

NATURAL KILLER (NK)-CELL ACTIVITY AND ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC) IN PRIMARY PRELEUKEMIC SYNDROME*

GITTE KERNDRUP, KARIN MEYER, JØRGEN ELLEGAARD and PETER HOKLAND

University Department of Medicine and Haematology, Aarhus Amtssygehus, Aarhus, Denmark

(Received 4 May 1983. Revision accepted 8 August 1983)

Abstract—In 13 patients with a multi-parameter based diagnosis of primary acquired preleukemic syndrome (PPS), natural killer (NK) cell activity and antibody-dependent cellular cytotoxicity (ADCC) were investigated on peripheral blood mononuclear cell (MNC) fractions. In all but two patients a defective NK activity was found. Lymphocyte-monocyte mixture experiments demonstrated that this was not due to suppressor monocytes. Furthermore, NK activity was defective when both myeloid and non-myeloid target cell lines were used. Addition of human leukocyte interferon to the NK cultures augmented the cytotoxicity, which exhibited the same kinetics as that of normal controls, but NK activity levels remained subnormal. These data strongly indicate that the decreased NK activity seen in the patients is due to a decreased number of circulating NK cells. In contrast ADCC was within the normal range both when MNC suspensions as well as when purified peripheral blood lymphocytes were used as effector cells thus ruling out subnormal lymphocyte ADCC masked by the presence of monocyte ADCC. These results demonstrate that PPS patients have a selective NK defect with an intact lymphocyte ADCC function. Whether this defect will prove to be valuable in the assessment of a malignant transformation in a given patient will await further longitudinal NK studies and clinical follow-up of the patients.

Key words: Preleukemic syndrome, NK activity, ADCC, α -Interferon.

INTRODUCTION

PRIMARY acquired preleukemic syndrome (PPS) is, in spite of intensive investigation, still an ill-defined entity when diagnosis is based on clinical and morphological criteria alone [2, 5, 16, 20, 26, 27]. Linman [16] defined PPS as an unexplained mono-, bi- or pancytopenia, often accompanied by a hypercellular bone marrow with megaloblastic morphological changes. With respect to bone marrow morphology PPS can be defined as a condition with less than 5% bone marrow myeloblasts and it is thus distinguished from smouldering leukemia or refractory anaemia with an excess of blasts, in which the percentage of myeloblasts by definition exceeds 5% but is less than 20% [2]. Since natural killer (NK) cells might play a role in the host defence against early neoplasia [18] and since some patients with PPS later develop acute myelogenous leukemia (AML) [15, 27], the purpose of this study was to elucidate whether patients with a diagnosis of PPS, as defined above, had significant alterations in NK activity. In normal human subjects and in experimental animal systems, the NK activity is, both *in vivo* and *in vitro*, enhanced by interferon or interferon inducers [6, 11, 13, 30]. In consequence of this, we also investigated the effect of human leukocyte interferon (α -IFN) on NK activity *in vitro*. The conflicting results concerning antibody-dependent cellular cytotoxicity (ADCC) in the defence against neoplasia [4, 22] likewise led us to investigate possible changes in ADCC in patients with PPS.

*Supported by the Danish Research Council Grant No. 12-3152.

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; α -IFN, human leukocyte interferon; AML, acute myelogenous leukemia; *e/t*, effector to target; MNC, mononuclear cell; NK, natural killer; PPS, primary preleukemic syndrome.

Correspondence to: Dr Gitte Kerndrup, Medicinsk afdeling II, Aarhus Amtssygehus, DK-8000 Aarhus C, Denmark.

