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NATURALLY OCCURRING CELL-MEDIATED CYTOTOXICITY IN MAN

BY

DIPONKAR BANERJEE

Thesis submitted to the School of Graduate Studies and Research of the University of Ottawa as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology.

ABSTRACT

Naturally occurring cellular cytotoxic (NOCC) activity of normal human peripheral leukocytes against rabbit spleen cells, rabbit, horse, sheep and bovine erythrocytes in vitro was shown to be mediated by mononuclear cells and polymorphonuclear leukocytes. Purified T lymphocytes were not cytolytic unless cultured for 48 to 72 hours prior to assay. The mononuclear cell mediating NOCC activity was found to be Fc receptor positive, with or without C3 receptors, phagocytic, plastic adherent and Concanavalin A receptor positive. Although non-adherent mononuclear cells were cytolytic to a lesser extent than adherent cells, both were required for optimal lysis.

NOCC was shown to be a reaction which differs from antibody dependent cellular cytotoxicity (ADCC) and similar to natural killing although mediated by different effector cells.

NOCC activity was found to be depressed in patients with chronic lymphocytic leukemia (CLL) as well as patients with breast cancer. As well, patients with fibrocystic disease of breast, a benign but potentially pre-malignant condition, were found to have low NOCC activity. The cell mediating NOCC may have a role in tumour-immunosurveillance but its activity may be influenced by multiple factors.

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LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cellular cytotoxicity
B cells	Bone marrow-derived lymphocytes
C3	Third component of the complement system
C3R	Receptor for third component of the complement system
Cis-DDP	Cis-diaminedichloroplatinum
CLL	Chronic lymphocytic leukemia
Con A	Concanavalin A
CR	Corrected release
CRBC	Chicken red blood cells
DNase	Deoxyribonuclease
E	Erythrocyte (usually sheep erythrocyte)
ERFC	Erythrocyte rosette forming cell
E_4	Erythrocyte rosettes formed at 4°C.
E_{29}	Erythrocyte rosettes formed at 29°C.
EA	Erythrocytes sensitized with IgG antibodies
EA-RFC	EA rosette forming cell
EAC	Erythrocytes sensitized with IgM antibodies and complement
EAC-RFC	EAC rosette forming cell
FBS	Fetal bovine serum
Fc	Crystallizable fragment of immunoglobulin
FcR	Receptor for Fc portion of immunoglobulin
HAGG	Heat aggregated gamma globulin
HBSS	Hank's balanced salt solution
HL60	Promyelocytic leukemia cell line
HRBC	Horse red blood cells

HuRBC	Human red blood cells
IF	Interferon
K cells	Killer cells
K562	Erythroleukemia cell line
LGL	Large granular lymphocytes
MAF	Macrophage activating factor
MC	Mononuclear cells
MDMC	Macrophage-depleted mononuclear cells
N cell	Non-specific cytotoxic cell
NAF	N cell activating factor
NIF	N cell inhibitory factor
NK cell	Natural killer cell
NOCC	Naturally occurring cellular cytotoxicity
NZW	New Zealand White
ORBC	Ox red blood cells
PBMNC	Peripheral blood mononuclear cells
PHA	Phytohemagglutinin
PMN	Polymorphonuclear leukocytes
PWM	Pokeweed mitogen
RNase	Ribonuclease
RRBC	Rabbit red blood cells
SLMC	Spontaneous lymphocyte-mediated cytotoxicity
SR	Spontaneous release
SRBC	Sheep red blood cells
T cell	Thymus-dependent lymphocyte
TCGF	T cell growth factor

T_G	T gamma cells: T lymphocytes bearing receptors for the Fc portion of IgG
T_M	T mu cells: T lymphocytes bearing receptors for the Fc portion of IgM
TPA	12-O-tetradecanoylphorbol-13-acetate
U937	Histiocytic lymphoma cell line

CHAPTER I

INTRODUCTION

Cytolytic activity by normal, unsensitized lymphocytes, against allogeneic cells in culture, without involvement of anti-target antibodies was noted by Holm (1967) and MacLennan and Loewi (1968). This phenomenon remained largely ignored until the early seventies when Behelak and Richter (1972) showed that normal, unsensitized rabbit lymphocytes are capable of lysing allogeneic lymphocytes spontaneously. Since then there has been a virtual explosion of knowledge in the field. The phenomenon has been variously termed as spontaneous cell-mediated cytotoxicity, spontaneous lymphocyte-mediated cytotoxicity, naturally-occurring cell-mediated cytotoxicity, natural killing and natural killer-cell activity. It was gradually appreciated that the effector cells in this system are heterogeneous, different cells having been implicated in a variety of experimental conditions. Some systems have been particularly well described, especially the natural killer (NK) cells.

The objectives of this study were to characterize the naturally occurring cell-mediated cytotoxicity of human leukocytes against xenogeneic target cells and to determine whether various neoplastic diseases can be associated with dysfunction of naturally-occurring cytotoxic cells as defined by our system.

In Chapter II, a literature review of the various forms of naturally-occurring cell-mediated cytotoxicity is presented, detailing the nature of the effector cells in various species, the spectrum of target cell selectivity and current data about the role of such cytotoxic cells in immunosurveillance and homeostasis of cell kinetics and differentiation. In Chapter III, the

materials and methods used for the study of naturally occurring cytotoxicity of human leukocytes in vitro are described. In Chapter IV, experimental results are presented, including the establishment of optimal conditions for the in vitro assay, identification of the effector cells, target cells, demonstration of the distinctness of the reaction from antibody-dependent cytotoxicity, and the changes in cytotoxicity associated with disease states. A general discussion follows in Chapter V with a summary of the main conclusions. In Chapter VI, the original contributions to knowledge, by the candidate, are listed.

CHAPTER II

REVIEW OF THE LITERATURE

Cytolytic activity mediated by freshly isolated peripheral blood mononuclear cells (PBMNC) against Chang liver cells was noted by Holm (1967) and MacLennan and Loewi (1968). This phenomenon was largely ignored until later when tumor immunologists became aware of this type of reaction while trying to study tumor-specific cytotoxic activity, in the 1970s. A similar reaction was described by Behelak and Richter (1972, 1974) and by Behelak, Shibata and Richter (1973), mediated by unsensitized rabbit lymphocytes against allogeneic lymphocytes. Behelak and Richter (1975) subsequently described a similar phenomenon in circulating human leukocytes, directed against rabbit spleen cells. Using K562 cells from an erythroleukemia cell line, Jondal and Pross (1975) described a phenomenon which they termed spontaneous lymphocyte mediated cytotoxicity (SLMC), in which freshly isolated peripheral blood lymphocytes could lyse K562 cells in vitro in a 22 hour assay. This was the beginning of the connection of natural cytotoxicity with tumour immunology. Prior to this, cytotoxicity against tumour cells had been regarded as a reaction mediated by sensitized cytotoxic lymphocytes, following the pioneering work of Hellstrom (1967) who developed the colony inhibition assay and observed cytotoxicity against experimental tumours and cultured cells from human tumours, with PBMNC from cancer patients. Hellstrom et al. (1968) found that normal relatives of patients with neuroblastoma showed positive cytotoxicity against neuroblastoma cells and attributed this to contact-induced sensitization against tumour antigens. Gradually, however, other investigators began to report cytotoxicity against leukemic cells or

cell lines derived from tumours, mediated by lymphocytes from normal individuals, unrelated to or without contact with cancer patients (Rosenberg et al., 1972, McCoy et al., 1973a, Oldham et al., 1973). Following a large scale study, involving 1099 tests, Takasugi et al. (1973) reported that cytotoxicity of lymphocytes from normal individuals was greater than that of lymphocytes from cancer patients. Kiuchi and Takasugi (1976) then characterized the spontaneously cytotoxic lymphocyte, which they called the non-selective cytotoxic cell (N cell), as a cell with neither T- nor B-cell markers.

By 1977, the concept of the naturally occurring cytotoxic cell became widely accepted and considerable effort then went into characterizing the nature of the cell and the mechanism of cytotoxicity. Using a cross-competition assay, Takasugi et al. (1977) showed that the apparent non-selectivity of the effector cell was, in fact, due to multiple effector cells, each with its own selective cytotoxicity, rather than multiple selectivity by the same effector cell. Akira and Takasugi (1977) suggested that natural antibodies present in human serum conferred the specificity upon the N cell since exposure of the effector cell to trypsin and low pH inhibited cytotoxicity and this could be reconstituted with serum. Following further studies, Takasugi et al. (1977) hypothesized that the N cell mediated cytotoxicity through an antibody dependent system, similar if not identical, to the antibody dependent cell-mediated cytotoxicity system (ADCC), except that the antibody was already present on the N cell and did not have to be added in the culture medium.

Spontaneous lymphocyte-mediated cytotoxicity (SLMC) against K562 cells was felt to be due to sensitization of the cytotoxic cell by a so-called "heterologous membrane antigen" acquired by cell lines maintained in fetal

calf serum-supplemented media (Sulit et al., 1976). Pross et al. (1978), however, showed that SLMC could be demonstrated against K562 cells maintained in cultures containing human serum instead of fetal calf serum.

