrophages and T cells, was present on 21–76% of the cells, and CR3/43, which recognizes MHC class II HLA-DR, -DP, -DQ, labelled 7–35% of the cells.

Double-antibody labelling

In order to characterize the cell populations more closely, cells from 11 of the same decidua were double labelled and analysed by flow cytometry. The results are summarized in Fig. 2.

Double labelling provided clear evidence of a population of cells positive for CD2 but negative for CD3; this population accounted for between 2% and 29% of the cells isolated from early decidua. These cells were also negative for another pan-T-cell marker, CD5.

Of the cells positive for NKH1, at least two types were present, one CD2-positive the other CD2-negative. The $CD2^+/NKH1^+$ cells, as a percentage of the total NKH1-positive population, varied from 14% to 100%, with a median value of 45%. Double-labelling with NKH1 and PE-conjugated antibody to CD3, or CR3/43 and PE-conjugated NKH1, indicated that all the NKH1-positive cells were CD3-negative and MHC class II-negative. In each individual tissue sample, the number of $CD2^+/NKH1^+$ cells was very similar to that of $CD2^+/CD3^-$ cells, the correlation coefficient being 0·757, with a probability of 0·007.

Virtually all the CD2-positive cells were also CD38-positive. This presumably included both the small numbers of $CD2^+/CD3^+$ T cells and the $CD2^+/CD3^-$ population, though there was also a substantial population of $CD2^-/CD38^+$ cells. Very few of the CD2-positive cells were also positive for MHC class II.

NKH1 and CD16, the two markers of peripheral blood LGL, appeared to define essentially separate decidual cell populations. Most CD16-positive cells were NKH1-negative, though small numbers were dimly positive for NKH1. Statistical analysis showed no correlation between the percentage cells positive in each tissue for NKH1 and those positive for CD16.

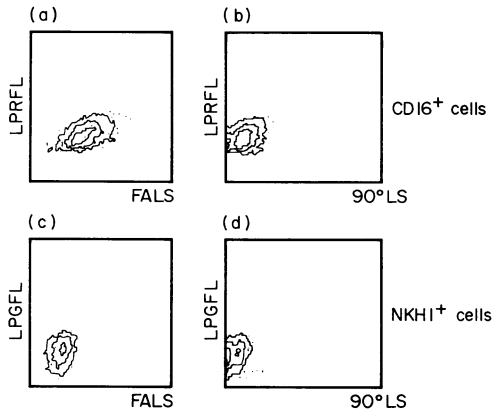

Figure 3. Characteristics of pure populations of decidual cells obtained by flow cytometry. Decidual cell preparations were labelled either with NKH1 followed by FITC-conjugated second antibody or with PE-conjugated antibody to CD16. Antibody-positive cells were selected by flow cytometry, and the pure cell populations re-analysed. Cell populations were either CD16-positive (a,b) or NKH1-positive (c,d) and were analysed for fluorescence intensity (log peak fluorescence) and forward angle light scatter (FALS), a measure of cell size (a,c) or fluorescence intensity and 90° light scatter (90°LS) a measure of cell granularity (b,d).

Sorting of pure cell populations

Decidual cells were either labelled with NKH1 followed by FITC-conjugated second antibody or with PE-conjugated antibody to CD16. The positive cells were selected on the basis of their fluorescence intensity compared with an appropriate control, and positive and negative populations were re-analysed after sorting to determine their purity.

Typically, preparations of NKH1-positive cells, 93–96% pure, were obtained from samples originally containing 30–61% NKH1-positive cells. The NKH1-negative preparations contained fewer than 5% NKH1-positive cells. A CD16-positive cell preparation, 94% pure, was obtained by sorting from a sample containing only 8% of cells positive for CD16. This sample was from a pregnancy of 8·5 weeks, and the fluorescence versus light scatter profiles of the NKH1-positive and CD16-positive preparations were compared (Fig. 3). The CD16-positive cells were larger and more granular than the NKH1-positive cells and more heterogeneous with respect to size. Giemsa staining of cytospins of the pure cell preparations demonstrated that both populations had the morphology of large lymphocytes.