MATERIALS AND METHODS

Patients

The material comprises 13 patients, five women and eight men, aged 36–84 years. The patients were admitted to the University Department of Medicine and Haematology, Aarhus Amtssygehus, during the period March 1979–October 1981. None had received cytostatics or radiotherapy prior to admission. Diagnosis was based on several abnormalities regarding clinical, chemical and morphological findings in each patient. The diagnostic criteria were unexplained mono-, bi- or pancytopenia of the blood with simultaneous presence of a hypercellular bone marrow. In addition, a number of other features were present in most patients at the time of diagnosis (Table 1). All patients had karyotypic abnormalities when examined by banding technique. All patients had less than 5% myeloblasts in their bone marrow and none had myeloblasts in peripheral blood at time of diagnosis as well as at time of investigation. At the end of the observation period four patients had died (one patient of overt AML, one of septicemia, one of ischaemic colitis and one of pneumonia).

Natural killer cell assay

Mononuclear effector cells were isolated on Isopaque-Ficoll gradients (Lymphoprep[®], Nygaard, Oslo) [3, 12]. As target cells the NK-sensitive erythroleukemia cell line K562 was used [12]. Culture conditions for the ⁵¹Cr release assay were as described in detail previously [12] and test systems were set up with and without the addition of 500 IU of partially purified α -IFN (kindly provided by Dr Kurt Berg, Institute of Microbiology, University of Aarhus). Specific lysis of target cells was calculated from the formula:

$$\frac{\text{Counts/min Exp} - \text{Counts/min SR}\%}{\text{Counts/min MR} - \text{Counts/min SR}}$$

TABLE 1. INITIAL BLOOD AND BONE MARROW FINDINGS IN 13 PATIENTS WITH A DIAGNOSIS OF PRIMARY ACQUIRED PRELEUKEMIC SYNDROME

Variable	Result
Sex	6F/7M*
Mean age	66 (range: 36–84)
Mean observation time (months)	52 (range: 3–144)†
Response to treatment	1/11‡
<u>Blood findings</u>	
Anaemia (F < 7.3 mmol/l; M < 8.2 mmol/l)	13/13
Macrocytosis (MCV > 102 fl)	6/13
Granulocytopenia (< 2 × 10 ⁹ /l)	9/13
Thrombocytopenia (< 50 × 10 ⁹ /l)	5/13
Pancytopenia	5/13
S-cobalamines I (normal: 200–800 pmol/l)	2/13
Granulocyte hyposegmentation	8/13
Karyotypic abnormalities	11/11
<u>Bone marrow findings</u>	
Overall cellularity	
(+ + + / + + / N / — / — / —)	7/4/0/1/1/0/0
Erythropoietic cellularity	
(+ + / + / N / — / —)	3/7/2/1/0
Granulopoietic cellularity	
(+ + / + / N / — / —)	3/8/1/1/0
Bone marrow iron content I	12/13
Sideroblasts I	10/12§
Myeloblasts (< 5%)	13/13

Included are the clinical data at time of diagnosis and the treatment.

*F = female, M = male.

†Four patients have died at end of observation time.

‡Response to treatment (folic acid, pyridoxine, anasterone and/or prednisone) measured in terms of diminished requirement for blood transfusions.

§In one case smear preparations of bone marrow were not available for revision.

where cour
cultured, c
only, and
results were
as routine i
each experi
In select
leukemia) li

Depletion of

The mon
adherence
staining for
specific for
percentage
purified mc

ADCC

Except for
assays. As
without the
tion of spec
compared to
In four p
c/t ratios at

Periphe
tested for
tests. As
that of 20

where counts/min Exp is the radioactivity of the supernatant when effector cells and target cells were co-cultured, counts/min SR the spontaneous release of radioactivity from cultures containing labelled target cells only, and counts/min MR the maximal release determined after detergent lysis of labelled target cells. The results were compared to a panel of normal controls ($n = 20$). According to earlier observations 16 h was chosen as routine incubation period [12]. Different effector to target (e/t) ratios (12.5:1, 25:1, 50:1, 100:1) were used in each experiment.

In selected experiments a panel of NK-sensitive target cells including the 1301 (acute T-lymphoblastic leukemia) line, and the MOLT-4 (acute T-lymphoblastic leukemia) line, was employed.

