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Cell populations in the human early pregnancy decidua: natural killer activity and response to interleukin-2 of CD56-positive large granular lymphocytes

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SUMMARY

Large granular lymphocytes (LGL) have been shown previously to be the most abundant cell type in the first trimester human decidua. Purified populations of decidual LGL were prepared by flow cytometry of cell dispersions labelled with NKH1 (CD56), an antibody specific for peripheral blood LGL, and the functional properties of CD56-positive cells, CD56-negative and unsorted decidual cells compared. Both CD56-positive cells and unsorted decidual cells have cytotoxic activity against the natural killer (NK) cell target K562 which was weak compared with that of peripheral blood mononuclear cells (PBMC). The CD56-negative cells had no cytotoxic activity against K562. All three decidual cell populations proliferated in response to recombinant human interleukin-2 (rIL-2), but none produced detectable levels of IL-2 in culture. When unsorted decidual cells were cultured for 7 days in rIL-2 the proportion of CD56-positive cells increased and NK activity against K562 was augmented. The NK activity of purified CD56-positive decidual cells was also augmented by culturing in rIL-2. The potential role of decidual LGL in regulating the development of the semi-allogeneic placenta is discussed.

INTRODUCTION

In human pregnancy, the decidua is the maternal tissue in closest contact with the foetal trophoblast cells of the placenta. First trimester decidua has been shown, by immunohistochemical (Bulmer & Johnson, 1985; Bulmer & Sunderland, 1984) and flow cytometric (Starkey, Sargent & Redman, 1988) analysis, to contain numerous cells of bone marrow origin, including a small number of classical T cells and many macrophages. The most abundant cell type, however, has been identified, flow cytometrically by its surface antigen expression, size and granularity, as large granula lymphocytes (LGL). These comprise 45% of all decidual cells and are mainly CD56-positive/CD16-negative/CD3-negative; with approximately 50% also expressing the CD2 antigen (Starkey *et al.*, 1988). This major decidual LGL population corresponds most closely to a minor population of peripheral blood LGL (Lanier *et al.*, 1986), and both populations have been reported to include some agranular CD56-positive cells (Lanier *et al.*, 1986; King *et al.*, 1989b).

The functions of decidual LGL are unknown, but LGL from peripheral blood have cytotoxic activity against certain tumour cell lines *in vitro* (Herberman & Ortaldo, 1981), and can regulate many immunohistological responses (Timonen *et al.*, 1981). They have been shown to have immunosuppressive activity *in vitro* (Abruzzo & Rowley, 1983; Arai *et al.*, 1983), and have been implicated in graft rejection (Nemlander, Saksela & Hayry, 1983). Peripheral blood LGL secrete various lymphokines, including interleukin-1 (IL-1), IL-2 (Scala *et al.*, 1984; Kasahara *et al.*, 1983) and interferon-gamma (IFN-γ) (Djeu *et al.*, 1982). Their activity is also modulated by lymphokines; thus both IL-2 and IFN-γ augment LGL cytotoxicity (Domzig, Stadler & Herberman, 1983; Ortaldo *et al.*, 1981) and IL-2 stimulates the proliferation of LGL *in vitro* (Timonen *et al.*, 1982).

Decidual LGL might therefore be expected to play a role in regulating placental growth and development by direct cytotoxic activity against trophoblasts or by regulating other local maternal immune responses to trophoblasts. Previous reports using unfractionated (King, Birkby & Loke, 1989a; Manaseki & Searle, 1989) or partially purified (Ritson & Bulmer, 1989) human decidual cells have demonstrated cytotoxic activity against K562 tumour targets. This paper, reports for the first

Abbreviations: BSA, bovine serum albumin; DRPMI-NHS, Dutch modified RPMI containing 10% NHS; FALS, forward angle light scatter; LGL, large granular lymphocyte; LPR, log peak red fluorescence; NHS, normal human serum; PBS, phosphate-buffered saline; PE, phycoerythrin; PGB, PBS containing 20 mM glucose and 5% NHS; PGN, PBS containing 20 mM glucose and 0·5% BSA; rIL-2, recombinant human interleukin-2.

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time the use in functional studies of populations of highly purified decidual LGL isolated by flow cytometry. This has enabled their natural killer (NK) activity and their response to rIL-2, to be characterized.