As reports of spontaneous or natural cytotoxicity began to accumulate, investigators began to examine the nature of the cytotoxic cell in detail. Although initial studies had indicated that the cell was a non-T lymphocyte with Fc and C3 receptors (Jondal and Pross, 1975; Pross and Jondal, 1975; Svedmyr and Jondal 1975; Peter et al 1975), West et al. (1977) challenged that concept by demonstrating that the majority of the natural cytotoxicity effector cells have Fc receptors but no detectable C3 receptors, and furthermore, express low affinity receptors for sheep erythrocytes, thus being a subset of the T lymphocytes. West et al. performed the assay at high effector to target ratios, for only 4 hours of incubation and without lysing of sheep erythrocyte rosettes after purification of T lymphocytes by a rosetting method. When methods for delineating T-helper and T-suppressor subsets by detection of Fc receptors for IgM and IgG became established (Moretta et al., 1976), Kay et al (1977) reported that natural cytotoxicity effector cells, by now known as natural killer (NK) cells, were preferentially enriched in T cell fractions containing mainly IgG Fc receptor bearing T cells ($T_G$cells) rather than fractions containing mainly IgM Fc receptor bearing T cells ($T_M$cells). Recently, with the development of monoclonal antibodies against lymphocyte differentiation antigens (Hoffman et al., 1980), further analysis has become possible. The majority of NK cells react with monoclonal antibodies 9.6 and 3A1 to T cell-associated antigens (Kamoun et al., 1981; Eisenbarth, 1980). NK cells also express an antigen reactive with a monoclonal antibody OKM1 which identifies monocytes (Zarling and Kung, 1980).

A monoclonal antibody has recently been developed against the NK cell (Leu7 or HNK1) and will help in further sorting out this apparent ambiguity (Abo and Balch, 1981).

NK cell enriched fractions obtained by Percoll-gradient centrifugation are also enriched for a certain mononuclear cell which has been termed a large granular lymphocyte (LGL)(Timonen and Saksela, 1980; Timonen et al., 1981). LGL are identical to the cells enriched by adherence to K562 cells (Timonen et al., 1979). Similar morphological features have been associated with mouse NK cells (Luini et al., 1981). Zagury et al. (1981) have studied several T lymphocyte clones maintained in the presence of T cell growth factor (TCGF; Interleukin 2) and were able to show NK activity in one of eleven clones. Similar findings were reported by Navarro et al. (1981), Timonen and Ortaldo (1981) and Nabel et al. (1981). Timonen and Ortaldo (1981) purified LGL on Percoll gradients, followed by further purification by depletion of high-affinity E-rosette forming cells and then cultured the LGL in the presence of TCGF. LGL were initially OKM1+, OKT3- and asialoGM1+ but became OKM1-, OKT3+ and asialoGM1-, suggesting differentiation into a more typical T lymphocyte phenotype during culture.

The distribution of NK effector cells in various lymphoid compartments has been studied. NK activity has been detected in the spleen and bone marrow (Lotzova and McCredie, 1978) in tonsils and lymph nodes (Eremin et al., 1978a). However, others have been unable to demonstrate NK cell activity in tonsils or lymph nodes (Nelson et al., 1977). In contrast to the circulating NK cell, Eremin et al. (1978b) found that tonsil or lymph node NK cells lack Fc and C3 receptors, and were trypsin resistant (Eremin et al., 1978a).

The relationship of NK cells to K cells, which mediate antibody-dependent cellular cytotoxicity (ADCC), has been examined closely and differences have been found. Whereas trypsin treatment of peripheral blood NK cells results in a reversible loss of activity, K cells are trypsin resistant (Kay et al., 1977; Koide and Takasugi, 1979; Eremin et al., 1978). Protein A or $F(ab')_2$ anti-IgG do not affect NK activity but inhibit K cell activity (Kay et al., 1977). Soluble immune-complexes have no effect on NK cells by inhibiting K cell activity (Bolhuis et al., 1978). Contradictory results, however, have been reported by others. Pape et al., (1979) found that Fab fragments of rabbit IgG anti-$F(ab')_2$ of human IgG inhibited NK activity, although the inhibition was incomplete. Troye et al. (1977) reported inhibition of NK activity against a transitional cell carcinoma cell line T24, by Fab fragments specific for either $F(ab')_2$ or Fc fragments of human IgG, but not by immunologically irrelevant Fab against ovalbumin. Perussia et al. (1979) have found that heat-aggregated IgG and soluble immune complexes inhibit NK cells when preincubated with the effector cells. These results led to the concept that NK cells and K cells are related and that NK cells are dependent on naturally occurring antibodies arming them in vivo. Koren et al. (1978), however, have raised serious doubts as to whether such a mechanism explains NK activity, by showing that patients with X-linked agammaglobulinemia have normal NK activity although they lack circulating antibodies. This was also reported by Pross et al. (1979).

Human NK activity can be modulated in vivo and in vitro by various substances, in particular, by interferon (IF) (Trinchieri and Santoli, 1978; Einhorn et al., 1978; Einhorn et al., 1978b). When IF is injected intramuscularly, there is a decrease in NK activity at 6 hours, followed by an increase which peaks at 24 hours and a decrease thereafter (Einhorn, 1980).

Repeated IF injections maintain NK activity at a high level (Einhorn, 1980). NK activity can be augmented in vitro by a soluble factor called N-cell activating factor (NAF), which is obtained by co-culturing lymphocytes with mitomycin C treated Raji cells or other B-cell lines (Koide and Takasugi, 1978). NAF is resistant to DNase, RNase, neuraminidase, but sensitive to trypsin and temperatures above 60°C (Koide and Takasugi, 1980). When NAF containing supernatants were concentrated by ultrafiltration and chromatographed on a Sephadex G100 column, activity was detectable in the protein peak eluted immediately following bovine serum albumin, thus indicating a molecular weight less than 67000 daltons (Koide and Takasugi, 1980). NAF may be related, if not identical, to IF, since both are heat sensitive, smaller than the albumin molecule, and have similar effects on NK cells. An inhibitory factor (N-cell inhibitory factor or NIF) has also been described (Koide and Takasugi, 1980). NIF is heat-stable and dialyzable, unlike NAF, and is produced by lymphocytes in culture.

The mechanism of action of IF on NK cells has been investigated. Pretreatment of lymphocytes with IF, followed by washing, prior to assay results in augmentation. The IF-NK interaction occurs as early as 5-10 minutes and is maximal at 10-30 minutes and is not temperature-dependent. Continued presence of IF in culture is not required (Ortaldo, Herberman and Djeu, 1980). Augmentation of NK activity by IF requires RNA and protein synthesis but not DNA synthesis (Ortaldo et al., 1980). The RNA synthesis is critical up to 4 hours after IF exposure since inhibition of NK activity is not seen if RNA synthesis is blocked 4 hours after IF exposure.

The significance of the NK cell in terms of its role in tumor immunosurveillance was appreciated largely due to data obtained from animal experiments. Athymic nude mice are unable to generate cytotoxic T lymphocytes

but have normal or decreased incidence of spontaneous tumours (Stutman, 1974; Rygaard and Povlsen, 1976; Sharky and Fogh, 1979). Chemically induced tumors are slow to develop and usually slower growing in athymic mice than in thymus-bearing mice of the same background strain (Gillette and Fox, 1974). Spleen cell preparations from athymic nude mice contain NK cells (Herberman et al., 1975) and it has been shown that there is a good correlation between NK activity in vitro and the resistance to inoculated tumour cells (Kiessling et al., 1975; Warner et al., 1977).

In man, the immunosurveillance role of NK cells has not been fully documented as yet. Patients with localized malignancies (Stage I) or malignancies with spread only to regional nodes (Stage II) have been found to have normal levels of NK activity in the blood (Oldham et al., 1975; Pross and Baines, 1976), whereas patients with widespread metastases may show depressed NK activity (McCoy et al., 1973b; Pross and Baines, 1976; Takasugi et al., 1977). When regional nodes were analyzed for NK activity, no signficant NK activity was found (Vose et al., 1977). However, Eremin et al (1978) have reported significant but variable NK activity in normal lymph nodes as well as tumor-draining nodes. Tumor-infiltrating lymphocytes do not show significant NK activity (Vose et al., 1977). Patients with familial melanoma and their relatives have been found to have low NK activity (Hersey, et al., 1979). Melanoma patients with low NK activity have a shorter period to recurrence than those with normal or high NK activity (Hersey et al., 1978). Patients with X-linked lymphoproliferative syndrome have been found to be NK cell deficient (Sullivan et al., 1980). Since the incidence of malignant lymphoma is high in these patients, the lack of NK mediated immunosurveillance may be an important factor.

Seaman et al. (1981) have shown that tumor-promoting phorbol diesters, especially 12-O-tetra decanoylphorbol-13-acetate (TPA), can suppress human NK activity in vitro. Inhibition can be achieved by pre-treatment of either target or effector cells with TPA and is related to the release of inhibitors from adherent cells in the presence of TPA in the culture medium. Sensitivity to NK-mediated lysis may be related to the stage of differentiation of the target cell. Gidlund et al. (1981) have shown that agents that induce differentiation in the target cells lead to decreased NK sensitivity. K562 cells, when induced to express erythroid differentiation markers by exposure to sodium butyrate or haemin become resistant to NK mediated lysis. Similarly, U937 cells (human histiocytic lymphoma cell line), when exposed to TPA or mixed lymphocyte culture supernatants differentiate into macrophage-like cells and become NK resistant. Seaman's results cannot be explained on the basis of differentiation of K562 cells induced by TPA since the exposure interval was too short. Metabolic inhibitors such as actinomycin D or cycloheximide can increase the sensitivity of target cells to NK-mediated lysis (Kunkel and Welsh, 1981), presumably due to impairment of membrane-repair mechanisms. Weinberg (1981) has reported that TPA can induce NK-like activity in tumor cell lines such as HL60 (promyelocytic leukemia), K562 as well as normal monocytes. Purified lymphocytes, however, cannot be induced by TPA to become cytolytic.

