Clin. exp. Immunol. (1982) **48**, 649–654.

Natural killer cell function of human neonatal lymphocytes

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(Accepted for publication 31 December 1981)

SUMMARY

Human natural killer cell (NK cell) activity against K-562 target cell line was evaluated in full term cord blood ($n=30$) and adult peripheral blood ($n=20$) using ^{51}Cr release assay. The level of NK cell activity was lower in cord blood compared to adult controls ($39{\cdot}6 \pm 11{\cdot}4\%$ vs $27{\cdot}4 \pm 11{\cdot}8\%$ at effector:target ratio 50:1). Adult males showed a significantly higher NK activity compared to females. No sex difference was observed in cord blood. Furthermore, partially purified human leucocyte interferon (IFNα) increased *in vitro* NK cell function of both adult and newborn lymphocytes. The present results indicate that the appearance and maturation of human NK cells occurs during the intrauterine life of the human fetus.

INTRODUCTION

Human natural killer (NK) cells are a recently described population of lymphocytes which is thought to be important in surveillance against tumours (Roder & Duwe, 1979; Kärre *et al.*, 1980; Roder *et al.*, 1980; Talmadge *et al.*, 1980). It is suggested that NK cell is not a special effector cell line but a certain maturation stage of either early monocyte or T-cell (Lohmann-Matthes, Domzig & Roder, 1979; Ortaldo *et al.*, 1979).

The appearance of lytically active NK cells in different organs has been studied mostly in mouse strains. The results show that NK cell activity is absent in newborn mouse, and during the first 6–8 weeks of life it reaches its maximum, decreasing thereafter (Herberman *et al.*, 1975; Kiessling *et al.*, 1975).

Instead, studies with human effector cells have demonstrated the presence of NK cell activity in human newborn lymphocytes. Human cord blood NK activity was observed to be lower compared to adult cells in different effector–target systems (Timonen & Saksela, 1977; Baines, Pross & Millar, 1978; Sato, Fuse & Kuwata, 1979), or to be equal to adult levels (Jondal & Pross, 1975).

In the present study NK function was evaluated in full-term neonates. Cord blood lymphocytes expressed ability to kill K-562 target cells. The level of cytotoxicity was lower when compared to adult controls. Cord blood cells responded with increased spontaneous killing to human leucocyte interferon.

MATERIALS AND METHODS

Target cells. The cell line K-562 was derived from a patient with chronic myeloid leukaemia in pleural blast crisis (Lozzio & Lozzio, 1973). K-562 cells which were kindly provided by Dr Leif Andersson, Department of Pathology, University of Helsinki, Helsinki, Finland, were grown as

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stationary cell suspension cultures in RPMI 1640 medium (GIBCO, New York) supplemented with 10% fetal calf serum (FCS) (GIBCO Europe Ltd, Glasgow, Scotland).

Effector cells. Cord blood from 30 full-term, healthy newborns and venous blood from 20 unrelated adults were collected into heparinized plastic tubes. Peripheral blood mononuclear cells were separated by using a Ficoll-Isopaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) gradient centrifugation (Böyum, 1968). The mononuclear cells were depleted of adherent cells as previously described (Koren & Williams, 1978). In brief, the mononuclear fraction was incubated for 60 min at 37°C in 5% CO_2 atmosphere on plastic tissue culture dishes (Sterilin Ltd, Teddington, England) in RPMI 1640 medium containing gentamycin (1 μg/ml) and 10% FCS. The non-adherent cells were harvested and resuspended in fresh RPMI 1640 medium with 10% FCS. After this procedure the non-adherent cells (macrophage depleted) contain fewer than 1% phagocytic cells as determined by latex particle uptake (Koren & Williams, 1978).