MATERIALS AND METHODS

Isolation of decidual cells

This study was approved by the Central Oxford Regional Ethics committee. Tissues were obtained from first trimester therapeutic abortions carried out for social not medical reasons. Gestational age was calculated from the first day of the last menstrual period. Samples of decidual tissue were obtained from 23 pregnant women with gestational ages from 7 to 12·5 weeks. All procedures were carried out using aseptic techniques. The medium used throughout was DRPMI—the Dutch modification of RPMI-1640 (Gibco-Europe, Uxbridge, Middlesex) supplemented with 1 mM glutamine (Gibco), 50 μg/ml gentamycin (David Bull Laboratories, Warwick, Warwickshire), 100 μg/ml streptomycin (Glaxo, Greenford, Middlesex) and 100 U/ml benzylpenicillin (Glaxo).

Single cell suspensions were obtained from decidual tissues by enzymic digestion at 37°, as described previously (Starkey *et al.*, 1988); the decidua was dissociated for 15 min in 1 mg/ml Dispase (Boehringer, Lewes, East Sussex) followed by a 1 hr digestion in 1·5 mg/ml type IV collagenase and 2 mg/ml type 1-S hyaluronidase (Sigma, Poole, Dorset). The cells were left at 4° overnight before being layered onto a 36%/62·5% Percoll (Pharmacia, Milton Keynes, Bucks) gradient to remove dead cells and the many large cells.

Monoclonal antibody labelling and flow cytometry

All procedures were carried out at 4°. Directly conjugated monoclonal antibodies (mAb) used were: phycoerythrin (PE)-conjugated NKH1 (1:30 dilution; Coulter Immunology, Luton, Beds), specific for CD56, the neural cell adhesion molecule found on most LGL (Griffin *et al.*, 1983; Lanier *et al.*, 1989); PE-conjugated T3 (1:40 dilution; Coulter), which defines the 22,000–28,000 molecular weight (MW) CD3 antigen (associated with the T-cell receptor) on T cells (Kung *et al.*, 1979); PE-conjugated Leu-11c (1:3 dilution; Becton-Dickinson, Oxford, Oxon), which defines the CD16 antigen (Fc receptor) on NK cells and neutrophils (Perussia *et al.*, 1983); and fluorescein (FITC)-conjugated T11 (1:40 dilution; Coulter), specific for the 45,000–50,000 MW CD2 antigen (E-rosette receptor) present on NK cells and T cells (Meuer *et al.*, 1984). The mAb CR3/43 (gift of Dr D. Y. Mason, John Radcliffe Hospital), which defines HLA-DR, -DP and -DQ (Dick, Steel & Dupont, 1984), was used at a dilution of 1:8 of the culture supernatant in indirect immunofluorescence, with FITC-conjugated mouse IgG $F(ab')_2$ (chromatographically purified in this laboratory) as the second antibody.

All antibodies were diluted in phosphate-buffered saline (PBS) containing 20 mM glucose and 5% normal human serum (PGN). Control antibodies, used to determine either red or green background immunofluorescence, were PE-conjugated mouse IgG1 (Coulter) or FITC-conjugated mouse IgG (Coulter) diluted 1:30 in PGN.

Samples were labelled for analysis as described previously (Starkey *et al.*, 1988). Briefly, 1×10^6 cells were incubated with antibody on ice for 30 min, centrifuged at 9000 *g* for 3 seconds, washed twice with 500 μl of PBS containing 20 mM glucose and 0·5% BSA (PGB), resuspended in 250 μl of PGB, and analysed in the cell sorter. In experiments where samples were fixed before being analysed, they were resuspended in 125 μl of PGB and fixed by mixing with an equal volume of PBS containing 2% formaldehyde and 0·5% BSA. Fixation had no effect on the antigenicity of the epitopes examined (data not shown).

For sorting, approximately 40×10^6 decidual cells, isolated as described above, were incubated with 2 ml of PE-conjugated CD56 for 30 min at 4°. The cells were then washed with 15 ml PGB, and centrifuged at 300 *g* for 10 min. The labelled cells were refiltered through 40 μm gauze, resuspended at 5×10^6/ml in PGB and sorted in an EPICS 541 Flow Cytometer (Coulter Electronics, Luton, Beds) using a 76 μm tip and the 488 nm line of the argon-ion laser.