The second cell type recognized to possess natural or spontaneous cytolytic activity is the mononuclear phagocyte (Fink, 1976). Mononuclear phagocytes originate in the bone marrow as promonocytes, circulate in peripheral blood as monocytes and migrate into various tissues where they become histiocytes, Kupffer cells, osteoclasts or microglia (Van Oud Alblas and van Furth, 1979). If peripheral blood mononuclear cells are precultured

for 6 to 7 days prior to addition of erythrocyte target cells, they become cytotoxic to human as well as chicken erythrocytes (Muchmore et al., 1977). The emergence of cytolytic activity is apparently due to a time-dependent loss of suppressor cell activity (Muchmore et al., 1977b). The suppressor cell is a radioresistant, heat-labile, non-T cell. Suppression of monocyte-mediated erythrocyte lysis can be enhanced by pokeweed mitogen (PWM) but the PWM induced suppressor cell is radiosensitive and it is not clear whether it is a T cell or a non-T cell since this question was not addressed (Muchmore et al., 1979).

Freshly isolated monocytes from peripheral blood or pleural effusions have been shown to be spontaneously cytotoxic to a number of tumor derived cell lines, with lysis seen from 6 hours of culture, peaking at 24 to 48 hours (Mukherji, 1981). Using K562 targets, Fischer et al. (1981) found freshly isolated monocytes are spontaneously cytolytic within 3-4 hours. Monocytes isolated by adherence to autologous serum coated plastic plates were more effective than those adherent to fetal calf serum treated plates, and this appeared to be due to selection of more cytolytic subpopulations by the autologous serum coated plates. Monocyte mediated tumor cell lysis was also reported by Hammerstrom (1981), who found that cytolysis is enhanced by macrophage activating factor (MAF). Even malignant cells of monocyte origin (malignant monoblasts) have been shown to possess tumor cytolytic properties (Ellegaard, 1981). Monocytes can also induce a reversible cell cycle block at G_0/G_1 in K562 cells (Bjorn, 1981).

Wood and Gillespie (1975) noted that intra-tumoral macrophages in murine solid tumors are able to limit the metastatic potential of the tumors. This has been observed by Birbeck and Carter (1972) and Mantovani et al. (1979) as well. Human tumors may contain from 0-56% macrophages as reported by

Alexander et al. (1976), Wood and Gollahon (1977) and Wood et al. (1978). Intratumoral macrophages can mediate spontaneous cytotoxic effects in vitro up to 24-48 hours after isolation (Evans, 1973). In some systems, macrophages isolated from regressing tumors demonstrate cytolytic activity in vitro (Russell et al., 1977) whereas those isolated from progressive tumors may lack cytolytic activity (Russell and McIntosh, 1977; Taniyama and Holden, 1979). Spontaneous monocyte-mediated cytotoxicity has also been reported to be low in patients with a variety of malignancies (none with mammary carcinoma were included in this study) by Kleinerman et al. (1980a). Kleinerman et al. (1980a) also noted that the cytotoxicity increased by three-fold in patients following six cycles of chemotherapy which included cis-diammine-dichlorplatinum (cis-DDP). In another report Kleinerman et al. (1980b) showed that cis-DDP can directly stimulate monocyte-mediated cytotoxicity in vitro.

The relationship between NK cells and spontaneously cytotoxic monocytes needs to be examined. NK cell enriched fractions obtained by Percoll-gradient centrifugation are also enriched for a certain mononuclear cell which has been termed a large granular lymphocyte (LGL) (Timonen and Saksela, 1980). LGL have low affinity sheep erythrocyte receptors, cytoplasmic alpha naphthyl acetate esterase reactivity with focal or diffuse staining, Fc receptors and azurophilic granules. These features have been accepted as proof of their T-lymphocyte origin. These features are also expressed by monocytes, except for the sheep erythrocyte receptor (however, certain leukemic monocytes may express sheep erythrocyte receptors (Tsukada et al., 1981). Could NK cells be a subset of non-phagocytic and non-adherent monocytes? Certainly the evidence for a monocytic-myeloid origin can be argued on the basis of NK cells expressing OKMI antigen (as defined by a monoclonal antibody against a monocyte antigen) and not T cell antigens OKT3, 4 or 8 (Kay and Horwitz,

1980). It is known that "activated" NK cells become adherent to nylon wool (Saksela et al., 1977). Kerbel et al (1981) have studied murine tumor cell line M9-78, derived from 3-methylcholanthrene treated DBA/2 mouse macrophages, which has NK activity and morphologically resembles promonocytes. Murine promonocytes possess NK-like activity (Lohmann-Matthes et al., 1979).

However, other studies published recently have again supported a T lymphocyte phenotype in NK cells (Zagury et al., 1981; Navarro et al., 1981; and Timonen and Ortaldo, 1981). These findings are difficult to reconcile with the hypothesis that NK cells are monocyte-related cells and need to be confirmed by other investigators. It should be noted that the lymphocyte cloning techniques of Timonen and Ortaldo (1981) have used unfractionated peripheral blood lymphocytes as the starting point and not highly purified T lymphocytes. The effects of T cell growth factor (TCGF) which was used to maintain T cell clones may not be confined to T lymphocytes since HL60 (promyelocytic leukemia) cells have been found to differentiate into monocytes if cultured in the presence of PHA-stimulated leukocyte conditioned medium (Todd et al., 1981), the standard source of "crude" TCGF (Strausser and Rosenberg, 1978). It is thus possible that NK cells, promonocytes or monocytes may be triggered to proliferate and differentiate by TCGF. The loss of OKMI and the acquisition of OKT3 antigens by NK clones remain to be explained (Timonen and Ortaldo, 1981). It is possible that small numbers of contaminating OKT3 T lymphocytes eventually take over from the OKMI NK cells. If NK cells are promonocytes and become monocytes in peripheral blood and histiocytes in tissues, this may account for the high NK activity in blood, low NK activity in tissues and high monocyte-spontaneous cytotoxicity in tissues, i.e. as NK cells differentiate, they may become less cytotoxic to NK-sensitive targets but more cytotoxic to monocyte-sensitive targets.

This thesis is based on work done on an in vitro model of spontaneous cytoxicity comparing different effector cells in relation to erythrocyte targets and K562 cells, examining similarities and differences between the systems and the relationship to antibody dependent cellular cytotoxicity.

CHAPTER III

MATERIALS AND METHODS

1. Materials

   a) chemicals and reagents

   b) apparatus

a) Chemicals and Reagents:

Tissue Culture Medium - RPMI 1640, CMRL 1066 and Med 199 were obtained from Microbiological Associates, Bethesda, Maryland, and stored at 4°C until use.

Hank's Balanced Salt Solution (HBSS) - was obtained from Microbiological Associates and stored at 4°C until use.

Antibiotics - Potassium penicillin G (5000 units/ml) and streptomycin sulphate (5000 ug/ml) were obtained from Microbiological Associates as premixed solutions and stored at -20°C until use.

Saline solution - 0.9% in pyrogen free sterile water was obtained from Abbot Laboratories Ltd., Montreal, Quebec and stored at room temperature.

Serum - Gammaglobulin-free human AB serum was obtained from Alpha-Gamma Laboratories, Sierra Madre, California. Human A,B, AB and O sera were obtained from venous blood of healthy volunteers. Fetal bovine serum was obtained from Grand Island Biological Co., Grand Island, New York. All sera were decomplemented and stored at -20°C until use.

Animals - Outbred, 4-5 lb., New Zealand White (NZW) rabbits were obtained from Rockland Rabbit Ranch, Rockland, Ontario.

Ficoll 400 - was obtained in 500g lots from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Hypaque (sodium diatriozate) 50% w/v, was obtained from Winthrop Laboratories, Aurora, Ontario.

Heparin sodium was obtained in concentrations of 1000 units/ml from Allen and Hanburys, Toronto, and stored at 4°C.

N-2-Hydroxyethylpiperazine -N'-2-ethanesulfonic acid (HEPES) buffer solution (1 molar) in Earle's balanced salt solution was obtained from Microbiological Associates and kept at 4°C.

51 Chromium-Sodium - was obtained (1mCi/ml, 270-350 Ci/g) from New England Nuclear, Boston, Massachusetts.

Erythrocyte target cells - Heparinized chicken, rabbit, ox, horse and sheep blood were obtained fresh weekly from Qualicum Laboratories, Ottawa, and stored at 4°C until use.

Carbonyl iron - was obtained from Sigma Chemical Co.

Anti-erythrocyte antibodies - Rabbit anti-ox erythrocyte antibody, IgG fraction, rabbit anti-ox erythrocyte antibody, IgM fraction, and goat anti-rabbit erythrocyte antibody, IgG fraction, were obtained from Cappel Laboratories, Cochranville, Pa.

