Clin. exp. Immunol. (1985) **62,** 121–127.

Natural killer cells in normal pregnancy: analysis using monoclonal antibodies and single-cell cytotoxicity assays

C. D. GREGORY,* HEATHER LEE,* G. B. REES,† I. V. SCOTT,† L. P. SHAH* & P. R. GOLDING* **Oncology Research Laboratory, Derby City Hospital, Derby, and †Department of Obstetrics and Gynaecology, Derby City Hospital/Derbyshire Women's Hospital, Derby, UK*

(Accepted for publication 18 April 1985)

SUMMARY

Peripheral blood lymphocytes (nylon wool non-adherent) from healthy pregnant women and normal non-pregnant females were tested for natural killer (NK) cell-mediated cytotoxicity against K562 target cells both by ^{51}Cr-release assay and single-cell cytotoxicity assay in agarose. The results indicated depression of NK cytotoxicity in pregnancy due to a decrease in the proportion of target-binding lymphocytes as well as a reduction in the lytic capacity of target-bound cells. The ability of active pregnancy-associated NK lymphocytes to recycle appeared to be unimpaired. Analysis of lymphocyte populations with monoclonal antibodies recognizing NK cell-associated antigens showed that the number of Leu-11$^+$ lymphocytes was reduced in pregnancy. Enumeration of Leu-7$^+$ cells and correlation of NK cell subpopulation data with cytotoxicity assay data suggest that pregnancy is associated with a reduction in the number of mature NK cells and probably also an inhibition of post-binding lytic activity.

Keywords natural killer cells pregnancy monoclonal antibodies single cell cytotoxicity assay

INTRODUCTION

The growth of the fetal allograft constitutes an immunological paradox and many mechanisms have been proposed to account for exemption of the fetus from rejection by the maternal immune system (see Billingham, 1981 for discussion). Natural killer (NK) cells may provide an important *in vivo* immunosurveillance mechanism against virally infected cells and tumour cells (reviewed in Herberman, 1982) and may also be involved in allograft rejection (Nemlander, Saksela & Hayry, 1983). In addition, it has been shown that murine trophoblast cells are highly susceptible to NK cell-mediated destruction *in vitro* (Tanaka & Chang, 1982). It is, therefore, conceivable that in the absence of effective regulation maternal NK cells could spontaneously attack the developing fetus *in vivo*.

Human studies have shown that maternal peripheral blood NK activity is significantly depressed in pregnancy (Baines, Pross & Millar, 1978). The present study was undertaken to determine whether decreased numbers of maternal NK cells might account for the observed depression of NK cell function.

Correspondence: Dr C. D. Gregory, Cancer Research Campaign Laboratories, Department of Cancer Studies, The Medical School, Birmingham B15 2TJ, UK.

MATERIALS AND METHODS

Blood samples and lymphocyte fractionation. Peripheral blood samples (10 ml) from 23 healthy pregnant volunteers (age range 19–41 years; 32–39 weeks gestation) attending the antenatal clinic at Derby City Hospital and 22 non-pregnant control females (age range 20–35 years) were collected into heparinized containers. All pregnancies were without complication; one twin pregnancy was included. 8/22 control females were receiving oral contraceptives. A further 2 ml of blood in EDTA were obtained from each subject for haematological analysis. Total white blood cell counts were determined automatically on a Model S Coulter counter (Coulter Electronics, Luton, England) and differential lymphocyte counts enumerated manually using stained smears. Mononuclear cells were isolated from undiluted heparinized blood by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden) at 400 ***g*** for 30 min. The mononuclear cell fraction was washed, resuspended in 20 ml pre-warmed (37°C) RPMI-1640 culture medium (GIBCO, Paisley, Scotland) containing 10% heat-inactivated fetal calf serum and antibiotics (RPMI-10) and placed in a 80 cm^2 plastic tissue culture flask (Nunc 153732, GIBCO). Following incubation at 37°C for 30 min, plastic non-adherent lymphocytes (NAL) were collected, washed and resuspended in 2 ml RPMI-10 at 37°C. Subsequently, nylon wool non-adherent lymphocytes (NNL) were fractionated according to Julius, Simpson & Herzenberg (1973) and resuspended in RPMI-10.