Cell populations were analysed on two-parameter histograms of FALS (forward angle light scatter) and LPR (log peak red fluorescence). CD56-positive and CD56-negative populations were defined by bit-map gating, before being sorted at 1500 cells/second using one droplet sorting with coincidence on. The sorted cells were collected into 10-ml tubes containing 2 ml of 50% fetal calf serum (FCS; Gibco) in DRPMI. Before using the cells in functional assays, they were washed twice in DRPMI containing 5% FCS and resuspended in an appropriate volume of assay medium.

Tissue culture

All assays were in round-bottomed microtitre plates unless otherwise stated, and culture was at 37° in 5% CO_2 in air.

NK cell cytotoxic activity

Effector cells were peripheral blood mononuclear cells (PMBC), obtained by Ficoll–Isopaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation, unsorted decidual cells (labelled or unlabelled with PE-conjugated CD56), or sorted CD56-positive or CD56-negative populations. These cells were resuspended in DRPMI (Gibco) containing 10% normal human serum (DRPMI-NHS).

Target cells were the leukaemia cell line K562 (Lozzio & Lozzio, 1973), maintained in culture in DRPMI containing 10% FCS. 3×10^6 cells were labelled with 300 μCi of ^{51}Cr (sodium chromate CJS.4; Amersham, Amersham, Bucks) in 100 μl of PBS at 37° for 60 min and then washed three times in DRPMI-NHS before being resuspended in the same medium at a concentration of 1×10^5 cells per/ml. ^{51}Cr-labelled target cells (5×10^3 in 50 μl DRPMI-NHS) were mixed with different concentrations of effector cells in a final volume of 200 μl in microtitre plates (Nunc, Roskilde, Denmark), giving effector: target ratios (E:T ratios) ranging from 50:1 to 3:1. The test combinations were set up in quadruplicate and control wells, containing target cells in medium alone, were included in each experiment to determine the spontaneous release. Maximum release was determined by incubating target cells with 200 μl of a 5% solution of Triton X-100 in PBS in the microtitre wells.

After other incubation, the cell-free supernatants were collected using a supernatant harvester (Skatron AS, Lier, Norway) and counted in a gamma counter (LKB, Milton Keynes, Bucks). The specific ^{51}Cr release (percentage cytotoxicity) was calculated as follows:

$$\text{cytotoxicity} = \frac{\text{test} - \text{spontaneous release}}{\text{maximum} - \text{spontaneous release}} \times 100\%.$$

Proliferation in rIL-2

Quintuplicate 200 μl cultures (10^5 cells/well in DRPMI-NHS) of sorted CD56-positive, CD56-negative or unsorted decidual cells were set up in microtitre plates. Human rIL-2 (20 U/ml; Boehringer Mannheim, Lewes, Sussex) was added to each well at the start of the Culture. Control wells contained cells in medium alone. After 2, 4 and 6 days of culture, 0·5 μCi [^{3}H]thymidine (TRK 120, 25 Ci/mMol; Amersham) was added to each well. Eighteen hours later, the cultures were harvested, using a cell harvester (Skatron AS), onto glass fibre filters and counted by liquid scintillation. Results are expressed as the median counts per minute (c.p.m.) of the quintuplicate cultures.

Measurement of NK activity after proliferation in rIL-2

Unsorted decidual cells or sorted CD56-positive cells were incubated in 24-well plates (Gibco) at 1×10^6 per well in 1 ml DRPMI-NHS containing 20 U/ml rIL-2. A further 5 U/ml were added on Day 3 of culture. After 7 days culture, the cells were harvested from the wells and tested for NK activity against K562 target cells as above.

Measurement of IL-2 production

Quintuplicate 200 μl cultures (1×10^5 cells/well in DRPMI-NHS) of sorted CD56-positive, CD56-negative or unsorted decidual cells were incubated in microtitre plates. After 2 and 6 days of culture, 100 μl of the cell-free supernatant were removed and added to microtitre wells containing 100 μl CTLL cells (3×10^4/ml in DRPMI-NHS), a mouse IL-2-dependent cell line (Gillis *et al.*, 1978) that had been growing in the absence of IL-2 for 3 days. After 48 hr incubation, each well was pulsed with 0·5 μCi [^{3}H]thymidine ([^{3}H]TdR) and 18 hr later the plates were harvested and counted as above. The response of CTLL cells to IL-2 was standardized by incubation with a range of concentrations (0·001–20 U/ml) of rIL-2; a significant response was detected only with 2·00 U/ml of rIL-2 or above.