DISCUSSION

In assessing the significance of our results, it is necessary to consider the limitations of the methodology. Firstly, the samples obtained were not necessarily representative of the entire decidua of the pregnancies studied. Secondly, the process of enzymic digestion used to prepare the cell dispersions could have caused the selective loss of some cell types, either by failing to release them from the tissue matrix, or by causing cell death, as has been indicated by Clark *et al.* (1986) for mouse decidua. The enzyme conditions used in our study however, consistently gave good yields of viable cells. Thirdly, we standardized our cell numbers with reference to cells expressing MHC class I antigens. Most glandular epithelial cells in pregnancy decidua

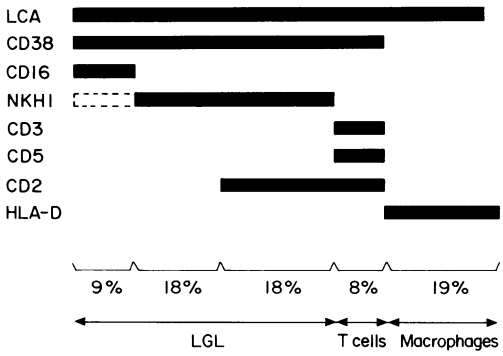

Figure 4. Summary of cell populations in first trimester human decidua. Data from Figs 1 and 2 are depicted schematically, with the length of the bars representing the percentage of cells positive for particular antigens. The percentage of decidual cells within each suggested subset is indicated at the bottom.

are negative for MHC class I (Johnson & Bulmer, 1984), and would have been excluded from our analyses. For these reasons, our results must be considered as semi-quantitative only, though comparison with immunohistology of tissue sections demonstrates that they give a reasonably accurate reflection of the *in vivo* situation, including the quite large variation between individuals.

We have defined, by single- and double-antibody labelling, several populations of bone marrow-derived cells in first trimester human decidua. Although the percentage of each cell type varies between individuals, an overall pattern is evident, and this is depicted schematically in Fig. 4. A small proportion, about 8%, appear to be classical T cells expressing CD2, CD3 and CD5 epitopes. Nearly a fifth, 19%, are positive for MHC class II antigens, of which few if any are also positive for CD2. On the basis of immunohistological observations (Bulmer & Johnson, 1985) it is probable that these are mostly macrophages, together with a small number of B cells.

The most abundant cell type, however, constituting 45% of the decidual cells positive for class I MHC, expressed surface antigens characteristic of large granular lymphocytes. At least three subsets could be distinguished. Most decidual LGL were strongly positive for NKH1 but negative for CD16, CD3 and MHC class II, and could be divided into two subsets, one CD2-positive the other CD2-negative. Only the $NKH1^+/CD2^+$ subset was shown directly to be CD38-positive but our immunohistological observations (P. M. Starkey, I. L. Sargent and C. W. G. Redman, unpublished observations) are consistent with the conclusion that all the NKH1-positive cells are CD38-positive. The pure NKH1-positive population isolated by flow cytometry was homogeneous, with an apparent size similar to that of lymphocytes, though with higher granularity.

Double-immunohistochemical labelling of decidual tissue sections with antibodies to NKH1 and CD2 confirmed that only some of the NKH1-positive cells are also CD2-positive (P. M. Starkey, I. L. Sargent and C. W. G. Redman, unpublished observations). The $NKH1^+/CD2^-$ subset is not therefore an artefact of cell dispersion.

A minor population of decidual LGL, accounting for on average 9% of class I MHC-positive decidual cells, was $CD16^+$ and negative or only dimly positive for NKH1. These were purified and found to be larger and more granular than the decidual NKH1-positive cells, with the size and granularity

expected of classical LGL. Most peripheral blood LGL are CD16-positive and weakly positive with NKH1 (Lanier *et al.*, 1986). The decidual CD16-positive cells seem to correspond to the $CD16^+/Leu7^-$ subset of peripheral blood LGL, which have the highest cytotoxic activity (Lanier *et al.*, 1983). We were unable to detect any decidual cells positive for Leu 7, and only very small numbers have been shown by immunohistology (Bulmer & Sunderland, 1984). By analogy with peripheral blood LGL the decidual CD16-positive cells would be expected to be CD38-positive and CD3-negative.

Peripheral blood LGL also include a minor subset that expresses high levels of the NKH1 antigen, is negative for both CD16 and CD3, and largely positive for CD2. This subset is a mixture of large granular and large agranular lymphocytes, and has only weak NK activity (Lanier *et al.*, 1986). The $NKH1^+/CD16^-$ decidual cells correspond most closely to this minor peripheral blood population, though only some are CD2-positive.