Cytotoxicity assays. (i) ^{51}Chromium release assay (CRA). NNL populations were tested for their ability to kill the erythroleukaemia target cell (TC) K562 (Andersson, Nilsson & Gahmberg, 1979) in 3-h cytotoxicity assays. TC labelling, and details of the cytotoxicity assay have been described elsewhere (Gregory & Atkinson, 1984). Briefly K562 TC, grown in continuous suspension culture in RPMI-10, were labelled with 100 μCi sodium ^{51}chromate (Amersham International) for 45 min at 37°C. NNL were incorporated in a final volume of 0·2 ml RPMI-10, with 10^4 labelled TC per well in U-shaped multiwell plates to give lymphocyte : TC (L : T) ratios ranging from 50 : 1 to 3 : 1. After centrifugation (50 ***g***, 5 min) the plates were incubated at 37°C for 3 h and percentage specific cytotoxicity of NNL was calculated according to Gregory & Atkinson (1984).

(ii) Single cell cytotoxicity assay (SCCA). The frequency of lymphocytes exhibiting the ability to bind and lyse K562 TC was assessed using a SCCA assay in agarose similar to that described by Bonavida, Bradley & Grimm (1983). Equal volumes (0·3 ml) of RPMI-10 containing lymphocytes (NNL) and K562 TC, each at a concentration of 2×10^6 ml were mixed in 16 × 125 mm round-bottomed polystyrene tubes (Lux 5101, Flow Laboratories, Rickmansworth, England) and centrifuged at 200 ***g*** for 5 min at 20°C. Following a further 10 min incubation at 20°C the supernatant was completely removed from the undisturbed pellet and 150 μl RPMI-10 (20°C) carefully added. The pellet was subsequently resuspended by aspirating once into the tip of the 150 μl pipette after which the tube was placed into a water bath at 37°C. Molten agarose (150 μl, 1·33% Sigma type VII (Sigma, Poole, England) at 37°C) was added to the cell suspension and, again, gently mixed by aspirating once into the pipette tip. Aliquots of the agarose mixture (50 μl) were then spread quickly onto agarose-coated slides which, after gelling (30 s at room temperature) were submerged in RPMI-10 and incubated at 37°C for 3 h in a humidified atmosphere of 5% CO_2 in air. The slides were subsequently stained for 5 min with 0·2% trypan blue in PBS, soaked for 10 min in two changes of 0·5% formaldehyde in PBS and finally stored in formaldehyde-PBS until examined. Four slides were analysed for each lymphocyte population; the following parameters were assessed: (i) Percentage conjugate-forming cells (% CFC), i.e. proportion of lymphocytes binding to K562 TC (per 200 lymphocytes). (ii) Percentage conjugate-forming cells attached to dead TC (% lytic conjugate-forming cells (LCFC) per 200 conjugates). (iii) Percentage dead, unbound TC (per 200 TC).

% LCFC included subtraction of background counts:

$$\% \text{ LCFC} = \text{Fraction of LCFC} \times (1 - \text{Fraction of background TC death}) \times 100.$$

The proportion of the total lymphocyte population capable of lysing K562 TC (% PLCFC) was calculated as:

$$\% \text{ PLCFC} = \% \text{ LCFC} \times \% \text{ CFC}.$$

SCCA results were calculated as arithmetic mean ± s.e.m. according to Rubin, Pross & Roder (1982).