Depletion of T cells with magnetic beads

Magnetic beads (100 μl) (Dynabeads M-450; Dynal U.K., Wirral, Merseyside) were coated with monoclonal anti-CD3 antibody (T3; Coulter) by mixing by slow end-over-end rotation overnight at 4°, with 20 μl of the antibody in 880 μl PBS. The beads were collected by placing the suspension in a magnetic particle concentrator (Dynal) for 30 seconds; the supernatant was discarded and the beads were given four 30-min washes, by end-over-end rotation at 4°, in PBS containing 1% fetal calf serum (PBS-FCS).

After the final wash, the anti-CD3 antibody-coated beads were resuspended in PBS-FCS containing unsorted decidual cells stimulated by rIL-2, to give a bead:cell ratio of 40:1 in a final volume of 300 μl. The beads and cells were mixed by end-over-end rotation for 30 min at 4° before being washed twice in PBS-FCS. The magnetic bead-coated T cells were then separated from free beads in the magnetic particle concentrator as above. The T lymphocyte-depleted cells were washed three times in PBS-FCS, and passed through the magnetic particle concentrator for a second time to remove any remaining CD3-positive cells. After washing in PBS-FCS, the cells were resuspended in DRPMI-NHS and used in NK cytotoxicity assays as above.

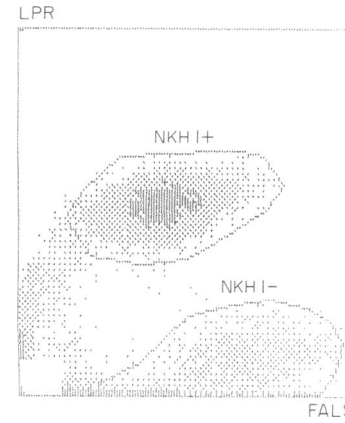

Figure 1. Cell sorting of CD56-positive LGL. Decidual cells were labelled with PE-conjugated CD56 antibody and analysed in the flow cytometer using FALS (forward angle light scatter) and LPR (log peak red fluorescence). CD56-positive and CD56-negative populations were defined by bit-map gating (dotted lines), which excluded dead and aggregated cells and debris, before being sorted using one droplet sorting.

Table 1. Decidual cells pre- and post-sorting with CD56 antibody

Sample	% CD56$^+$*	% viability†	Yield‡ (cells $\times 10^{-6}$)
Pre-sort§	47 (25–64)	81 (64–91)	45 (25–109)
Post-sort¶			
CD56$^+$ population	98 (86–99)	83 (55–89)	5 (2–7)
CD56$^-$ population	6 (2–13)	80 (50–93)	6 (2–10)

* Percentage CD56-positive cells determined by flow cytometry

† Viability assessed by phase-contrast microscopy and trypan blue exclusion.

‡ Cell yield before and after sorting with CD56 antibody.

§ Decidual cells before sorting with CD56 antibody.

¶ Cell populations obtained after sorting approximately 40×10^6 decidual cells with CD56 antibody.

All values are expressed as median values (range) from 23 decidual cell preparations.

Statistical analysis

The differences within groups were tested by the Wilcoxon test for pair differences, and between groups by the Wilcoxon rank sum test. Non-parametric tests were chosen because the observations were not normally distributed.

RESULTS

Isolation of decidual LGL

A typical two-parameter flow cytometric profile of decidual cells labelled with PE-CD56 is shown in Fig. 1. Forward angle light scatter (FALS), a measure of cell size, is plotted against log peak red fluorescence (LPR). Cell populations, positive or negative for CD56, were defined by bit-map gating, as shown in Fig. 1, to exclude dead and aggregated cells, and sorted using one droplet sorting.

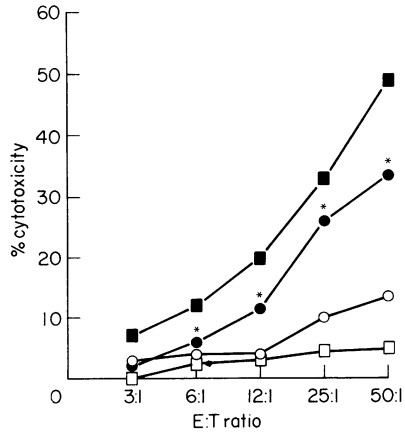

Figure 2. Cytotoxicity of CD56-positive cells against K562 targets. Unsorted (○) and sorted CD56-positive (●) and CD56-negative (□) decidual cells, together with PBMC from the same patients (■) were used as effector cells in 6-hr ^{51}Cr-release assays against K562 target cells. Values are the median percentage cytotoxicity from 12 experiments. Cytotoxic activity of CD56-positive cells was significantly greater than both the CD56-negative and unsorted populations (*$P < 0{\cdot}01$).