Cytotoxicity assay. K-562 cells were used in 4 and 18 hr microcytotoxicity assay (West *et al.*, 1977). Briefly, 2×10^6 target cells in 1 ml were labelled with 400 μCi ^{51}Cr isotope (The Radiochemical Centre, Amersham, England) for 1 hr at 37°C and then washed twice with the medium and resuspended in RPMI 1640 with 10% FCS at a concentration of 10^5 cells/ml. The cytotoxicity assay was performed in round bottom microplates (Nunc, Roskilde, Denmark). Ten thousand ^{51}Cr-labelled target cells in 0·1 ml were mixed with 0·1 ml effector cells in different effector:target ratios ranging from 6:1 to 50:1. The test combinations were set up in triplicates, and control wells containing only target cells were included in each experiment to determine the maximum and spontaneous release. The microplates were incubated at 37°C in 5% CO_2 atmosphere for 4 or 18 hr. After incubation the cell free supernatants were collected using the Titertec Supernatant Collection System (Flow Laboratories, Irvine, Scotland) and counted in a well-type gamma counter (Wallac, Turku, Finland). The specific ^{51}Cr release was determined according to the following formula: (experimental release − spontaneous release/maximal release − spontaneous release) × 100. Maximal release was determined by incubating labelled K-562 cells alone in water containing 5% sodium dodecyl sulphate, SDS. The comparison of specific cytotoxicity levels were carried out also in terms of lytic units (LU). One LU in an effector cell population was defined as the number of cells required to produce 20% specific cytotoxicity (LU_{20}) using 10^4 labelled target cells. Results are expressed as $LU_{20}/10^6$ effector cells.

Interferon. The partially purified *Sendai virus*-induced human leucocyte interferon (IF; IFNα) was kindly provided by Dr Kari Cantell, Central Public Health Laboratory, Helsinki, Finland. It was produced and purified as previously described (Cantell & Hirvonen, 1978). Human leucocyte interferon was added to cytotoxicity assays at the beginning of 4 hr incubation, the final concentration being 1000 units/ml in each well.

Statistics. Student's *t*-test was employed in the comparison of mean values.

RESULTS

Twenty adult and 30 newborn peripheral blood samples were tested for their NK activity. Fig. 1 shows that positive natural killing was observed in almost all neonates. In only two cases was specific cytotoxicity less than 15% at the highest E:T ratio used. Although the ranges of cytotoxicity in both groups were about the same, the mean NK levels differed significantly. Human cord blood lymphocytes expressed a lower level of spontaneous cytotoxicity compared to adult lymphocytes ($P < 0{\cdot}02$ at 6:1 of E:T ratios, and $P < 0{\cdot}01$ at higher E:T ratios).

Table 1 shows the results analysed according to the sex of the effector cell donor. In adult group natural killing was significantly higher in males than in females ($P < 0{\cdot}05$). Also the difference of NK activity between adults and neonates became more evident when cord blood NK cytotoxicity was compared to adult male values. Instead, adult females did not show a clearly higher level of natural cytolytic activity than newborns. Cord blood NK cytotoxicity did not show any difference in respect to donor's sex. Cord blood NK cell activity was also evaluated according to the management of the delivery, mother's parity, and the weight of the newborn (Table 2). NK cell function of the neonatal lymphocytes after Caesarean section or induced labour did not differ significantly from the values

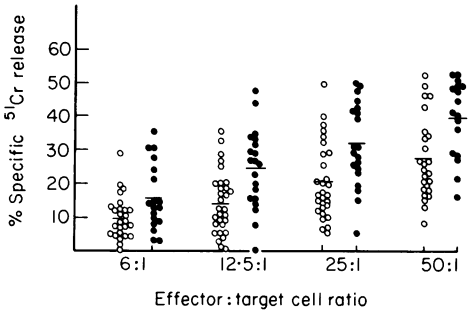

Fig. 1. NK cell function of human full-term cord blood (○) and adult peripheral blood (●) lymphocytes. Individual cytotoxicity values are given, and the arithmetic mean values at different E:T ratios are expressed with horizontal lines.