Monoclonal antibodies. Immunofluorescence of lymphocyte populations was performed on cells immobilized in agarose. Lymphocytes (NAL and NNL) were suspended at a concentration of $3{\cdot}75 \times 10^7$/ml in 40 μl 0·67% agarose (Sigma type VII) in RPMI-10 at 37°C. Aliquots (10 μl) of the suspensions were spread onto agarose-coated slides which were subsequently immersed in RPMI-10 at 4°C. These preparations permitted immunostaining of cells through the agarose. Monoclonal antibodies (anti-Leu-7 and anti-Leu-11b, Becton Dickinson, Mountain View, California, USA; OKMI, Ortho Diagnostics, Rariton, New Jersey, USA) diluted 1/10 in PBS, were applied to the lymphocyte-agarose preparations at 4°C. After 15–20 min the slides were washed for 10 min in two changes of RPMI-10 at 4°C and subsequently incubated with fluorescein isothiocyanate-conjugated goat $F(ab')_2$-anti-mouse Ig (Tago, Inc, Burlingame, CA, USA) for a further 15 min at 4°C. The slides were finally washed in two changes of RPMI-10 (4°C). The proportion of fluorescing lymphoid cells was determined from a total of 200 cells for each marker. Control preparations lacking the primary antibody gave consistently negative results.

Statistical Analyses. Data from pregnancy and control groups were compared using student's *t*-test and, in some cases, correlation of results was assessed by regression analysis using the method of least squares.

RESULTS

CRA

Differences between pregnancy and control NNL NK activity in 3-h CRA are shown in Fig. 1. It can be seen that, at all L-T ratios tested, lymphocytes from control females were more efficient at killing K562 TC than those from pregnant women ($P < 0{\cdot}001$). At the highest L:T ratio analysed (50:1) lymphocytes from control females were found to exhibit 53% cytotoxicity compared to only 32% during pregnancy.

SCCA in agarose

Enumeration of individual K562-killer cells was achieved using 3-h assays of lymphocyte-TC conjugates immobilized in agarose. The results are shown in Table 1. It is apparent that both

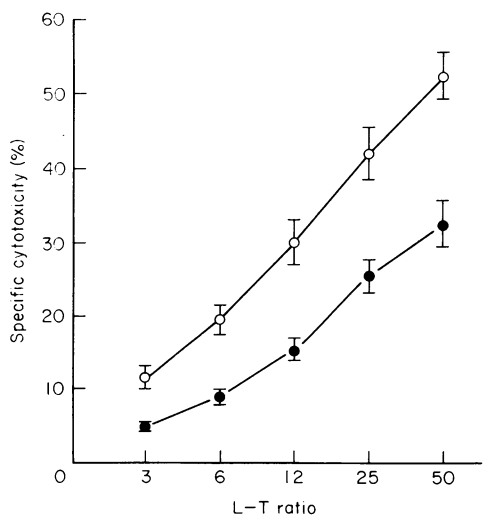

Fig. 1. Cytotoxic activity of nylon wool non-adherent lymphocytes from pregnant women (●) and non-pregnant controls (○) tested in 3 h ^{51}Cr-release assays against K562 TC. Each point represents mean ± s.e.m. All pregnancy points are significantly lower than the corresponding control ($P < 0{\cdot}001$).

Table 1. Single-cell cytotoxicity assay of lymphocytes from pregnant and non-pregnant females. Results represent mean percent ± s.e.m.

Parameter	Control	Pregnancy
CFC*	12·32 ± 0·62	9·93 ± 0·54§
LCFC†	13·53 ± 1·37	8·98 ± 0·89§
PLCFC‡	1·61 ± 0·16	0·88 ± 0·10¶

* Conjugate-forming cells (percentage per 200 lymphocytes).

† Lytic conjugate-forming cells (percentage per 200 conjugates).

‡ Lytic conjugate-forming cells (percentage of lymphocyte population).

Student's *t*-test: control versus pregnancy § $P < 0{\cdot}01$, ¶ $P < 0{\cdot}001$.

percentage conjugate-forming cells (CFC) and percent lytic CFC (LCFC) were significantly reduced in pregnancy ($P < 0{\cdot}01$). Moreover, the percentage PLCFC (i.e. the proportion of lytic lymphocytes in the total NNL population) was reduced from 1·6% in controls to 0·9% in pregnant women ($P < 0{\cdot}001$).