Details from 23 preparations are summarized in Table 1. The proportion of CD56-positive cells varied, but was unrelated to gestational age, confirming earlier findings (Starkey *et al.*, 1988). The CD56-positive cells were 98% pure, and after sorting always exceeded 2×10^6 and usually provided 5×10^6 cells in each population, enough for functional studies.

NK activity of CD56-positive LGL

NK activity was measured using CD56-positive, CD56-negative, and unsorted decidual cells. The patient's own PBMC were included as a control. Figure 2 summarizes the results from 12 experiments. Unsorted decidual cells demonstrated weak NK activity, which was the same whether cells had been labelled with PE-conjugated CD56 (data not shown) or not (Fig. 2). The CD56-positive cells showed significantly higher NK activity than unsorted decidual cells ($P < 0{\cdot}01$) and the CD56-negative population did not kill K562 targets. The NK activity of PBMC was consistently higher than that exhibited by any of the decidual populations. To check that the enzymes used to disperse the decidual cells did not affect cytotoxic activity, PBMC were exposed to the same enzymes and their cytotoxicity was found not to be significantly altered; the average cytotoxicity of two preparations at an effector to target ratio of 50:1 was 36·2% before enzyme treatment and 38·5% after.

The unsorted decidual cells would be expected to include about 20% macrophages (Starkey *et al.*, 1988), which might produce prostaglandins and thereby inhibit the NK activity of the LGL (Droller, Schneider & Perlmann, 1978). However, in three experiments, there was negligible prostaglandin E_2 production by unsorted decidual cells (mean = 1·194 pmol/ml, measured by radioimmunoassay, as described by Lopez Bernal *et al.*, 1987), and preincubation for 4 hr at 37° in DRPMI-NHS containing 10^{-7} M indomethacin, an inhibitor of prostaglandin synthesis, gave no increase in the cytotoxic activity of unsorted decidual cells against K562 targets (data not shown).

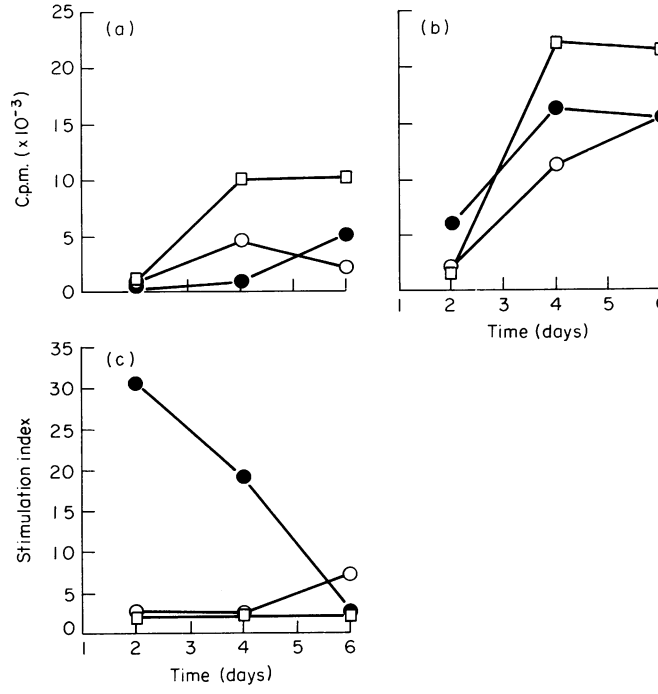

Figure 3. Proliferation of CD56-positive cells in rIL-2. Proliferation was measured after 2, 4 and 6 days incubation with and without rIL-2. (a) The response of CD56-positive (●), CD56-negative (□) and unsorted (○) decidual cells in medium alone; (b) their responses to 20 U/ml rIL-2. The stimulation index shown in (c) is a ratio of the c.p.m. in the presence of rIL-2 to the c.p.m. in the absence of rIL-2. The values are median c.p.m. from six experiments.