Depletion and purification of monocytes

The mononuclear cells isolated from the blood were separated into lymphocytes and monocytes by the plastic adherence method as previously described in detail [15]. Purity of the isolated suspensions was estimated after staining for non-specific esterase enzyme activity and by reactivity with the monoclonal antibody Mo2, which is specific for monocytes [29]. Monocyte contamination of the lymphocyte suspension never exceeded 2%, and the percentage of monocytes in the adherent cell fraction in all instances exceeded 80%. In selected experiments, purified monocytes were added in graded numbers to NK cultures.

ADCC

Except for the target cells and the α -IFN addition, the ADCC cultures were set up similar to the NK activity assays. As target cells the mastocytoma cell line P815 was used (e/t ratio 50:1). Assays included cultures with and without the addition of rabbit anti-P815 anti-serum (final dilution 1:2500). Incubation, harvesting and calculation of specific chromium release was done as described for the NK activity assays. The results were likewise compared to a panel of normal controls ($n = 20$).

In four patients ADCC assays were carried out on purified lymphocyte and monocyte cell fractions at various e/t ratios and the results compared to results obtained with the unseparated mononuclear cell fractions.

RESULTS

Peripheral blood mononuclear cells isolated from 13 patients with preleukemia were tested for spontaneous as well as antibody-dependent cytotoxicity in the NK and ADCC tests. As will be seen from Fig. 1, in which the NK activity of the patients is compared to that of 20 normal donors, only two of the 13 patients had a normal level of NK activity.

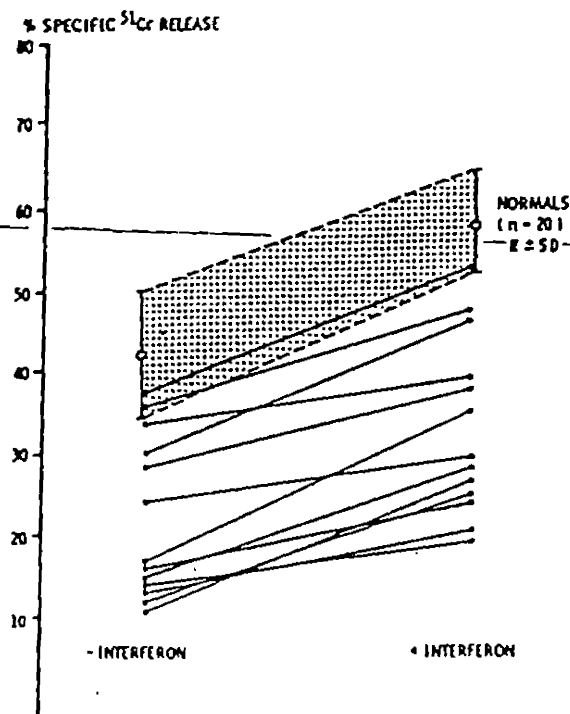


FIG. 1. NK activity of 13 preleukemia patients both with and without the addition of 500 units of α -IFN. The dotted area indicates the normal range $\pm$ S.D.

After addition of 500 units of α -IFN to the cultures, an increase in the NK activity comparable to that of the normal donors was observed. However, only one patient remained within the normal range, indicating that the unstimulated, but not necessarily the interferon activated NK cells, possessed severely depressed cytotoxic activity. In order to evaluate whether the depressed NK activity was due to a decreased level of NK activity by a normal number of circulating NK cells, or alternatively to a decreased number of NK cells in the peripheral blood, different effector-to-target ratios were used in all assays. From Fig. 2, which shows the results from a representative experiment, it is evident that the kinetics of K562 target cell lysis are similar in the patient and the normal donor. This may indicate that the defective NK activity is due to a reduced number of NK cells in the blood and not to an intrinsic defect in the NK cells.