Comparison of CRA and SCCA data in both pregnant and control females is shown in Fig. 2. It is clear that NNL cytotoxicity as assessed by 3-h CRA correlates with % PLCFC data obtained from appropriate SCCA. In addition, it would appear that the degree of correlation is similar in both control and pregnancy groups suggesting either that the CRA is measuring only one cycle of lytic events (since, in the SCCA, active lymphocytes are permitted to kill only one TC) or, more probably, that the recycling capacity of active NK cells is related to the number of 'first-hit' NK cells

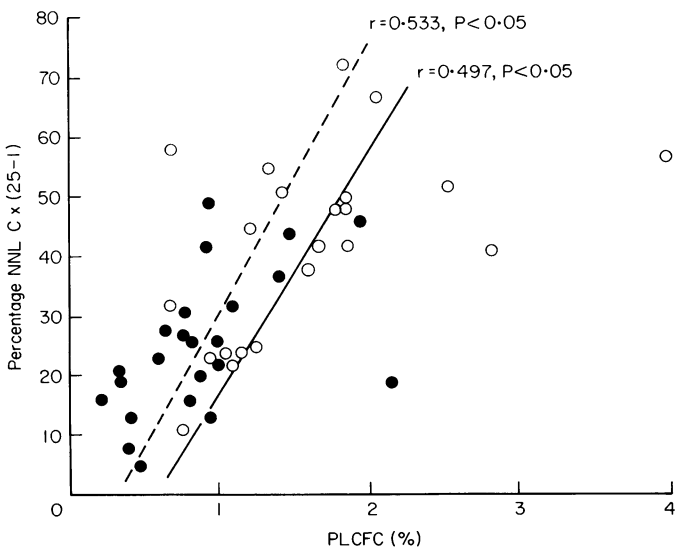

Fig. 2. Correlation between ^{51}Cr-release and single-cell cytotoxicity assays for pregnant women (●– – –●; $r = 0{\cdot}533$, $P < 0{\cdot}05$) and non-pregnant control females (○——○); $r = 0{\cdot}497$, $P < 0{\cdot}05$) ^{51}Cr-release assay data were taken as percentage specific cytotoxicity of nylon non-adherent lymphocytes (% NNL Cx) at a L:T ratio of 25:1 and single-cell assay data were percent lytic conjugate-forming cells (% PLCFC). r = regression coefficient (method of least squares).

Table 2. Monoclonal antibody labelling of lymphocytes from pregnant women and non-pregnant controls. Results represent mean ± s.e.m.

Monoclonal antibody	Specificity	Control female			Pregnancy		
		% NAL*	No./μl†	% NNL‡	% NAL	No./μl	% NNL
Anti-Leu-7	Large granular lymphocytes	11·7 ± 1·3	300 ± 30	11·9 ± 1·3	10·1 ± 1·2	270 ± 40	10·2 ± 1·2
Anti-Leu-11b	NK cells and neutrophils	15·8 ± 1·7	420 ± 50	17·5 ± 1·9	10·6 ± 1·5§	260 ± 40§	11·0 ± 1·8§
OKMI	NK cells, monocytes and granulocytes	10·1 ± 1·2	270 ± 40	12·9 ± 1·9	10·0 ± 1·7	250 ± 50	8·8 ± 1·2

* Percent positive lymphocytes in plastic non-adherent mononuclear cell fraction.

† Number of positive lymphocytes/μl peripheral blood. Value obtained by relating proportion of positive lymphocytes to absolute lymphocyte count.

‡ Percent positive nylon wool non-adherent lymphocytes.

Student's *t*-test: § control versus pregnancy $P < 0{\cdot}05$.

(estimated by % PLCFC) and, moreover, this relationship is similar in both control and pregnant females (Fig. 2).

Distribution of lymphocytes bearing NK-associated markers

Lymphocytes bearing NK-associated antigens were assessed by indirect immunofluorescence using monoclonal antibodies OKMI, anti-Leu-7 and anti-Leu-11b. The results, together with the specificities of these antibodies are shown in Table 2. Lymphocytes bearing Leu-7 (HNK-1) and OKMI antigens were found to be present in similar proportions and absolute numbers in both pregnancy and control groups. However, Leu-11$^+$ lymphocytes were found to be reduced from 16% (420 cells/μl) in controls to around 11% (260 cells/μl) in pregnant women. The proportion of Leu-11$^+$ NNL was similarly reduced in pregnancy. Lymphocytes bearing NK-associated antigens were not concentrated or diluted to any significant extent by nylon wool fractionation.