Proliferation of CD56-positive LGL in rIL-2

The ability of unsorted and sorted decidual cells to proliferate in medium alone and in response to rIL-2 was examined. Figure 3a shows that, without rIL-2, CD56-positive cells did not proliferate for the first 4 days in culture, but thereafter their [^{3}H]TdR incorporation increased fivefold. Unsorted cells proliferated slowly until Day 4, and less after this time. CD56-negative cells proliferated more than either CD56-positive or unsorted cells, thymidine incorporation reaching a peak at Day 4 with an 11-fold increase compared to Day 2 (Fig. 3a). When 20 U/ml of rIL-2 were added at the beginning of the culture period, the proliferation of all cell types was greater than that observed in medium alone (Fig. 3b). CD56-positive cells showed the greatest response to IL-2, peaking at Day 4 and declining slightly thereafter. The response of unsorted cells continued to increase until Day 6 of culture, whereas that of CD56-negative cells peaked at Day 4, when there was a 13-fold increase in [^{3}H]TdR uptake compared to Day 2 of culture.

A stimulation index was calculated as the ratio of [^{3}H]TdR uptake of cells cultured in rIL-2 to that of cells cultured in medium alone (Fig. 3c). CD56-positive cells had the highest index, their response peaking at Day 2 with a 30-fold increase in proliferation, and declining thereafter. The index for unsorted cells was low until Day 4, but had increased slightly by Day 6. CD56-negative cells showed a twofold increase in proliferation, which remained constant throughout the 6-day culture period.

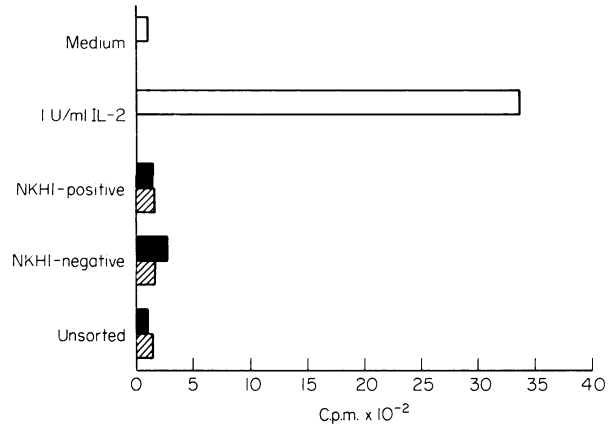

Figure 4. Production of IL-2 by CD56-positive cells. The proliferation of the IL-2-dependent CTLL cell line is shown in medium alone, 1 U/ml rIL-2 and cell-free supernatants from CD56-positive, CD56-negative and unsorted decidual cells, cultured in medium alone for 2 (black bars) and 6 (hatched bars) days. The values are the median c.p.m. from six experiments.

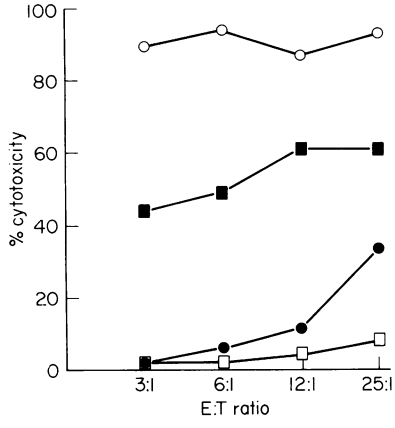

Figure 5. Cytotoxicity of decidual cells after proliferation in rIL-2. The NK activity of unsorted decidual cells before (□) and after (■) proliferation for 7 days in rIL-2 (20 U/ml rIL-2 on Day 0, followed by 5 U/ml rIL-2 on Day 3 of culture) was measured against K562 target cells in a 6-hr ^{51}Cr-release assay. The values are the median percentage cytotoxicity from seven experiments. Similarly the NK activity of CD56-positive cells before (●) and after proliferation in rIL-2 (○) was assessed in two experiments.

Production of IL-2 by CD56-positive LGL

Both the unsorted and sorted cells proliferated in medium alone (Fig. 3a), suggesting that they might themselves produce IL-2. The secretion of IL-2 into culture supernatant by the CD56-positive, CD56-negative and unsorted decidual cells was measured using the IL-2-dependent CTLL mouse cell line. No IL-2 was detected in the conditioned medium of any of the three cell populations (Fig. 4).