Table 1. NK cell function in adult peripheral blood and newborn cord blood lymphocytes. Effect of sex

	% cytotoxicity*			
	Adult		Newborn	
E:T ratio	male ($n=7$)	female ($n=13$)	male ($n=15$)	female ($n=15$)
12·5:1	31·2 ± 7·4	20·6 ± 13·1	13·1 ± 8·0	15·5 ± 10·6
25:1	40·1 ± 7·6†	27·2 ± 12·3	21·2 ± 11·3	20·7 ± 11·4
50:1	47·0 ± 5·0†	35·5 ± 12·0	28·1 ± 11·7	26·9 ± 12·7

*Results are expressed as mean ± s.d.
†$P < 0·05$ when compared to adult female values.

Table 2. NK cell function of human full-term cord blood lymphocytes. Effect of delivery, parity and weight of the newborn

	% cytotoxicity*
Delivery	
Spontaneous labour ($n=21$)	21·7 ± 12·4†
Induced labour ($n=5$)	20·0 ± 9·1
Caesarean section ($n=4$)	17·3 ± 7·9
Parity	
Primipara ($n=9$)	22·9 ± 9·4
Multipara ($n=21$)	19·9 ± 12·1
Weight	
< 3500 g ($n=15$)	19·8 ± 8·0
> 3500 g ($n=15$)	21·8 ± 14·1

*E:T ratio 25:1.
†Mean ± s.d.

obtained after spontaneous labour. Newborns of primi- and multipara mothers expressed about the same level of spontaneous cytotoxicity against K-562 targets. No difference was observed between newborns under 3,500 g or over 3,500 g birth weights.

We also studied the effect of human leucocyte interferon (IFNα) on NK cell cytotoxicity. IFNα increased the cytotoxicity of both adult and neonatal lymphocytes. The augmentation was observed after 4 hr of incubation. Also the longer incubation period (18 hr) without IFNα treatment resulted in increase of NK cell cytotoxicity (results not shown).

DISCUSSION

The results of the present study demonstrate that human full-term cord blood lymphocytes express a positive natural killer cell activity against the K-562 target cell line. The range of cytotoxicity was large although all newborns were full-term and healthy. Such aspects as the management of delivery (spontaneous or induced labour, Caesarean section), parity, or the weight of the newborn did not effect the level of cytotoxicity.

All adults tested showed a positive dose–response effect and specific cytotoxicity over 15%. Adult males expressed higher NK activity against K-562 targets than adult females. Similar results have been reported earlier (Santoli *et al.*, 1978; Hoffman, 1980; Penschow & MacKay, 1980). In newborns no difference was observed according to the sex. Steriod hormones, especially oestrogens, have been suggested to be responsible for the lower NK activity in women, and studies with mice have demonstrated that exogenous oestrogen reduces NK cytotoxicity (Seaman *et al.*, 1978; Seaman, Merigan & Talal, 1979). Fluctuations in oestrogen level could also explain the high range of cytotoxicity observed in females. It is possible that the high maternal oestrogen level in late pregnancy has an effect also on cord blood lymphocytes reducing NK activity.

In our experiments both adult peripheral blood and newborn cord blood lymphocytes expressed increased cytotoxicity against K-562 cell line when effector and target cells were incubated with human leucocyte interferon (IFNα). Activation of spontaneous killing was seen already after 4 hr of incubation. Both adult and cord blood lymphocytes responded with a relative equal increase of NK activity after interferon treatment. Results very similar to ours have recently been reported by Antonelli, Stewart & Dupont (1981) and Kohl *et al.* (1981). It is obvious that cord blood lymphocytes contain NK cells which are cytotoxic against K-562 targets, and pre-NK cells which become cytolytic under the influence of interferon (Saksela, Timonen & Cantell, 1979). Cantell *et al.* (1968) have shown that human leucocyte interferon is constantly produced throughout intrauterine and postnatal life. Recently, Bryson *et al.* (1980) have reported that human cord blood lymphocytes are capable of producing type I interferon but not immune interferon. This could also partly explain the lower NK values of cord blood lymphocytes observed in the present study.