The proportions of Leu-7$^+$ and Leu-11$^+$ cells were found to correlate, in normal females, with the cytotoxic activity of the lymphocyte population—measured both by CRA and SCCA (Table 3). On the other hand, no correlation was observed between either of these markers and either method of cytotoxicity measurement in lymphocyte samples from pregnant women (Table 3). This suggests impairment of lytic activity of Leu-7$^+$ and Leu-11$^+$ cells during pregnancy.

Table 3. Correlation between NK cell subsets and cytolytic activity in lymphocyte populations (nylon non-adherent, NNL) from control and pregnant females

Correlation	Control		Pregnancy	
	r	*P*	*r*	*P*
% Specific cytotoxicity versus % Leu-7$^+$ NNL	0·493	< 0·05	0·121	NS
% Specific cytotoxicity versus % Leu-11$^+$ NNL	0·737	< 0·01	0·285	NS
% PLCFC versus % Leu-7$^+$ NNL	0·650	< 0·05	0·205	NS
% PLCFC versus % Leu-11$^+$ NNL	0·658	< 0·01	0·274	NS

r = regression coefficient (method of least squares).

Percentage specific cytotoxicity was taken from CRA at L:T ratio 25:1.

DISCUSSION

The results presented in this report confirm the original demonstration (Baines *et al.*, 1978) that pregnancy is associated with a depression of NK activity as assessed by short-term CRA using K562 TC. In addition analysis of individual NK cells using the SCCA indicates that the depression of NK activity is due both to a decrease in the % CFC and an impairment of CFC in exerting their cytolytic effects. These observations differ from those of Toder, Nebel & Gleicher (1984a) which indicated depression of post-binding lysis amongst CFC without impairment of binding capacity. In contrast to NK cells from cancer patients which may suffer a depressed recycling ability (Steinhauer *et al.*, 1982), it has been suggested that pregnancy-associated NK cells have uninhibited recycling capacity (Toder *et al.*, 1984a). Our results also suggest effective recycling of NK cells in pregnancy, since cytotoxicity, as assessed by CRA was found to correlate with % PLCFC in both normal and pregnant females (Fig. 2). A more direct assessment of this point would be of interest.

Using the monoclonal antibodies OKMI, anti-Leu-7 (HNK-1) and anti-Leu-11b, all of which recognize epitopes associated with NK cells (Zarling *et al.*, 1981; Abo & Balch, 1981; Phillips & Babcock, 1983; Thompson *et al.*, 1982) we have shown here that, whereas OKMI$^+$ and Leu-7$^+$ cells remain unchanged in both proportion and number during pregnancy, Leu-11$^+$ cells are deficient in maternal peripheral blood. Whether the reduction in Leu-11$^+$ cells accounts entirely for the reduction of % CFC in pregnancy awaits more direct assessment. Moreover, it will be of interest, using two-colour immunofluorescence, to define further the subset(s) of NK cells affected by pregnancy. In this context, Lanier *et al.* (1983) have shown that lymphocyte populations exhibiting NK activity may be divided into highly active Leu-11$^+$, Leu-7$^-$ cells, Leu-11$^-$, Leu-7$^+$ cells having weaker activity, and Leu-11$^+$, Leu-7$^+$ cells showing variable activity between individuals. Investigation of the tissue distribution of these subsets has led to the suggestion that maturation of human NK cells involves the expression of the Leu-11 antigen by Leu-7$^+$ precursor cells, the most mature (and highly cytolytic) NK cells belonging to the subset defined as Leu-11$^+$, Leu-7$^-$ (Abo, Miller & Balch, 1984). It is therefore possible that pregnancy interferes with the normal maturation of NK cells (from Leu-11$^-$, Leu-7$^+$ to Leu-11$^+$, Leu-7$^+$ or Leu-11$^+$, Leu-7$^-$), since our results indicate no change in the proportion or number of maternal Leu-7$^+$ cells. Of relevance to this proposition are studies in mice which indicate that chronic administration of estrogen affects the generation of NK cells, probably at the level of the bone marrow (Seaman *et al.*, 1978).