LGL are sometimes described as 'null cells', and many null cells of bone marrow origin, lacking B- and T-cell antigens, are found in mouse decidua in allogeneic pregnancies (Kearns & Lala, 1985). These cells appear to reach the decidua via the lymph nodes draining the uterus (Chatterji-Hasrouni, Santer & Lala, 1980). Functional studies have shown mouse decidual cells to have NK activity (Gambel *et al.*, 1985; Croy *et al.*, 1985) and the ability to suppress the *in vitro* generation of cytotoxic T cells in the mixed lymphocyte reaction (Clark *et al.*, 1983). The suppressor cells are small granular lymphocytes, distinct from the larger NK cells, though both are included in the null cell population (Slapsys, Richards & Clark, 1986). The absence of suppressor cells in the decidua and uterine lymph node has been implicated in pregnancy failure in the mouse (Clark *et al.*, 1983). Immunosuppressive activity has also been detected *in vitro* with unfractionated preparations of human first trimester decidual cells (Daya *et al.*, 1985a,b; Nakayama *et al.*, 1985).

Peripheral blood LGL have cytotoxic activity against a variety of tumour cell lines and virally infected cells (Herberman & Ortaldo, 1981). They are implicated in graft rejection (Gregory & Atkinson, 1984) and have immunosuppressive activity both *in vivo* and *in vitro* (Abruzzo & Rowley, 1983; Arai *et al.*, 1983).

The decidual LGL characterized in this paper therefore might be expected to modulate placental development in several ways. Cytotoxic activity, if directed against placental cells might limit the invasion of decidua by trophoblast that is essential for proper placentation. Alternatively, immunosuppressive activity could modulate the maternal immune response in the decidua and promote placental development. Finally, it has been suggested that the bone marrow-derived cells of the decidua, through secretion of various cytokines such as interleukin-2 and interferon-gamma, which are secreted by peripheral blood LGL (Herberman & Ortaldo, 1981), may stimulate directly placental growth (Athanassakis *et al.*, 1987). Direct evidence of a role for NK cells in pregnancy failure has been obtained from studies in mouse (Fougerolles & Baines, 1987).

Functional studies of pure populations of decidual LGL are in progress to elucidate their role *in vivo*.

ACKNOWLEDGMENTS

This work was funded by Birthright. We thank Ms Jan Hector and Mrs Gillian Watt for their excellent technical assistance, Mr Mike Jackson for his expert operation of the flow cytometer and our consultant colleagues for their permission to study their patients.

REFERENCES

Abruzzo L.V. & Rowley D.A. (1983) Homeostasis of the antibody response: immunoregulation by NK cells. *Science*, **222**, 581.

Arai S., Yamamoto H., Itoh K. & Kumagai K. (1983) Suppressive effect of human natural killer cells on pokeweed mitogen-induced B cell differentiation. *J. Immunol.* **131**, 651.

Athanassakis I., Bleackley R.C., Paetkau V., Guilbert L., Barr P.J. & Wegmann T.G. (1987) The immunostimulatory effect of T cells and T cell lymphokines on murine fetally derived placental cells. *J. Immunol.* **138**, 37.

Barnstable C.J., Bodmer W.F., Brown G., Galfre G., Milstein C., Williams A.F. & Ziegler A. (1978) Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. *Cell*, **14**, 9.

Bulmer J.N. & Johnson P.M. (1984) Macrophage populations in the human placenta and amniochorion. *Clin. exp. Immunol.* **57**, 393.

Bulmer J.N. & Johnson P.M. (1985) Immunohistological characterisation of the decidual leucocytic infiltrate related to endometrial gland epithelium in early human pregnancy. *Immunology*, **55**, 35.

Bulmer J.N. & Sunderland C.A. (1984) Immunohistological characterization of lymphoid cell populations in the early human placental bed. *Immunology*, **52**, 349.

Chatterjee-Hasrouni S., Santer V. & Lala P.K. (1980) Characterisation of maternal small lymphocyte subsets during allogeneic pregnancy in the mouse. *Cell. Immunol.* **50**, 290.

Clark D.A., Brierley J., Slapsys R., Daya S., Damji N., Chaput A. & Rosenthal K. (1986) Trophoblast-dependent and trophoblast-independent suppressor cells of material origin in murine and human decidua. In: Proceedings of the 3rd International Congress of Reproductive Immunology (eds D.A. Clark and B.A. Croy), pp. 219–226. Elsevier, Amsterdam.