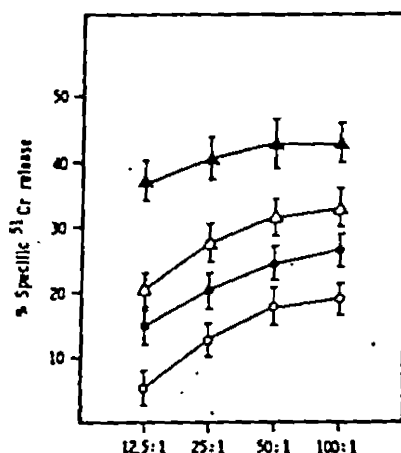


FIG. 2. Effector/target ratio curves of a representative normal donor (Δ) and a preleukemia patient ($\circ$) in NK assay against K562 cells (closed symbols, after the addition of 500 IU α -IFN).

To elucidate further the NK defect, NK activity assays were carried out with two T-ALL cell lines as target cells, simultaneously with the routinely used K562 target cell line. Decreased NK activity was also demonstrated against these target cells (Fig. 3).

Since the mononuclear cell fraction harvested from the gradient interface contained from 5 to 25% monocytes (as detected by the monoclonal antibody Mo2), we found it important to evaluate the influence of monocytes upon NK activity and ADCC. In the NK assay, where monocytes in high percentages could be imagined to inhibit cytotoxicity, lymphocytes and monocytes were separated and graded numbers of purified monocytes were added to the fixed numbers of lymphocytes and target cells in NK cultures. As will be seen from Fig. 4, addition of up to 40% monocytes did not significantly influence the NK activity of either patient or normal donor lymphocytes (monocytes alone gave no lysis). Thus, it seems unlikely that the decreased NK activity of the preleukemia patient is due to the presence of peripheral blood suppressor monocytes.

In contrast to the NK results Fig. 5 shows that nine patients had normal ADCC and only three had decreased cytotoxic activity against sensitized P815 mastocytoma cells. In one patient a significantly increased cytotoxic activity was found.

In the ADCC assay using IgG-sensitized P815 cells, which are also susceptible to lysis by monocytes, we tested the effect of monocyte depletion of the effector cell suspensions. In Table 2 data from four such experiments are given and it is apparent that non-adherent lymphocytes from the patients retained their cytotoxic activity in the ADCC assay, indicating that the ADCC results (Fig. 5) are not due to cytotoxic monocytes, but can be attributed to the patient lymphocytes only.

FIG. 3.
S.D. 1.4

FIG. 4
MONO
CYTES

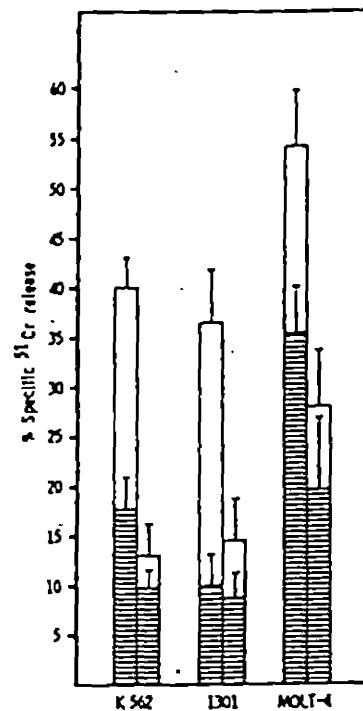


FIG. 3. NK activity of three preleukemia patients (right columns) and three normal donors ($x \pm$ S.D.) against three different target cell lines without α -IFN and with (total columns) the addition of 500 α -IFN units/ml.

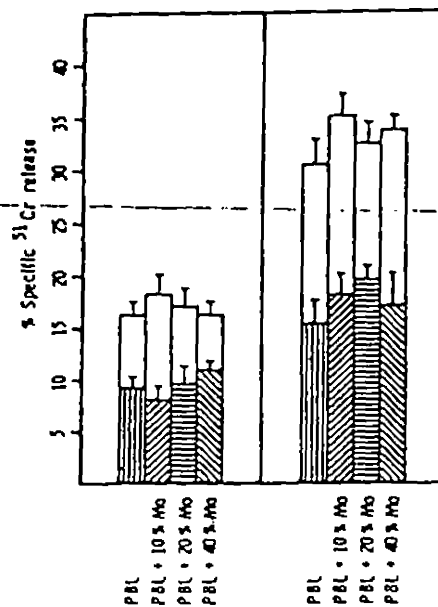


FIG. 4. NK activity of peripheral blood lymphocytes from a preleukemia patient (left panel) and a normal donor (right panel) after addition of graded numbers of monocytes (monocytes alone did not result in lysis). Cytotoxicity without α -IFN and with (total columns) the addition of 500 α -IFN units/ml. $x \pm$ S.D. is shown for simultaneous assays in each experiment.