Taken together, the results presented here indicate that pregnancy is associated with both a reduction in number (Leu-11$^+$ and CFC) and an impaired post-binding lytic potential of maternal NK cells. The observed loss of correlation between % Leu-7$^+$ cells and cytolytic activity in pregnancy might be due to an excess of Leu-11$^-$, Leu-7$^+$, weakly cytolytic NK cells. Such cells coincide with the Leu-7$^+$, T3$^+$ M$^-$ subset of NK cells (Abo *et al.*, 1984) and may possess an immature cytolytic machinery, since, although they have the morphological appearance of large granular lymphocytes (LGL), they contain fewer cytoplasmic granules than the highly-cytolytic LGL (Abo *et al.*, 1983). In addition, loss of correlation between % Leu-11$^+$ cells and cytotoxicity in pregnancy indicates that the activity of mature NK cells is suppressed, probably after binding to TC. However, more direct evidence is required to confirm this proposition.

Serum factors are likely to be involved in mediating post-binding NK cell regulation in pregnancy. Indeed, pregnancy serum has been shown to inhibit normal adult NK activity *in vitro* (Barrett, Rayfield & Brent, 1982) without affecting the proportion of CFC (Toder *et al.*, 1984b). Amniotic fluid and diethystilboestrol have been shown to have similar, though more potent, effects (Toder *et al.*, 1984b; Kalland & Campbell, 1984). These findings are in line with serum-mediated inhibition of NK activity in mice bearing progressively growing mammary adenocarcinoma (Nair *et al.*, 1980). It is particularly interesting that soluble factors, released from the surface of murine trophoblast cells *in vitro* may be effective in blocking NK cytotoxicity at the level of the effector cell (Kolb & Chaouat, 1982; Kolb, Chaouat & Chassoux, 1984). Continued investigation of the regulation of NK activity in pregnancy, particularly with regard to the identification of serum-associated inhibitory factors, should yield important information, of relevance to tumour immunology as well as the fetal-maternal relationship.

This study was supported by the people of Derby through their Cancer Immunology Fund. Grateful thanks are due to Dr Stewart Mayne and staff of the Haematology Department, Derby City Hospital, for kindly performing the white blood cells counts, and Wendy Field for help in collection of blood specimens. We also thank Mrs Susan Gregory and Deborah Williams for typing the manuscript.

REFERENCES

ABO, T. & BALCH, C.M. (1981) A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* **127,** 1024.

ABO, T., MILLER, C.A. & BALCH, C.M. (1984) Characterization of human granular lymphocyte subpopulations expressing HNK-1 (Leu-7) and Leu-11 antigens in the blood and lymphoid tissues from fetuses, neonates and adults. *Eur. J. Immunol.* **14,** 616.

ABO, T., MILLER, C.A., GARTLAND, G.L. & BALCH, C.M. (1983) Differentiation stages of human natural killer cells in lymphoid tissues from fetal to adult life. *J. exp. Med.* **157,** 273.

ANDERSSON, L.C., NILSSON, K. & GAHMBERG, C.G. (1979) K562—a human erythroleukaemic cell line. *Int. J. Cancer*, **23,** 143.

BAINES, M.G., PROSS, H.F. & MILLAR, K.G. (1978) Spontaneous human lymphocyte-mediated cytotoxicity against tumour target cells. IV. The suppressive effect of normal pregnancy. *Am. J. Obstet. Gynecol.* **130,** 741.

BARRETT, D.S., RAYFIELD, L.S. & BRENT, L. (1982) Suppression of natural cell-mediated cytotoxicity in man by maternal and neonatal serum. *Clin. exp. Immunol.* **47,** 742.