Human gammaglobulin (Fraction II) - was obtained from Pentex Inc., Kanakee, Ill.

b) Apparatus:

Culture tubes: 17x100 mm plastic sterile tubes were obtained from Falcon Plastics, Oxnard, California.

Microtitre plates, V-bottomed, 0.3 ml capacity per well, were obtained from Linbro.

Universal Containers (20 ml) were obtained from Sterlin Ltd., Richmond, Surrey, England.

Syringes of all sizes were Plastipak sterile disposable plastic obtained from Becton and Dickinson Co., Canada Ltd., Mississauga, Ontario.

Pipettes - 1, 5, and 10 ml Pyrex disposable serological pipettes were obtained from Corning Glass Works, Corning, New York.

Incubator - A water jacketed, humidified, CO2 incubator (National Co.) was used for all cell cultures. A CO2 concentration of 5% was maintained by adjusting the air:CO2 flow rate ratio to 95:5. Compressed air was passed through a Norgen filter (Littleton, Colorado) to remove suspended oil and water.

Isolation of buffy coat cells:

Heparinized blood was allowed to stand at 37°C, resulting in sedimentation of red blood cells. The plasma layer was collected with minimal contamination by red blood cells. The plasma fraction was centrifuged at 3000 RPM for 10 minutes. The supernatant was discarded unless required for experiments. The cell pellet was washed twice in Medium 199 by centrifugation and resuspended in Medium 199 at the required concentration.

Isolation of peripheral blood mononuclear cells:

Heparinized blood was diluted 1:1 with sterile normal saline and layered on Ficoll-Hypaque gradients (sp. gr. 1.077) and centrifuged at 400G for 30 minutes at 18°C. The interface band of cells (lymphocytes and monocytes) was collected, washed thrice in Medium 199 and resuspended at the required concentration.

Isolation of highly purified polymorphonuclear leukocytes (PMN) and mononuclear cells simultaneously:

Buffy coat cells were obtained as described above, diluted 1:1 with sterile normal saline and layered on Ficoll-Hypaque gradients (sp. gr. 1.077) and centrifuged at 400G for 30 minutes. The interface cells (mononuclear cells) were collected, washed and resuspended in Medium 199. The Ficoll-Hypaque pellet (PMN enriched) was collected, washed and resuspended in Medium 199.

Depletion of phagocytic cells:

Mononuclear cells were incubated with carbonyl iron (2 mg/ml) in Medium 199 with 10% AB serum for 30 minutes at 37°C in 10 ml Falcon test tubes. The test tubes were then held next to a powerful magnet so that the free carbonyl iron particles and cells containing the particles were pulled to one side of the tube by the magnetic force. The supernatant was collected with a Pasteur pipette. The process was repeated untl no carbonyl iron particles could be seen in the supernatant. Monocytes before and after phagocyte depletion were enumerated by counting non-specific esterase positive cells (Yam et al., 1970).

Preparation of indicator cells for E-rosetting:

Sheep blood was washed in Hank's balanced salt solution (HBSS) thrice and resuspended at a concentration of 10^8 sheep red blood cells (SRBC) per ml in HBSS. They were maintained at a temperature of 4°C until used.

Enumeration of E-rosette forming cells (ERFC):

Mononuclear cells at a concentration of $3x10^6$ cells per ml in HBSS were mixed with washed SRBC (10^8 per ml) in equal volumes (200 ul each) in 5 ml Falcon tubes, incubated for 15 minutes at 37°C in the presence of 20% fetal bovine serum (FBS), which had been previously absorbed with washed SRBC extensively (to remove any antiSRBC antibodies which may be present). The tubes were then centrifuged at 200G for 5 minutes and then stored overnight at 4°C. The pellets were then gently disrupted. A drop of 0.1% Trypan blue was added. A sample of the resuspended cells was then placed in a Neubauer

counting chamber and the E-RFC were enumerated under a microscope. Rosettes were identified as a morula-like cluster of red cells around a lymphocyte. Only viable cells were included in the total cell count.

Preparation of EA indicator cells:

The procedure was essentially that described by Froland et al. (1974). Equal volumes of ox red blood cells (ORBC) at $5x10^8$ ORBC per ml in HBSS were mixed with rabbit anti-ORBC antibody, IgG fraction at a subagglutinating concentration, in 5 ml Falcon tubes, at 37°C for 60 minutes. The antibody-sensitized ORBC (EA) were washed thrice and resuspended in HBSS at a concentration of 10^8 EA per ml.

Enumeration of EA-RFC:

Mononuclear cells ($3x10^8$/ml) were mixed with EA indicator cells (10^8/ml) in equal volumes (0.2 ml each) in 5 ml Falcon tubes. The tubes were then incubated at 37°C for 15 minutes and then centrifuged at 200G for 5 minutes. The pelleted cells were gently resuspended and EA-RFC were enumerated in a counting chamber. Mononuclear cells with 3 or more adherent EA indicator cells were identified as EA-RFC.

Preparation of EAC indicator cells:

The method of Ross et al. (1973) was followed. Equal volumes of ORBC ($5x10^8$ per ml in HBSS) and rabbit anti-ORBC, IgM fraction at a sub-agglutinating concentration were mixed and incubated at 37°C for 30 minutes. The antibody sensitized ORBC (EA) were washed thrice in HBSS and resuspended in HBSS ($5x10^8$ per ml). An equal volume of normal human serum (stored frozen

at -70°C) was added at a sub-lytic concentration, as the source of complement (C). The mixture was incubated at 37°C for 30 minutes, washed thrice and resuspended at a concentration of 10^8 cells per ml in HBSS.

Enumeration of EAC-RFC:

The method was identical to that used for EA-RFC.

Preparation of E-RFC-enriched and E-RFC-depleted fractions:

SRBC indicator cells were prepared as described previously but then treated with 0.14 M 2-amino ethyl iso-thiouronium bromide hydrobromide (AET) by mixing 1 volume of packed SRBC and 4 volumes of AET, incubating for 30 minutes at 37°C. After washing thrice, the AET-treated SRBC were resuspended at 10^8 per ml in HBSS. Equal volumes of mononuclear cells and AET-SRBC were mixed and incubated at 37°C for 15 minutes in the presence of 20% absorbed FBS. The cells were then centrifuged at 200G for 5 minutes and placed in an ice-bath for 2 hours. The pellets were gently disrupted and resuspended in HBSS (original volume). The resuspended cells were layered on Ficoll-Hypaque gradients (equal volumes) and centrifuged at 400G for 30 minutes. The interface cells (E-RFC depleted) and pellet cells (E-RFC enriched) were collected and washed. The E-RFC enriched fraction was treated with Boyle's solution to lyse the rosetted SRBC.

Preparation of EA-RFC enriched and EA-RFC depleted fractions:

Three ml of HBSS containing 10^7 mononuclear cells were mixed with 3 ml of EA indicator cells (10^8 cells per ml HBSS). The cells were incubated at 37°C for 15 minutes and then centrifuged at 200G for 5 minutes. The pelleted cells were resuspended in HBSS and the rosetted cells were separated from the

unrosetted cells by centrifugation on Ficoll-Hypaque discontinuous density gradients as for E-RFC separation. The rosetted and the unrosetted cells were treated with tris-buffered ammonium chloride (Boyle's solution), washed three times in HBSS and kept at 4°C until tested.

Preparation of EAC-RFC enriched and EAC-RFC depleted fractions:

The procedure used for rosetting the mononuclear cells with the EAC indicator cells, the separation of rosetted from non-rosetted cells and their treatment with ammonium chloride was identical to that described in the preceding paragraph for the preparation and isolation of EA-rosetting and non-rosetting cells.

Preparation of FcR+/C3R+, FcR-/C3R+, FcR+/C3R- and FcR-/C3R- fractions:

Peripheral blood mononuclear cells were rosetted with EA indicator cells as described previously, separated into non-rosetting (FcR-) and rosetting (FcR+) fractions by Ficoll-hypaque centrifugation. Following lysis of rosettes with Boyle's solution, each fraction was rosetted with EAC indicator cells. The rosetted (C3R+) and non-rosetted (C3R-) fractions were separated on Ficoll-hypaque gradients, washed and treated with Boyle's solution. This resulted in four fractions: 1) FcR+/C3R+ cells; 2) FcR-/C3R+ cells; 3) FcR+(C3R- cells); and 4) FcR- and C3R- cells. These fractions were kept at 4° C until used for assays.

Preparation of plastic-adherent and non-adherent fractions:

Peripheral blood mononuclear cells were obtained as previously described and incubated in 35 mm plastic Petri dishes at 37°C for 60 minutes in Medium 199 with 20% AB serum at a cell concentration of 10^7 per ml. At the end of the incubation, non-adherent cells were harvested by gentle washing with warm Medium 199 several times, pooling all the wash supernatants. Adherent cells were then harvested by flooding the dishes with cold (4°C) Medium 199 and dislodging adherent cells with a rubber policeman. The resulting cell suspension was transferred into 10 ml Falcon tubes with a Pasteur pipette.