The present results are in agreement with earlier studies on the immunological competence of the human neonate. Cord blood lymphocytes have been shown to express lower cell-mediated lympholysis after mixed leucocyte culture (Granberg, Manninen & Toivanen, 1976), lower capacity of antibody-dependent cell-mediated cytotoxicity (ADCC) (Campbell *et al.*, 1974), and lower phytohaemagglutinin-induced lymphocyte cytotoxicity (Campbell *et al.*, 1974; Eife *et al.*, 1974) compared to adults. On the basis of these results it is evident that different types of cellular killing mechanisms are functioning in the newborn although not reaching the adult levels.

Clinically it is important to note a positive NK activity in cord blood because recent studies have suggested that NK cells may be an important factor against viral infections, e.g. human cytomegalovirus and *herpes simplex* virus (Ching & Lopez, 1979) which can cause severe infections especially in newborns. Our results show that full-term cord blood contains interferon-inducible NK cells and therefore exogenous interferon in clinical use could improve host responses of newborns against viral infections.

In conclusion, human newborn cord blood contains a population of lymphocytes which is cytotoxic against the K-562 cell line without a prior sensitization. The capacity to kill NK sensitive target cells is lower compared to adult lymphocytes but can be augmented with human leucocyte interferon. The appearance and maturation of human NK cells occurs during intrauterine life. Studies on fetal development of NK cells and the NK capacity of premature infants are in progress.

We wish to thank Professor Paavo Toivanen for his comments on the manuscript, and Dr Kari Cantell, Central Public Health Laboratory, Helsinki, Finland, for supplying us with samples of human leucocyte interferon. This work was supported by grants from the Research and Science Foundation of Lääke Oy, the Sigrid Jusélius Foundation, and the Finnish Cancer Union.

REFERENCES

ANTONELLI, P., STEWART, W. & DUPONT, B. (1981) Distribution of natural killer cell activity in peripheral blood, cord blood, thymus, lymph nodes, and spleen and the effect of *in vitro* treatment with interferon preparation. *Clin. Immunol. Immunopathol.* **19,** 161.

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

BRYSON, Y.J., WINTER, H.S., GARD, S.E., FISCHER, T.J. & STIEHM, E.R. (1980) Deficiency of immune interferon production by lymphocytes of normal newborns. *Cell. Immunol.* **55,** 191.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human peripheral blood. *Scand. J. clin. lab. Invest.* **21** (suppl. 97), 97.

CAMPBELL, A.C., WALLER, C., WOOD, J., AYNSLEY-GREEN, A. & YU, V. (1974) Lymphocyte subpopulations in the blood of newborn infants. *Clin. exp. Immunol.* **18,** 469.

CANTELL, K. & HIRVONEN, S. (1978) Large-scale production of human leucocyte interferon containing 10^8 units per ml. *J. gen. Virol.* **39,** 541.

CANTELL, K., STRANDER, H., SAXÈN, L. & MEYER, B. (1968) Interferon response to human leucocytes during intrauterine and postnatal life. *J. Immunol.* **100,** 1304.

CHING, C. & LOPEZ, C. (1979) Natural killing of *herpes simplex* virus type 1-infected target cells: normal human responses and influence of antiviral antibody. *Infect. Immun.* **26,** 49.

EIFE, R.F., EIFE, G., AUGUST, C.S., KUHRE, W.L. & STAEHR-JOHANSEN, K. (1974) Lymphotoxin production and blast cell transformation by cord blood lymphocytes: dissociated lymphocyte functions in newborn infants. *Cell. Immunol.* **14,** 435.

GRANBERG, C., MANNINEN, K. & TOIVANEN, P. (1976) Cell-mediated lympholysis by human neonatal lymphocytes. *Clin. Immunol. Immunopathol.* **6,** 256.

HERBERMAN, R.B., NUNN, M.E., HOLDEN, H.T. & LAVRIN, D.H. (1975) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int. J. Cancer,* **16,** 216.

HOFFMAN, T. (1980) Natural killer function in systemic lupus erythematosus. *Arthrit. Rheum.* **23,** 30.