BILLINGHAM, R.E. (1981) Immunobiology of the maternal-fetal relationship. In *Progress in Clinical and Biological Research* Volume 70 *Reproductive Immunology* (ed. by N. Gleicher), pp. 63–75. Alan R. Liss, Inc., New York.

BONAVIDA, B., BRADLEY, T.P. & GRIMM, E.A. (1983) Frequency determination of killer cells by a single-cell cytotoxic assay. *Methods in Enzymol.* **93,** 270.

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

HERBERMAN, R.B. (1982) *NK Cells and Other Natural Effector Cells.* Academic Press, London.

JULIUS, M.H., SIMPSON, E. & HERZENBERG, L.A. (1973) A rapid method for isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.

KALLAND, T. & CAMPBELL, T. (1984) Effects of diethylstilbestrol on human natural killer cells *in vitro*. *Immunopharmacology*, **8,** 19.

KOLB, J.-P. & CHAOUAT, G. (1982) Immunoregulation by placenta of maternal and anti-fetal reactions. *Immunobiology*, **163,** 113.

KOLB, J.-P., CHAOUAT, G. & CHASSOUX, D. (1984) Immunoactive products of placenta. III. Suppression of natural killing activity. *J. Immunol.* **132,** 2305.

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.

NAIR, P.N.M., FERNANDES, G., ONOE, K., DAY, N.K. & GOOD, R.A. (1980) Inhibition of effector cell functions in natural killer cell activity (NK) and antibody-dependent cellular cytotoxicity (ADCC) in mice by normal and cancer sera. *Int. J. Cancer*, **25,** 667.

NEMLANDER, A., SAKSELA, E. & HAYRY, P. (1983) Are 'natural killer' cells involved in allograft rejection? *Eur. J. Immunol.* **13,** 348.

PHILLIPS, J.H. & BABCOCK, G.F. (1983) NKP-15: a monoclonal antibody reactive against purified natural killer cells and granulocytes. *Immunol. Lett.* **6,** 143.

RUBIN, P., PROSS, H.F. & RODER, J.C. (1982) Studies of human natural killer cells. II. Analysis at the single cell level. *J. Immunol.* **128,** 2553.

SEAMAN, W.E., BLACKMAN, M.A., GINDHART, T.D., ROUBINIAN, J.R., LOEB, J.M. & TALAL, N. (1978) β-Estradiol reduces natural killer cells in mice. *J. Immunol.* **121,** 2193.

STEINHAUER, E.H., DOYLE, A.T., REED, J. & KADISH, A.S. (1982) Defective natural cytotoxicity in patients with cancer: normal number of effector cells but decreased recycling capacity in patients with advanced disease. *J. Immunol.* **129,** 2255.

TANAKA, K. & CHANG, K.S.S. (1982) Modulation of natural killer cell sensitivity of murine trophoblast cells by tumor promoter and interferon. *Int. J. Cancer*, **29,** 315.

THOMPSON, J.S., GOEKEN, N.E., BROWN, S.A. & RHOADES, J.L. (1982) Phenotypic definition of human monocyte/macrophage subpopulations by monoclonal antibodies II. Distinction of non-T, non-B natural killer cells (NK) from antigen-presenting stimulating cells in the mixed lymphocyte response (MLR). American Association for Clinical Histocompatibility Testing, 9th Annual Meeting, p A23.

TODER, V., NEBEL, L. & GLEICHER, N. (1984a) Studies of natural killer cells in pregnancy. I. Analysis at the single cell level. *J. Clin. Lab. Immunol.* **14,** 123.

TODER, V., NEBEL, L., ELRAD, H., BLANK, M., DURDANA, A. & GLEICHER, N. (1984b) Studies of natural killer cells in pregnancy. II. The immunoregulatory effect of pregnancy substances. *J. Clin. Lab. Immunol.* **14,** 129.

ZARLING, J.M., CLOUSE, K.A., BIDDISON, W.E. & KUNG, P.C. (1981) Phenotypes of natural killer cell populations detected with monoclonal antibodies. *J. Immunol.* **127,** 2575.