Separation of Concanavalin A (Con A) binding and non-binding cells:

ORBC were incubated with a sub-agglutinating concentration of Concanavalin A for 30 minutes at room temperature, washed thrice, adjusted to 10^8/ml and then mixed with an equal volume of (3 ml each) of mononuclear cells ($3x10^6$ cells/ml). The cell mixture was incubated at room temperature for 15 minutes, centrifuged at 200G for 5 minutes and the resulting cell pellet was gently resuspended. The resuspended cells were layered on a Ficoll-hypaque gradient and the rosetted (Con A receptor positive) and non-rosetted (Con A receptor negative) cells were separated using identical procedures described for E-RFC, EARFC and EACRFC. The adherent ORBC were lysed with Boyle's solution.

Preparation of soluble aggregates of human gammaglobulin (HAGG):

Human gammaglobulin (Fracton II, Pentex Inc., Kankakee, Ill.) was dissolved in M199 to a concentration of 50 mg per ml. Soluble aggregates were prepared by heating in a water bath at 63°C for 30 minutes. Precipitates were removed by centrifugation at 10,000 RPM for 15 minutes and the HAGG solution was kept at -20°C until used.

Trypsin treatment of effector cells:

The mononuclear cells were treated with trypsin (2 mg per ml or 0.5 mg per ml) for 30 minutes at 37°C in Medium 199. They were then washed three times with Medium 199 and suspended in culture medium for analysis of their cytotoxic activity in the NOCC and ADCC assays.

Preparation of rabbit target cells:

Rabbits were sacrificed by the intravenous injection of nembutal. The spleen was quickly excised, chopped up with a fine-pointed scissors and the cells were expressed from the tissue fragments by vigorous shaking in a sterile Falcon tube containing 10 ml of culture medium. The cells were washed twice and resuspended in Medium 199 at the desired cell concentration.

Spleen mononuclear cells were purified by centrifuging the spleen cell suspension on a discontinuous gradient of Ficoll-Hypaque, (Sp.Gr.1.1, with 6% methyl cellulose) at 900G. Rabbit erythrocytes were obtained by bleeding into a syringe containing heparin and dextran (5%). The blood-heparin-dextran mixture was transferred to glass cylinders which were placed upright in a 37°C incubator to allow sedimentation of the red blood cells. The buffy coat was

pipetted off and the red cells were suspended in 4 volumes of culture medium. The red cells were washed thrice in Medium 199 and resuspended in culture medium to the desired cell concentration.

Chicken, ox and sheep erythrocytes:

These were obtained fresh every week from Qualicum Laboratories, Ottawa, in Alsever's solution or as heparinized blood.

Labelling of target cells with ^{51}Chromium:

Cell suspensions were adjusted to 10^8 cells/ml. The cells were labelled by incubating 10^7 cells with 0.1 ml ^{51}Cr solution (sodium chromate 1mCi/ml, New England Nuclear, Boston, Mass.) at 37°C for 2 hours in a humidified atmosphere of 5% CO2 in air. The labelled cells were washed 3 times and resuspended in Medium 199 (10^6 cells/ml) and kept at 4°C until used.

The NOCC cytotoxicity assay:

The cultures were set up in V-bottomed microplates with a capacity of 0.3 ml per well. Aliquots of the human effector cells (0.1 ml), suspended in the culture medium, were distributed into the designated wells ($5x10^5$ or 10^6 cells per well) and doubling dilutions were then made in 0.1 volumes of culture medium. Aliquots (0.1 ml) of the ^{51}Cr-labelled target cells, also suspended in culture medium, were then added to each well (10^5 cells per well). The plates were gently shaken to ensure complete intermixing of the effector and target cells in the wells. The plates were centrifuged at 50 g for 5 minutes at room temperature and then placed in an incubator for 20 hours (unless otherwise stated) at 37°C in a humidified atmosphere of 5% CO2 in air. The plates were then centrifuged at 500 g for 10 minutes at 18°C following

which a 0.1 ml sample from each well was transferred into a biogamma counting vial. The radioactive content of the vials was determined by analysis in a Beckman Biogamma counter.

When the ADCC reaction was carried out, 0.05 ml of the anti-target cell antiserum was added to the wells containing 0.1 ml effector cells and 0.1 ml target cells. The medium used was M199 fortified with fetal calf serum (FCS) to a final concentration of 6%. The duration of culture was 20 hours.

Inhibition of ADCC and NOCC with HAGG was carried out by adding 0.05 ml of the HAGG solution to the wells containing the effector cells 2 hours before the addition of the target cells. The cytotoxic activity was expressed as percent corrected Chromium release as calculated by the following formula:

$$\text{Percent corrected Chromium release} = \frac{\text{Test - spontaneous release}}{\text{Total - spontaneous release}} \times 100$$

Where Test = Cpm in supernatant from wells containing effector cells and ^{51}Cr-labelled target cells.

Spontaneous release = Cpm in supernatant from wells containing ^{51}Cr-labelled target cells and unlabelled target cells in a number equivalent to the effector cells used in the parallel test culture.

Total = Cpm in 0.1 ml of resuspended ^{51}Cr-labelled target cells.

Experimental Subjects:

1) Normal controls were healthy volunteers, laboratory personnel, graduate students and supervisors.

2) Patients with chronic lymphocytic leukemia (CLL). Patients with hematologically documented CLL attending outpatient clinics were the source of effector cells. Informed consent was obtained for venipuncture.

3) Patients with breast diseases:

The cases in the present study included women admitted as inpatients for surgical biopsy of breast masses. Informed consent was obtained for venipuncture. The age range was 19 to 74 years with an average age of 48.5 years. Controls were healthy volunteers in the same age range. Blood samples were obtained from each patient on the morning of surgery prior to pre-anaesthetic medication. All breast disease patients and controls studied had normal peripheral blood leucocyte counts and normal differential counts at the time of the study.

Histological assessment of mammary lesions:

Surgical pathology reports which were routinely issued following histological examination of the biopsies were used to identify those patients with mammary carcinoma, fibroadenoma or fibrocystic disease of breast. The cases were reviewed to classify the lesions according to tumor type, size, stromal reactions and changes in regional nodes. Stromal reactions included diffuse and perivenous lymphocytic infiltration. Axillary lymph nodes were examined for metastases, sinus histiocytosis, follicular hyperplasia, diffuse (paracortical) hyperplasia and lymphocyte depletion.

Statistical analysis:

Mean corrected ^{51}chromium release was calculated for each group and compared with the control group using the Student's "t"-test.

CHAPTER IV

RESULTS

1. ESTABLISHMENT OF OPTIMAL CONDITIONS FOR THE ASSESSMENT OF NOCC ACTIVITY OF NORMAL HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS:

1.1 The choice of nutrient medium for the NOCC assay:

Medium 199, CMRL 1066 and RPMI 1640 were compared in their ability to support the NOCC reaction. Peripheral blood mononuclear cells were isolated and suspended in PBS until placed in culture in the appropriate medium. All other conditions were identical, including serum and HEPES buffer supplementation. Table I summarizes the results. All three media were capable of supporting the NOCC reaction but Medium 199 consistently gave the best results. Medium 199 was, therefore, the medium of choice for all subsequent experiments.

1.2 The optimal incubation period:

Mononuclear effector cells were cultured with target cells at a ratio of 5:1 for varying lengths of time after which supernatants were collected. Each set of plates also had wells with target cells alone for measuring the total label and spontaneous release to avoid low results due to gradual isotope decay in the long term cultures. Fig. 1 shows the mean of three experiments. Significant cytotoxicity was seen by 2 to 6 hours with peak cytotoxicity at 20-48 hours. However, by 48 hours, the spontaneous release was unacceptably high (37% to 63% with a mean of 52%). For all subsequent experiments, cultures were terminated at 20 hours.