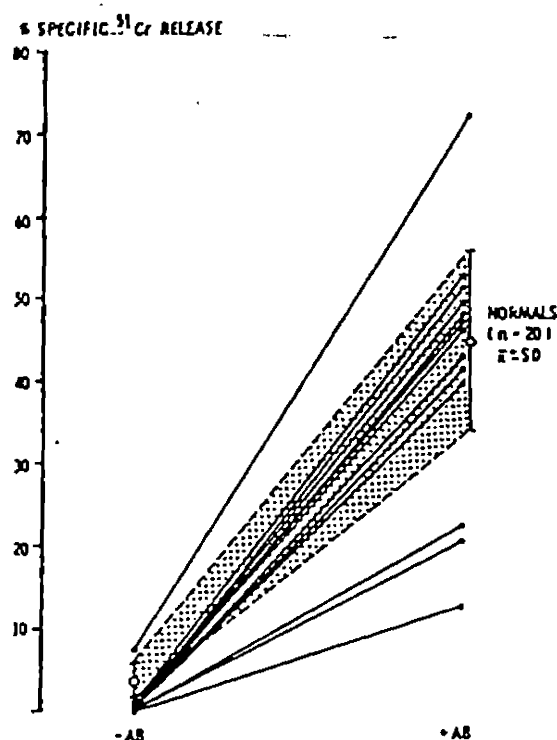


FIG. 5. Antibody-dependent cellular cytotoxicity (ADCC) in 13 preleukemia patients compared to that of normal controls (dotted area). The figure depicts the specific chromium release with and without the addition of an anti-P815 antiserum (final dilution 1:2500).

TABLE 2. ADCC OF UNSEPARATED AND SEPARATED CELL FRACTIONS IN FOUR PATIENTS WITH PRIMARY PRELEUKEMIC SYNDROME

E/t ratio	Ab*	Patient No. 4		Patient No. 11		Patient No. 42		Patient No. 1	
		Unsep.†	Non-adh.‡	Unsep.	Non-adh.	Unsep.	Non-adh.	Unsep.	Non-adh.
12.5:1	—	2.2‡	2.6	0.8	0.5	0.3	2.5	—0.5	1.2
	+	13.5	14.2	45.1	35.3	13.0	23.3	26.3	27.3
25:1	—	0.8	2.8	0.6	2.8	—2.9	9.8	0.0	2.6
	+	21.0	22.5	57.9	54.5	32.9	36.3	42.8	40.3
50:1	—	4.9	5.3	0.7	1.6	—3.3	21.6	n.t.	5.2
	+	36.0	32.6	58.0	60.7	40.2	43.1	n.t.	49.9

*Addition of anti-P815, final dilution 1:2500.

†Isopaque-Ficoll separated mononuclear cells.

‡Plastic non-adherent cells.

‡Median value of triplicate determination.

n.t.: Not tested.

DISCUSSION

Cell-mediated immune reactions have been thought to play an important role in the rejection of experimental tumors or grafts. The effector cell mediating these cytotoxic reactions was originally thought to be the cytotoxic T lymphocyte (CTL). Whereas serious

doubts have been cast on the role of these cells in surveillance against malignancy, review, ser with the lymphoma: found [9, individual of which 14]. An in malignant growth. I capacity investigate against c activity a

In 11 significant activity c defect of was simi monocy amount non-mye defect is with a : showed

Reduced Heimpe defect n reduced to the : longitu heterog cytogetr stantly change primar