Cytotoxicity and phenotype of decidual cells after culture in rIL-2

Decidual cells, either unsorted or sorted CD56-positive cells, were used as effector cells against K562 target cells after 7 days culture in rIL-2. rIL-2 significantly augmented the NK activity of unsorted ($P < 0.05$) and CD56-positive cells (Fig. 5). The increase in cytotoxic activity was more striking at lower E:T ratios in both populations of cells. PBMC grown in rIL-2 also exhibited increased cytotoxic activity against K562 cells (data not shown).

Unsorted cells were analysed by antibody labelling followed by flow cytometry before and after 7 days culture in rIL-2. There were increases in the proportions of CD56-, CD3- and CD2-positive cells after 7 days (Table 2), but none was statistically significant. The enhanced cytotoxicity of unsorted decidual cells after stimulation with rIL-2 could have been due to an increase in T cells rather than LGL. In two experiments, unsorted decidual cells were proliferated in rIL-2 and then depleted of CD3-positive cells using magnetic beads coated with anti-CD3 antibody. Analysis of these CD3-depleted samples on the flow cytometer showed that the proportion of CD3-positive cells had been reduced from 8% and 10·7%, respectively, to 0%. The cytotoxicity against K562 of these CD3-depleted samples was only marginally decreased (Fig. 6) suggesting that CD56-positive LGL were responsible for virtually all of the enhanced NK activity of the decidual cells.

Table 2. Decidual cell phenotype before and after culture in rIL-2

Time of assay	Cell no. $\times 10^{-6}$ (% viability)	% antibody-positive cells				
		CD56	CD2	CD3	HLA-D	CD16
Day 0	24 (81)	36 (33–66)	30 (18–54)	2 (0–4)	15 (9–19)	0·5 (0–1)
Day 7 (+rIL-2)	15 (67)	50 (30–60)	47 (35–53)	8 (0–11)	12 (6–18)	0 (0)

The percentage of antibody-positive cells was assessed by flow cytometry before (Day 0) and after (Day 7) culture in rIL-2. Values are the median values (range) from six decidual cell preparations. Viability was assessed by trypan blue exclusion and phase-contrast microscopy.

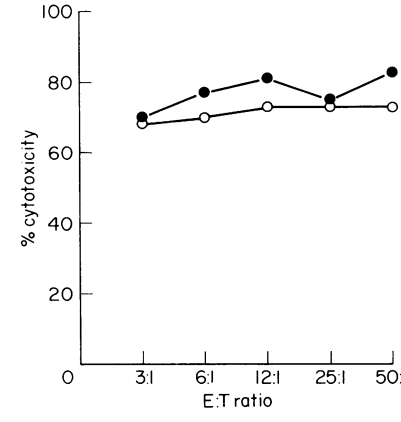

Figure 6. Cytotoxicity of IL-2 stimulated decidual cells after depletion of T cells. The NK activity of unsorted decidual cells before (○) and after (●) depletion of CD3-positive T cells was measured against K562 target cells in a 6-hr ^{51}Cr-release assay. The values are the mean percentage cytotoxicity from two experiments.

DISCUSSION

There are numerous bone marrow-derived cells in the early pregnancy decidua of both mouse (Kearns & Lala, 1985) and human (Bulmer & Johnson, 1985; Bulmer & Sunderland, 1984; Starkey *et al.*, 1988), which include tissue macrophages and LGL, with T cells as a minor component. The largest population during the first trimester of pregnancy is CD56-positive/CD3-negative LGL (Starkey *et al.*, 1988). The cell populations isolated by enzymic digestion and flow cytometry correspond closely to those identified by immunohistology.

While it is important to examine whole decidual populations functionally, it is essential to isolate and characterize antigenically defined subpopulations in order to understand the precise interactions between different cell types. It is shown here that this is possible. Viable populations of CD56-positive and CD56-negative cells can be purified by flow cytometry in large enough yields for their functions to be examined *in vitro*.

Unfractionated or partially purified preparations of cells from mouse (Croy *et al.*, 1985) and human (King *et al.*, 1989a; Manaseki & Searle, 1989; Ritson & Bulmer, 1989) decidua have been reported to have NK activity. The latter observation has been confirmed here and it is shown that the activity is confined to the subset of CD56-positive LGL. Decidua has previously been shown to contain a minor but variable population of CD16-positive cells, negative or only weakly positive for CD56 (Starkey *et al.*, 1988), that would, under the conditions of flow cytometric sorting used in this paper, have been included in the CD56-negative population which appeared to have no NK activity.