TABLE 1.
THE EFFECT OF DIFFERENT MEDIA ON NOCC

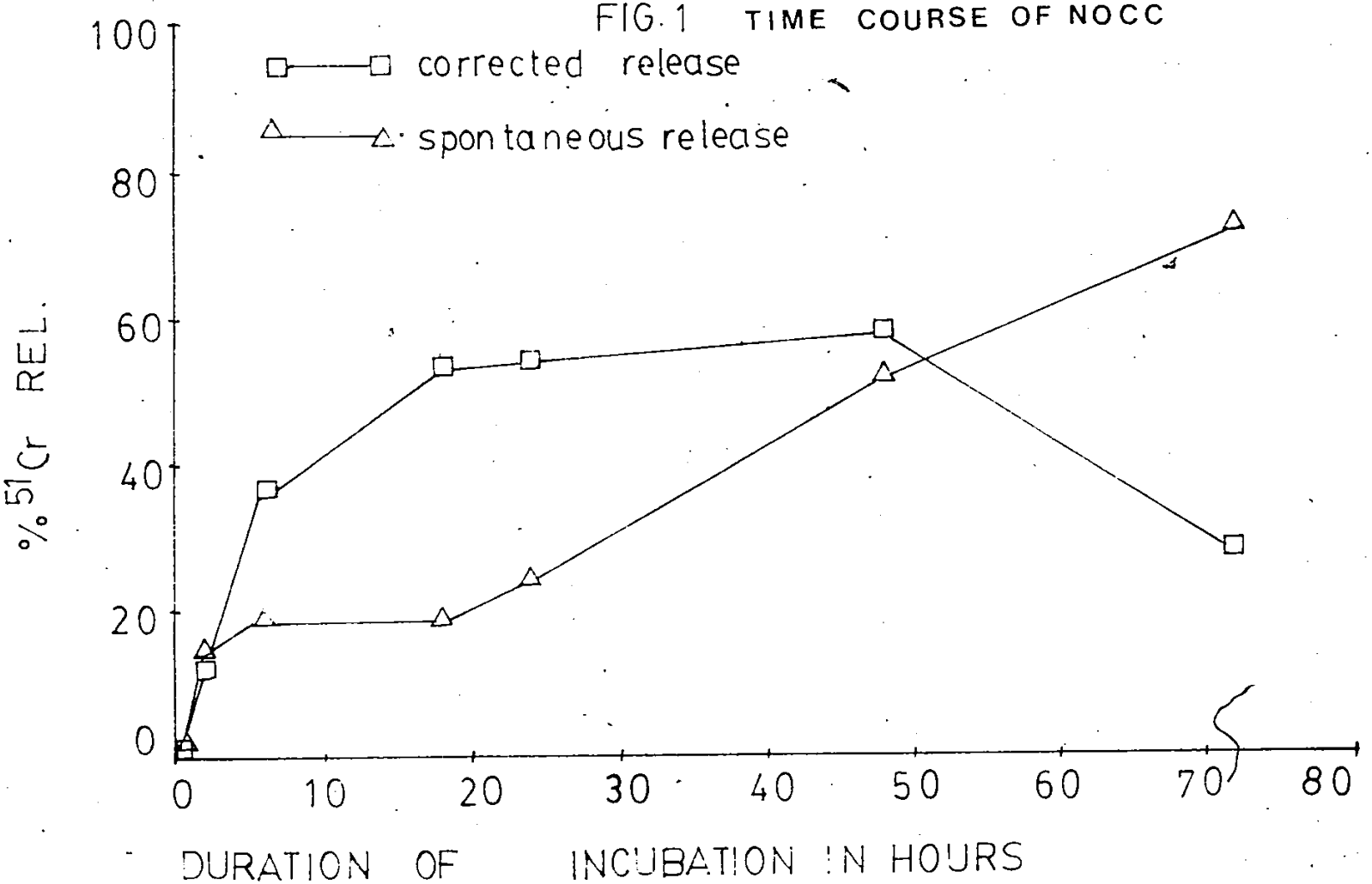
Effector: Target ratio	MED 199		CMRL 1066		RPMI 1640	
	CR*	SR**	CR	SR	CR	SR
20:1	38 ± 5	9 ± 3	37 ± 1	7 ± 3	14 ± 4	15 ± 7
10:1	42 ± 4	9 ± 4	35 ± 2	7 ± 3	22 ± 5	17 ± 8
5:1	44 ± 8	10 ± 4	38 ± 3	7 ± 4	25 ± 5	22 ± 10
2.5:1	36 ± 10	14 ± 5	36 ± 7	12 ± 6	23 ± 8	28 ± 12
1.25:1	24 ± 9	16 ± 5	29 ± 11	16 ± 7	15 ± 7	33 ± 12
0.6:1	9 ± 5	20 ± 6	13 ± 6	22 ± 11	9 ± 5	39 ± 12
0.3:1	-7 ± 8	30 ± 11	-4 ± 9	32 ± 15	-13 ± 14	50 ± 13

*CR = corrected ^{51}Cr release (percent) + S.D.

**SR = spontaneous release (percent) + S.D.

Target cells were ^{51}Cr labelled rabbit erythrocytes (10^5/well). All media were supplemented with penicillin and streptomycin, 25 mM HEPES and 10% agamma human serum.

FIG. 1 TIME COURSE OF NOCC



1.3 The effect of serum on NOCC:

1.3.1. Human Serum: Although NOCC was observed in the total absence of any serum in the culture medium, human serum was able to enhance the cytotoxicity consistently. Table 2 shows that pooled human AB serum produced an enhanced cytotoxic response at lower effector:target ratios in comparison to serum-free cultures. The nature of the enhancing factor was investigated and these experiments will be detailed later. For subsequent experiments, 10% human serum was used to supplement the culture medium routinely unless otherwise indicated.

1.3.2. Fetal bovine serum: In contrast to human serum, fetal bovine serum did not enhance NOCC activity at concentrations between 2.5% and 50% (Fig. 2). In fact, the addition of fetal bovine serum to cultures containing human serum produced an inhibitory effect on NOCC activity (Table 3).

1.3.3. Rabbit Serum: Autologous or pooled rabbit serum did not augment the NOCC reaction and was slightly inhibitory at a concentration of 10% (Fig. 3).

1.4 The optimal target cell number per well:

Keeping effector:target ratios constant at 5:1, $6x10^3$ to 10^5 target cells were cultured with appropriate numbers of effector cells. A plateau of cytotoxicity was found with $2.5x10^4$ to 10^5 target cells per well (Fig. 4). The use of fewer than $2.5x10^4$ target cells per well resulted in significant (>20%) spontaneous release of the ^{51}Cr label. In subsequent experiments, 10^5 target cells per well were routinely used, to have a wide margin of safety, avoiding unacceptably high spontaneous release.

TABLE 2.
EFFECT OF WHOLE AB SERUM ON NOCC

Effector: Target Ratio	Percent corrected chromium release (± 1.S.D.)at Serum Concentrations of				
	0%	5%	10%	20%	30%
20:1	25 ± 10	21 ± 4	19 ± 8	4 ± 9	15 ± 2
10:1	14 ± 6	25 ± 12	26 ± 9	26 ± 13	28 ± 18
5:1	9 ± 5	39 ± 13	39 ± 11	41 ± 16	41 ± 14
2.5:1	1 ± 3	43 ± 14	50 ± 12	47 ± 16	52 ± 8
1.25:1	1 ± 4	41 ± 9	46 ± 11	46 ± 16	48 ± 5
0.6:1	-1 ± 4	25 ± 5	32 ± 12	28 ± 10	29 ± 2
0.3:1	-2 ± 4	12 ± 7	9 ± 13	13 ± 10	15 ± 3

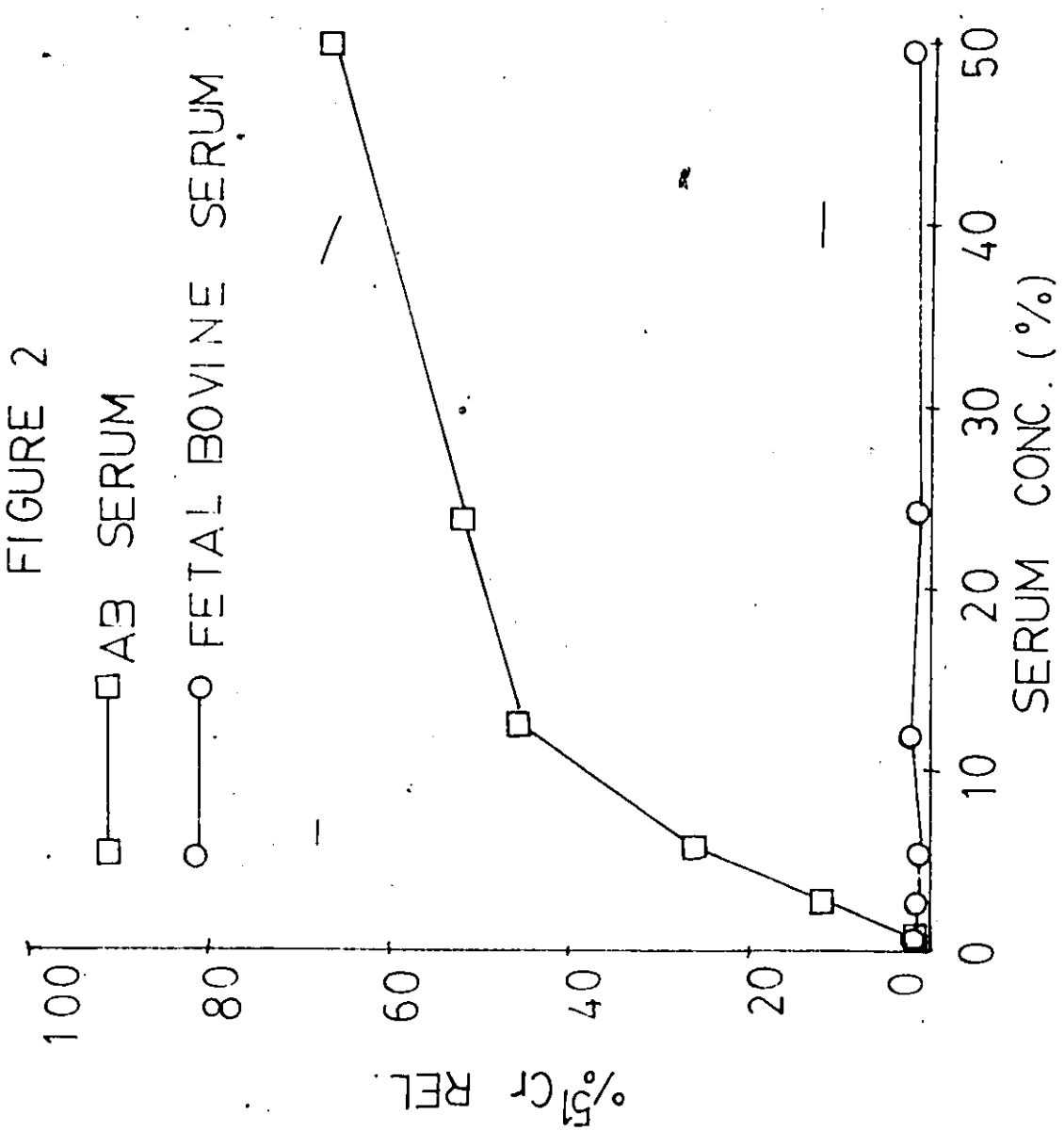

EFFECT OF SERUM ON NOCC
FIGURE 2
AB SERUM
FETAL BOVINE SERUM
% 51Cr REL.
100
80
60
40
20
0
0
10
20
30
40
50
SERUM CONC. (%)