The several inhibit tumor system The la again: a recr

*A syndr 10: have t betw

doubts have lately been cast on the role of CTL in immune defence against cancer, NK cells are now, based on extensive data, thought to play an important role in the immune surveillance against early neoplastic growth in both humans and rodents (for a recent review, see Herberman [10]). This is perhaps best exemplified by investigations in patients with the Chediak-Higashi immunodeficiency [1, 25], who often die in malignant lymphomatous states, and in whom a low NK activity (and ADCC) against tumor cells is found [9, 25].* Earlier observations comparing NK activity in normal and tumor-bearing individuals have shown a significant decrease in NK activity in tumor patients, the extent of which could be positively correlated to the degree of dissemination of the disease [7, 14]. An important fact to bear in mind, however, is that earlier studies on NK activity in malignant disease have been concerned with patients already harbouring a malignant growth. In contrast, patients with PPS provide an opportunity to test the immunological capacity in individuals with a potentially malignant condition [16, 27], a situation where investigations would seem to yield more information concerning the early immunity against cancer. These considerations have led us to the present investigation of NK activity and ADCC in patients with PPS.

In 11 of 13 patients with a multiparameter-based diagnosis of PPS we found a significantly reduced NK activity.† Several factors indicate that this decrease in NK activity could be due to a low number of circulating effector cells and not to an intrinsic defect of the NK cells. Firstly, the correlation between the e/t ratio and target cell killing was similar in the patients and the normal donors (Fig. 2). Secondly, the addition of monocytes, which could be expected to suppress NK cells [32] did not influence the amount of lysis (Fig. 4). Thirdly, the decreased NK activity was demonstrable against non-myeloid cell lines as well as myeloid cell lines (Fig. 3) indicating that the observed NK defect is not selective towards myelogenous cells. Finally, enumeration of cells reactive with a monoclonal antibody (901) specific for NK cells (manuscript in preparation) showed significantly reduced numbers of 901-positive cells.

Reduced NK activity in PPS has also recently been demonstrated by Porzolt and Heimpel [21] and may be either an effect of a subclinical malignant condition or the NK defect may indeed be the primary advent. Favoring the first hypothesis is the finding of reduced NK activity in tumor-bearing individuals [7, 14] which also might be comparable to the demonstration of cytogenetical abnormalities in PPS [19]. On the other hand longitudinal cytogenetical studies performed in our patients with PPS indicate a certain heterogeneity among these, and several of our patients do not show any major cytogenetical abnormalities (to be published), whereas their NK activity is more constantly reduced. This might speak in favour of an NK defect preceeding the cytogenetical change. A prospective study on these patients might give some clues as to what is the primary defect triggering evolution towards leukemic proliferation.

The *in vivo* action of α -IFN against tumor cells is under intensive investigation and several mechanisms for an anti-tumoral effect have been considered including a direct inhibitory effect on tumor cell multiplication [8] or an alteration in the surface antigens of tumor cells and a subsequent increase in the susceptibility to the action of the immune system. Finally, a direct enhancement of anti-tumor host responses could be envisaged. The latter two possibilities require the existence of a functioning host immune defence against tumors. The *in vitro* action of interferon on NK cells is thought to be due partly to a recruitment of killer cells [28] and partly to an increased recycling capacity of the NK

*A similar impaired NK activity has been observed by Purtilo in patients with X-linked lymphoproliferative syndrome [24].

†Our controls were not age-matched (younger mean age for the controls). However, Pross and Baines [23] have recently shown that aged individuals exhibit higher NK activity than the young. Therefore the comparison between our groups would tend to weaken the statistical significance of the NK differences.

cells [31]. *In vitro* assays for NK activity in our patients with PPS with the addition of α -IFN resulted in augmentation of the cytotoxicity to the same extent and following the same kinetics as for normal controls, but overall cytotoxicity remained subnormal in 12 patients. From this it may be concluded that the number of circulating NK cells is reduced in PPS, whereas the number of "pre-NK" cells, the cells recruitable by interferon, or their activity is normal.