Decidual NK activity was therefore found to be confined to the CD56-positive population, which is also CD3- and CD16-negative. This corresponds to a minor subset of PBMC with only weak NK activity (Lanier *et al.*, 1983). In peripheral blood, most CD56-positive cells are also CD16-positive and have high NK activity (Lanier *et al.*, 1983), which could explain the lower cytotoxic activity of purified decidual CD56-positive cells compared with unpurified PBMC. The CD56-positive cells are heterogeneous (CD2-positive of CD2-negative) and the possibility that the subpopulations have differing NK activity is under investigation.

Clearly, the decidual LGL must be derived from peripheral blood, but whether they can proliferate or differentiate *in situ* in the decidua is unclear. There is evidence of *in situ* proliferation of CD56-positive LGL in non-pregnant endometrium, though none was detected in early pregnancy decidua (Pace, Morrison & Bulmer, 1989). CD56-positive decidual cells proliferate *in vitro* in response to rIL-2, but after 4 days the proliferation declines, suggesting that there is no endogenous production of IL-2. This was confirmed by the failure to detect IL-2 production by isolated CD56-positive cells.

Whether in certain circumstances, such as intrauterine infection, CD56-positive cells can respond to IL-2 released by decidual T cells in unknown. Stimulation of NK activity in this way may be part of the natural protective immunity of the decidua. The relatively small increase in proliferation demonstrated by the CD56-negative cells in the presence of IL-2 probably reflects the fact that T cells would be only a minor component of these cells (Starkey *et al.*, 1988).

In contrast to the CD56-positive population, unsorted decidual cells only proliferate in response to IL-2 after 4 days in culture. The reasons for this are unclear; it may result from the reduced numbers of CD56-positive cells within the unsorted population or may indicate that other lymphokines, such as IL-4, are being released which suppress IL-2-induced proliferation. IL-4 has been shown to suppress IL-2-induced LAK activity (Nagler, Lanier & Phillips, 1988).

The NK activity of unsorted decidual cells that have been grown in rIL-2 for 7 days is greatly augmented. This confirms the results of Manaseki & Searle (1989). In contrast, Ritson & Bulmer (1989), using partially purified decidual LGL, were unable to demonstrate any proliferation in response to IL-2, possibly because of the low viability of their preparations ($< 30\%$ after 3 days *in vitro*). Although in the present experiments the percentage of CD3-positive cells increased after culture of unfractionated decidual cells in rIL-2, depletion experiments showed that most cytotoxicity after IL-2 incubation was associated with CD56-positive/CD3-negative cells. Similar IL-2-induced increases in NK activity were found with purified CD56-positive cells. Whether this augmented NK activity includes LAK activity directed against NK-resistant cell targets is not yet known. A more physiologically relevant target for decidual cytotoxic activity would be trophoblasts. Preliminary data suggest that human trophoblasts are not killed by decidual LGL (King *et al.*, 1989a; B. L. Ferry, I. L. Sargent, P. M. Starkey and C. W. G. Redman, manuscript in preparation).

The *in vivo* functions of decidual cells derived from the bone marrow are unknown. Whether or not they are relevant to pregnancy success is a matter for speculation. It should be remembered that ectopic pregnancies can survive in non-decidualized tissues, and in the abdominal cavity can even progress to term (Paterson & Grant, 1975). The decidual immune cells may have a primary role in preventing intrauterine infections, but other functions have also been suggested.

In the mouse, granular lymphocytes which suppress T-cell functions have been identified in the uterus and have been invoked to explain the survival of the placental graft (Slapsys & Clark, 1982), although this is disputed (Croy *et al.*, 1985). Unfractionated preparations of human decidua have been reported to have similar immunosuppressive activity *in vitro* (Daya *et al.*, 1985a, b; Nakayama *et al.*, 1985), so it is possible that decidual LGL are the source of this activity. Another concept is that of immunotrophism (Athanassakis *et al.*, 1987) in which it is postulated that interaction between trophoblast and immune cells causes the synthesis and secretion of growth factors which enhance proliferation.

In the light of these hypotheses it will be of great interest to purify other subsets of human decidual LGL (CD16-positive/CD56-negative, CD56-negative/CD2-negative, CD56-positive/CD2-negative), and to examine their functions, particularly with respect to cytokine production and to trophoblast cytotoxicity. It may be that the more minor decidual LGL populations, for example, CD16-positive/CD56-negative, which do not have high NK activity *in vitro*, fulfil some of these suggested immunoregulatory functions.

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