JONDAL, M. & PROSS, H. (1975) Surface markers on human B and T lymphocytes. VI. Cytotoxicity against cell lines as a functional marker for lymphocyte subpopulations. *Int. J. Cancer,* **15,** 596.

KIESSLING, R., KLEIN, E., PROSS, H. & WIGZELL, H. (1975) 'Natural' killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur. J. Immunol.* **5,** 117.

KOHL, S., FRAZIER, J.J., GREENBERG, S.B., PICKERING, L.K. & LOO, L.-S. (1981) Interferon induction of natural killer cytotoxicity in human neonates. *J. Pediatr.* **98,** 379.

KOREN, H.S. & WILLIAMS, M.S. (1978) Natural killing and antibody-dependent cellular cytotoxicity are mediated by different mechanisms and by different cells. *J. Immunol.* **121,** 1956.

KÄRRE, K., KLEIN, G.O., KIESSLING, R., KLEIN, G. & RODER, J. (1980) Low natural *in vivo* resistance to syngeneic leukaemais in natural killer-deficient mice. *Nature,* **284,** 624.

LOHMANN-MATTHES, M.-L., DOMZIG, W. & RODER, J. (1979) Promonocytes have the functional characteristics of natural killer cells. *J. Immunol.* **123,** 1883.

LOZZIO, C.B. & LOZZIO, B.B. (1973) Cytotoxicity of a factor isolated from a human spleen. *J. Natl. Cancer Inst.* **50,** 535.

ORTALDO, J.R., MACDERMOTT, R.P., BONNARD, G.D., KIND, P.D. & HERBERMAN, R.B. (1979) Cytotoxicity from cultured cells: analysis of precursors involved in generation of human cells mediating natural and antibody-mediated cytotoxicity. *Cell. Immunol.* **48,** 356.

PENSCHOW, J. & MACKAY, I.R. (1980) NK and K cell activity of human blood: differences according to sex, age, and disease. *Ann. rheum. Dis.* **39,** 82.

RODER, J.C. & DUWE, A.K. (1979) The beige mutation in the mouse selectively impairs natural killer cell function. *Nature,* **278,** 451.

RODER, J.C., HALIOTIS, T., KLEIN, M., KOREC, S., JETT, J.R., ORTALDO, J., HERBERMAN, R.B., KATZ, P. & FAUCI, A.S. (1980) A new immunodeficiency disorder in humans involving NK cells. *Nature,* **284,** 553.

SAKSELA, E., TIMONEN, T. & CANTELL, K. (1979) Human natural killer cell activity is augmented by interferon via recruitment of 'pre-NK' cells. *Scand. J. Immunol.* **10,** 257.

SANTOLI, D., TRINCHIERI, G., MORETTA, L., ZMIJEWSKI, C.M. & KOPROWSKI, H. (1978) Spontaneous cell-mediated cytotoxicity in humans. Distribution and characterization of the effector cell. *Clin. exp. Immunol.* **33,** 309.

SATO, T., FUSE, A. & KUWATA, T. (1979) Enhancement by interferon of natural cytotoxic activities of lymphocytes from human cord blood and peripheral blood of aged persons. *Cell. Immunol.* **45,** 458.

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.

SEAMAN, W.E., MERIGAN, T.C. & TALAL, N. (1979) Natural killing in estrogen treated mice responds

poorly to poly I:C despite normal stimulation of circulating interferon. *J. Immunol.* **123,** 2903.

TALMADGE, J.E., MEYERS, K.M., PRIEUR, D.J. & STARKEY, J.R. (1980) Role of NK cells in tumour growth and metastasis in beige mice. *Nature*, **284,** 622.

TIMONEN, T. & SAKSELA, E. (1977) Human natural cell-mediated cytotoxicity against fetal fibroblasts. I. General characteristics of cytotoxic activity. *Cell. Immunol.* **33,** 340.

WEST, H.W., CANNON, G.B., KAY, H.D., BONNARD, G.D. & HERBERMAN, R.B. (1977) Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. *J. Immunol.* **118,** 355.