Both myeloid and lymphoid cells can act as effector cells in the ADCC assay, but lymphocytes seem to be more potent than myeloid cells in the lysis of sensitized tumor cells [17]. Even though this reaction is of importance in the defense against a variety of microorganisms, its *in vivo* relevance in tumor immunology is not yet resolved. It is interesting that the ADCC against the P815 mastocytoma cell line was normal (Fig. 5 and Table 2) while the NK activity was decreased since these functions are generally assumed to be exerted by the same lymphoid subset. This dissociation may seem surprising but it is in accordance with the hypothesis that the lytic mechanism in the NK and ADCC reactions are different.

In conclusion, our studies have shown that peripheral blood lymphocytes from preleukemia patients have a severely depressed NK activity, probably due to the low number of circulating NK cells in the blood. Longitudinal studies are now in progress to further elucidate this finding through measurements of peripheral blood and bone marrow NK functions of purified lymphocyte subsets against a variety of myeloid target cells and against autologous bone marrow cells.

REFERENCES

1. ARGYLE J. C., KJELDSBERG C. R., MARTY J., SHIGEOKA A. O. & HILL H. R. (1982) T-cell lymphoma and the Chediak-Higashi syndrome. *Blood* 60, 672.
2. BENNETT J. M., CATOVSKY D., FLANDRIN G., GALTON D. A. G., GRALNICK H. R. & SULTAN C. (1982) Proposals for the classification of the myelodysplastic syndromes. *Br. J. Haemat.* 51, 189.
3. BOYUM A. (1968) Separation of leukocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* 21 (suppl 97).
4. CAVALLO G., GIOVARELLI M., FORMI G. & LANDOLFO S. (1978) Is antibody dependent cellular cytotoxicity an important mechanism of resistance to tumors *in vivo*? *Immunochimistry* 15, 801.
5. CLEMMESSEN L., HANSEN M. M., MORTENSEN L., THIEDE T. & VIDERBAEK A. (1981) The applicability of simple blood and bone marrow variables in prediction of acute leukaemia in patients with acquired bi- or pancytopenia. *Scand. J. Haemat.* 27, 193.
6. EINHORN S., BLONGREN H. & STRANDER H. (1978) Interferon and spontaneous cytotoxicity in man. I. Enhancement of the spontaneous cytotoxicity of peripheral lymphocytes by human leukocyte interferon. *Int. J. Cancer* 22, 405.
7. FORBES J. T., GRECO A. F. & OLDHAM R. K. (1981) Human natural cell-mediated cytotoxicity. II. Levels in neoplastic disease. *Cancer Immun. Immunother.* 11, 147.
8. GRESSER L., MAURY C. & BROUTY-BOYE D. (1972) Mechanism of the antitumor effect of interferon in mice. *Nature, Lond.* 239, 167.
9. HALIOTIS T., ROOER J., KLEIN M., ORTALDO J., FAUCI A. S. & HERBERMAN R. B. (1980) Chediak-Higashi gene in humans. I. Impairment of natural-killer function. *J. exp. Med.* 151, 1039.
10. HERBERMAN R. B., Ed. (1982) *NK Cells and Other Natural Effector Cells*. Academic Press, New York.
11. HERON I., HOKLAND M., MOLLER-LARSEN A. & BERG K. (1979) The effect of interferon on lymphocyte-mediated effector cell functions: selective enhancement of natural killer cells. *Cell Immun.* 42, 183.
12. HOKLAND P. & ELLEGAARD J. (1981) Immunological studies in chronic lymphocyte leukemia. II. Natural killer- and antibody dependent cellular cytotoxicity potentials of malignant and non-malignant lymphocyte subsets and the effect of α -interferon. *Leukemia Res.* 5, 349.
13. HOKLAND P., ELLEGAARD J. & HERON I. (1980) Immunomodulation by *Corynebacterium parvum* in normal humans. *J. Immun.* 124, 2180.
14. KADISH A. S., DOYLE A. T., STEINHAEUER E. H. & GHOSSEIN N. A. (1981) Natural cytotoxicity and interferon production in human cancer: deficient natural killer activity and normal interferon production in patients with advanced disease. *J. Immun.* 127, 1817.
15. KRAGBALLE K., ELLEGAARD J. & HERLIN T. (1980a) Antibody-dependent monocyte-mediated cytotoxicity. Isolation and identification by esterase staining of human monocytes detached from monolayers by lowering the temperature. *Scand. J. Haemat.* 24, 399.
16. LINMAN J. W. & SAARNI M. I. (1974) The preleukemic syndrome. *Semin. Hemat.* 11, 93.
17. MACDONALD H. R., BONNARD G. D., SORDAT B. & ZAWOONIK S. A. (1975) Antibody-dependent cell-mediated cytotoxicity: heterogeneity of effector cells in human peripheral blood. *Scand. J. Immun.* 4, 487.
18. MARX J. L. (1980) Natural killer cells helps defend the body. *Science* 210, 624.
19. PANANI A., the G-bani
20. PIERRE R.
21. PORZSOLT
22. PRATHER C. cellular cyt
23. PROSS H. I. normal cyt
24. PURTILLO D. agammaglob
25. ROOER J. C. FAUCI A. S.
26. ROTHSTEIN
27. SAARNI M. *Am. J. Me*
28. SAKSELA E. via recruit
29. TODD R. F. monodona
30. TRINCHIERI derived or antagonisti
31. ULLBERG N. *exp. Med.*
32. DE VRIES J. cytotoxic ci