TABLE 3.
EFFECT OF FETAL BOVINE SERUM ON NOCC

Effector: Target Ratio	Percent Corrected ^{51}Chromium Release (± S.D.) in Cultures Containing the Following: 10% AB serum	10% AB serum + 10% FBS
20:1	33 ± 1	17 ± 2
10:1	39 ± 2	21 ± 4
5:1	47 ± 1	18 ± 1
2.5:1	41 ± 3	9 ± 1
1.25:1	27 ± 1	7 ± 1
0.62:1	10 ± 0	4 ± 1
0.3:1	-22 ± 1	-4 ± 1

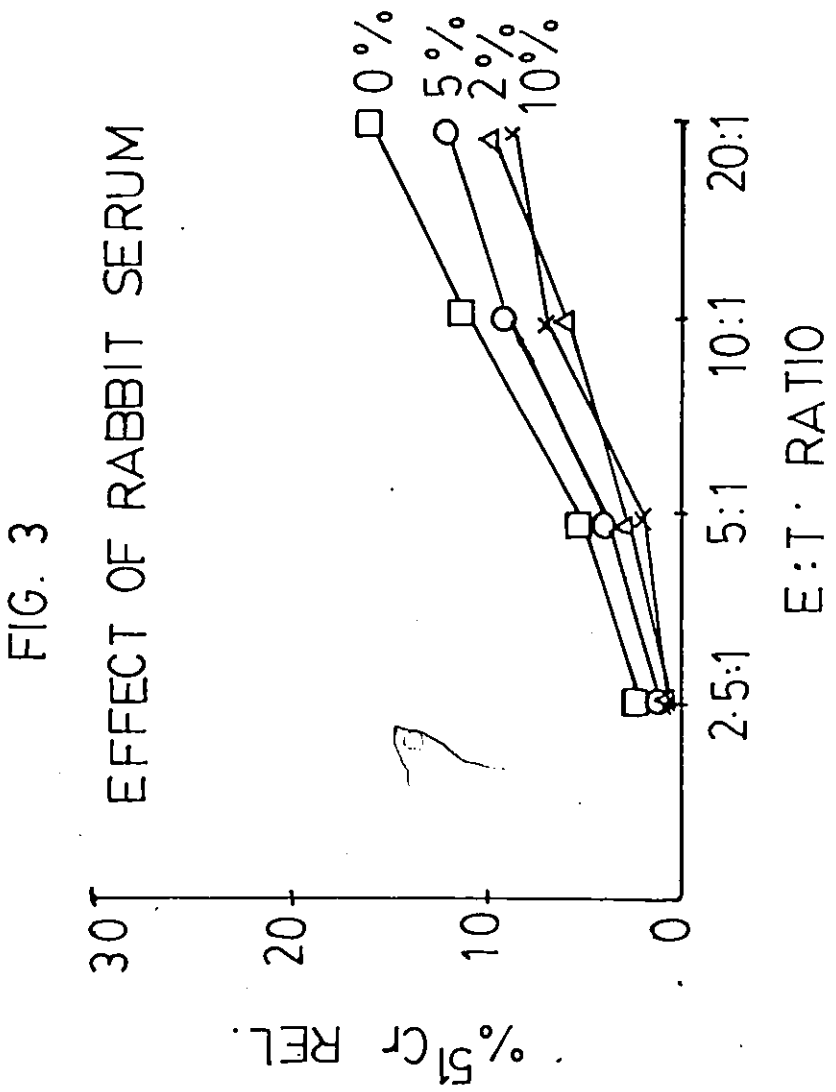
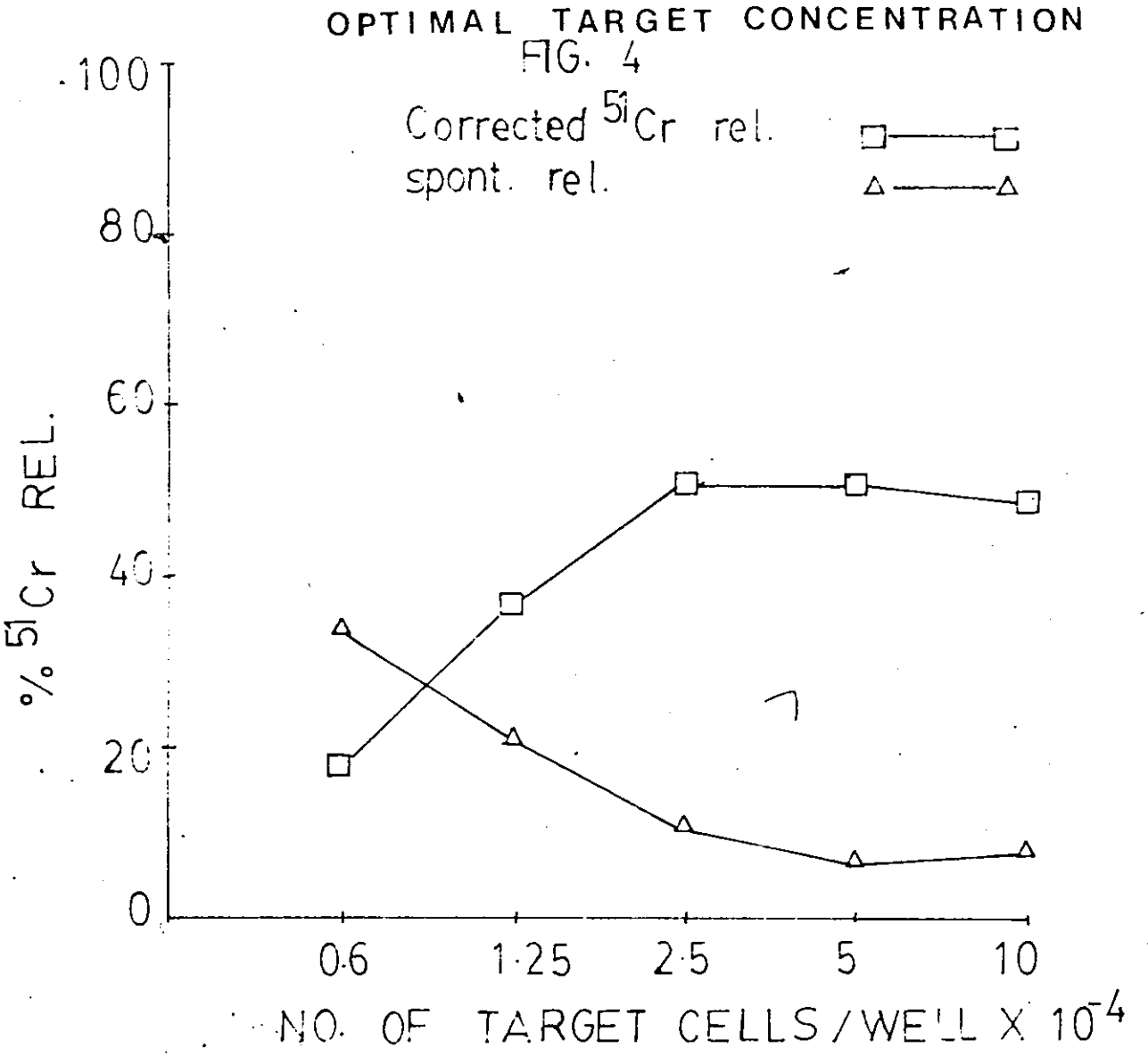

EFFECT OF RABBIT SERUM
% ^{51}Cr REL.
30
20
10
0
0%
5%
2%
10%
2.5:1
5:1
10:1
20:1
E:T RATIO

FIG. 3


OPTIMAL TARGET CONCENTRATION
FIG. 4
Corrected 51Cr rel.
spont. rel.
100
80
60
40
20
0
% 51Cr REL.
0·6
1·25
2·5
5
10
NO. OF TARGET CELLS / WELL X 10-4

1.5 The optimal Effector:Target ratio:

Keeping target cell numbers constant at 10^5 per well, various numbers of effector cells were added, with resulting effector:target cell ratios of 0.3:1 to 20:1. Cytotoxicity was usually optimal at a ratio of 5:1 (Table 1). In most experiments, however, several effector:target ratios were used, since it was observed that peak cytotoxicity occurred at different effector:target ratios in some indivduals and this could vary in the same individual, as shown in Fig. 5. Normal donor (H.H.), a fellow graduate student had been tested on several occasions and usually showed a consistent cytotoxicity curve. However, following an attack of coryza, his curve was found to have shifted to the left. Whether this was due to the viral infection or due to medications is unknown.