Clark D.A., Slapsys R., Croy B.A., Krcek J. & Rossant J. (1984) Local active suppression by suppressor cells in the decidua: a review. *Am. J. Reprod. Immunol.* **5**, 78.

Clark D.A., Slapsys R.M., Croy B.A. & Rossant J. (1983) Suppressor cell activity in uterine decidua correlates with success or failure of murine pregnancies. *J. Immunol.* **131**, 540.

Croy B.A., Gambel P., Rossant J. & Wegmann T.G. (1985) Characterization of murine decidual Natural Killer (NK) cells and their relevance to the success of pregnancy. *Cell Immunol.* **93**, 315.

Dalchau R., Kirkley J. & Fabre J.W. (1980) Monoclonal antibody to a human leukocyte-specific membrane glycoprotein probably homologous to the leukocyte-common (L-C) antigen of the rat. *Eur. J. Immunol.* **10**, 737.

Daya S., Clark D.A., Devlin C., Jarrell J. & Chaput A. (1985a) Preliminary characterisation of two types of suppressor cells in the human uterus. *Fertil. Steril.* **44**, 778.

Daya S., Clark D.A., Devlin C., Jarrell J. & Chaput A. (1985b) Suppressor cells in human decidua. *Am. J. Obstet. Gynecol.* **151**, 267.

Fougerolles A.R. de & Baines M.G. (1987) Modulation of the natural killer cell activity in pregnant mice alters the spontaneous abortion rate. *J. Reprod. Immunol.* **11**, 147.

Gambel P., Croy B.A., Moore W.D., Hunziker R.D., Wegmann T.G. & Rossant J. (1985) Characterization of immune effector cells present in early murine decidua. *Cell. Immunol.* **93**, 303.

Gregory C.D. & Atkinson M.E. (1984) Large agranular lymphocytes: early non-specific effector cells in allograft rejection in the mouse. *Immunology*, **53**, 257.

Griffin J.D., Hercend T., Beveridge R. & Schlossman S.F. (1983) Characterization of an antigen expressed by human natural killer cells. *J. Immunol.* **130**, 2947.

HERBERMAN R.B. & ORTALDO J.R. (1981) Natural killer cells: their role in defenses against disease. *Science*, **214**, 24.

JOHNSON P.M. & BULMER J.N. (1984) Uterine gland epithelium in human pregnancy often lacks detectable maternal MHC antigens but does express fetal trophoblast antigen. *J. Immunol.* **132**, 1608.

KEARNS M. & LALA P.K. (1985) Characterization of hematogenous cellular constituents of the murine decidua: a surface marker study. *J. Reprod. Immunol.* **8**, 213.

LANIER L.L., LE A.M., CIVIN C.I., LOKEN M.R. & PHILLIPS J.H. (1986) The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J. Immunol.* **136**, 4480.

LANIER L.L., LE A.M., PHILLIPS J.H., WARNER N.L. & BABCOCK G.F. (1983) Subpopulations of human natural killer cells defined by expression of the Leu 7 (HNK-1) and Leu 11 (NK-15) antigens. *J. Immunol.* **131**, 1789.

NAKAYAMA E., ASANO S., KODO H. & MIWA S. (1985) Suppression of mixed lymphocyte reaction by cells of human first trimester pregnancy endometrium. *J. Reprod. Immunol.* **8**, 25.

PERUSSIA B., STARR S., ABRAHAM S., FANNING V. & TRINCHIERI G. (1983) Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J. Immunol.* **130**, 2133.

RITSON A. & BULMER J.N. (1987) Endometrial granulocytes in human decidua react with a natural-killer (NK) cell marker, NKH1. *Immunology*, **62**, 329.

SLAPSYS R.M., RICHARDS C.D. & CLARK D.A. (1986) Active suppression of host-versus-graft reaction in pregnant mice. VIII. the uterine decidua-associated suppressor cell is distinct from decidual NK cells. *Cell. Immunol.* **99**, 140.

SUNDERLAND C.A., NAIEM M., MASON D.Y., REDMAN C.W.G. & STIRRAT G.M. (1981) The expression of major histocompatibility antigens by human chorionic villi. *J. Reprod. Immunol.* **3**, 323.