19. PANANI A., PAPATANNIS A. G., KYRKU K. & GARDIKAS C. (1977) Cytogenetic studies in preleukaemia using the G-banding technique. *Scand. J. Immun.* 18, 301.
20. PIERRE R. V. (1974) Preleukemic states. *Semin. Hemat.* 11, 73.
21. PORZOLT F. & HEIMPEL H. (1982) Impaired T-cell and NK-cell function. *Blut* 45, 243.
22. PRATHER O. S., GELLER R. W. & LAUSCH R. N. (1979) Inability of antiscrum active in antibody-dependent cellular cytotoxicity and arming tests to protect against simian virus 40 tumor cell challenge. *J. natn. Cancer Inst.* 62, 1273.
23. PROSS H. F. & BAINES M. G. (1982) Studies of human natural killer cells. I. *In vivo* parameters affecting normal cytotoxic functions. *Int. J. Cancer* 29, 383.
24. PURILO D. T. (1981) Linked lymphoproliferative syndrome. An immunodeficiency disorder with acquired agammaglobulinemia, fatal infectious mononucleosis, or malignant lymphoma. *Arch. Path. Lab. Med.* 105, 119.
25. ROOER 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 involving NK cells. *Nature, Lond.* 284, 553.
26. ROTHSTEIN G. & WINTROBE M. M. (1975) Preleukemia. *Adv. int. Med.* 29, 20.
27. SAARNI M. I. & LIMMAN J. W. (1973) Preleukemia. The hematologic syndrome preceding acute leukemia. *Am. J. Med.* 55, 38.
28. SAKSELA E., TIMONEN T. & CANTELL K. (1979) Human natural killer cell activity is augmented by interferon via recruitment of "Pre-NK" cells. *Scand. J. Immun.* 10, 257.
29. TODD R. F., NADLER L. M. & SCHLOSSMAN S. F. (1981) Antigens on human monocytes identified by monoclonal antibodies. *J. Immun.* 126, 1435.
30. TRINCHIERI G. & SANTOLI D. (1978) Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J. exp. Med.* 147, 1314.
31. ULLBERG M. & JONDAL M. (1981) Recycling and target binding capacity of human natural killer cells. *J. exp. Med.* 153, 615.
32. DE VRIES J. E., MENDELSON J. & BONT W. S. (1980) Requirement for monocytes in the spontaneous cytotoxic effects of human lymphocytes against non-lymphoid targets. *Nature, Lond.* 285, 574.