1.6 The effect of centrifugation of the micro-plate prior to effector:target co-culture:

It was felt that cytotoxicity may be enhanced if close effector-target contact occurred early during incubation rather than over a period of several hours if they were allowed to gradually sediment and settle on the bottom of the culture-wells. Thus, a comparison was made between cultures which had been subjected to gentle centrifugation (500 rpm) immediately following the admixture of effector and target cells and those without centrifugation. Table 4 summarizes the results. Higher levels of cytotoxicity were usually obtained by including a pre-centrifugation step.

1.7 The effect of resuspension/centrifugation of effector-target cell mixtures prior to harvest of supernatants:

It was speculated that ^{51}Cr labelled macromolecules may be released from lysed target cells but many remain trapped in the cell-pellet by a mesh-work of cytoskeletal elements and cell-membrane fragments, thus free diffusion

EFFECT OF VIRAL INFECTION
FIG. 5

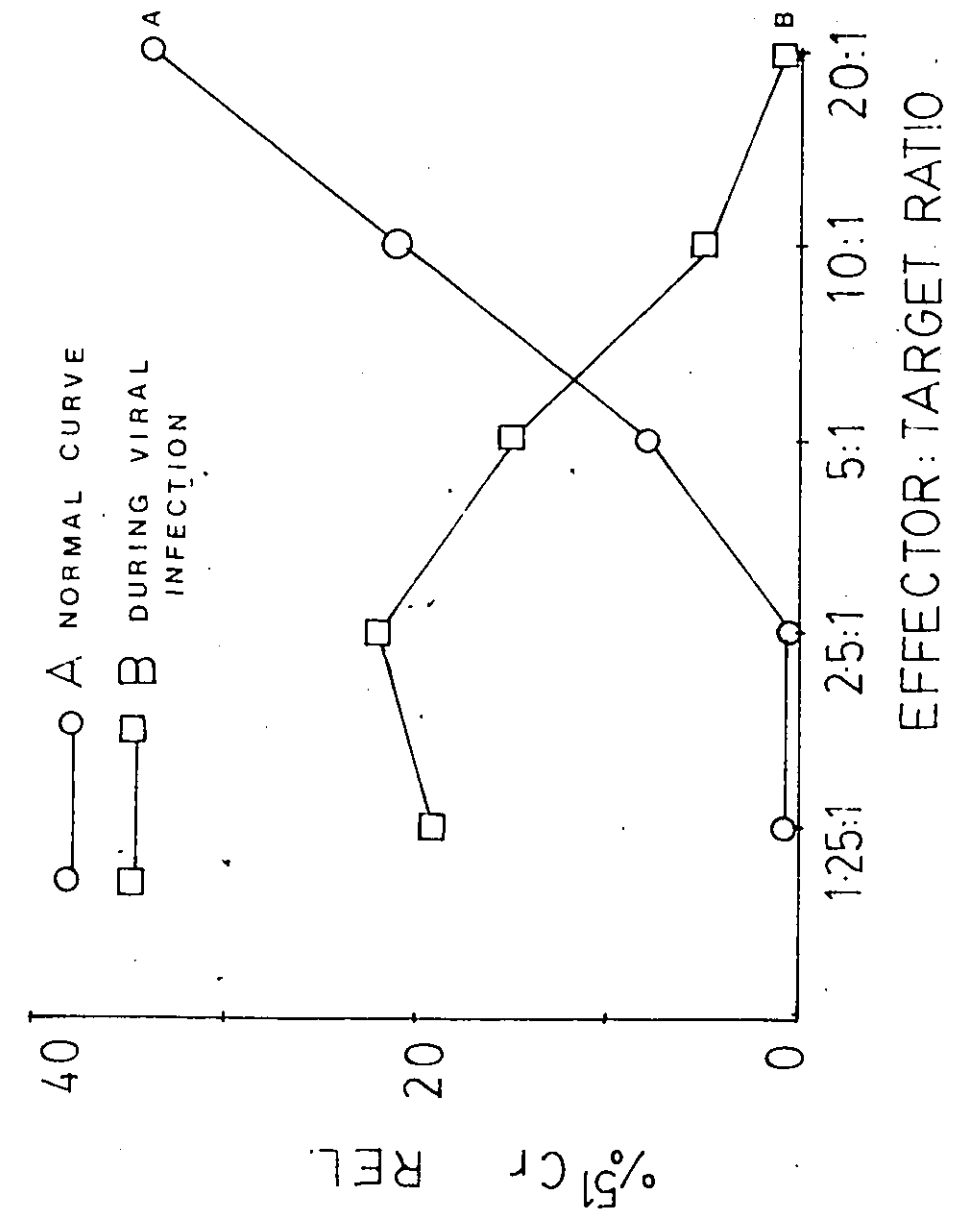


TABLE 4.
EFFECT OF CENTRIFUGATION OF MICROPLATE PRIOR TO CULTURE

Effector:Target Ratio	Percent corrected ^{51}Cr Release	
	Without Pre-centrifugation	With Pre-centrifugation
10:1	51 ± 13	55 ± 8
5:1	55 ± 10	64 ± 2
2.5:1	50 ± 7	57 ± 8

into culture supernatants may be hindered. Disruption of cell pellets followed by centrifugation was thought to be a possible method of releasing the ^{51}Cr labelled macromolecules. Table 5 shows that pellet disruption followed by centrifugation usually yielded a better cytotoxicity figure as compared to supernatants collected from cultures containing undisrupted pellets. Although levels of spontaneous release were also higher, this was not usually a problem. In subsequent experiments, cell pellets were routinely disrupted at the end of the culture period, followed by centrifugation to pellet unlysed cells, after which supernatants were harvested for gamma-counting.

2. IDENTIFICATION OF THE TARGET CELL IN THE NOCC REACTION.

2.1 Serum-free System:

In the original assay described by Behelak and Richter (1972) rabbit spleen cells were used as targets. Lysis of erythrocytes in the rabbit spleen cell preparation by incubation with Tris-buffered ammonium chloride solution prior to ^{51}Cr labelling resulted in enhanced cytotoxicity (Table 6) but unacceptable spontaneous release. Furthermore, severe target cell clumping was occasionally encountered following the lysis of erythrocytes.

2.2 Serum-supplemented System:

In the presence of 10% AB serum, untreated spleen cells were lysed but target cells devoid of erythrocytes were resistant to lysis (Table 7). Subsequent experiments using unfractionated spleen cells and Ficoll-hypaque purified splenic or peripheral blood mononuclear cells (rabbit) or erythrocytes as targets showed clearly that, in the presence of serum, it is the erythrocyte that is susceptible to lysis and not the mononuclear cell fractions (Table 8).

TABLE 5.
EFFECT OF RESUSPENSION/CENTRIFUGATION PRIOR TO HARVEST OF SUPERNATANTS

Effector: Target Ratio	Percent Corrected ^{51}Cr Release (± S.D.) Without Resuspension/ Centrifugation	With Resuspension/ Centrifugation
10:1	49 ± 2	55 ± 8
5:1	49 ± 1	64 ± 2
2.5:1	42 ± 14	57 ± 8

TABLE 6.
EFFECT OF TREATMENT OF TARGET CELLS[a] WITH BOYLE'S SOLUTION[b], ON NOCC[c]

	Untreated Targets		Treated Targets	
	Spont. Rel. (%)	Corr. ^{51}Cr. Rel. (%)	Spont. Rel. (%)	Corr. ^{51}Cr. Rel. (%)
20:1	36 ± 3	23 ± 2	47 ± 3	30 ± 2
10:1	37 ± 2	11 ± 1	52 ± 3	28 ± 2
5:1	37 ± 3	4 ± 1	55 ± 5	14 ± 1
2.5:1	38 ± 4	1 ± 1	54 ± 4	7 ± 1

[a]Rabbit spleen cells.

[b]Tris-buffered 0.83% ammonium chloride solution

[c]Serum-free system

TABLE 7.
EFFECT OF TREATMENT OF TARGET CELLS[a] WITH BOYLE'S SOLUTION[b], ON NOCC[c]

Effector: Target Ratio	Percent Corrected ^{51}Cr Release (± S.D.) with the Following Targets	
	Control[d] Targets	Treated Targets[e]
Expt. 1		
5:1	21 ± 1	10 ± 2
2.5:1	19 ± 2	5 ± 4
1.25:1	16 ± 1	7 ± 3
0.6:1	15 ± 1	5 ± 3
Expt. 2		
5:1	13 ± 3	4 ± 6
2.5:1	13 ± 0	1 ± 3
1.25:1	17 ± 1	5 ± 2
0.6:1	15 ± 2	5 ± 3
Expt. 3		
5:1	22 ± 0	7 ± 2
2.5:1	20 ± 2	5 ± 5
1.25:1	18 ± 0	7 ± 7
0.6:1	11 ± 1	8 ± 2

[a]Rabbit spleen cells

[b]Tris-buffered 0.83% ammonium chloride solution

[c]10% AB serum used in assay.

[d]Incubated in Hank's balanced salt solution for 10 min. then washed.

[e]Incubated with Boyle's solution for 10 min. and washed.

TABLE 8.
COMPARISON OF ERYTHROCYTE AND MONONUCLEAR CELL TARGETS IN NOCC[a]

Effector: Target Ratio	Percent Corrected ^{51}Cr Release (± S.D.) with the Following Targets: Erythrocyte Targets[b]	Mononuclear Cell Targets[c]
Expt. 1		
5:1	22 ± 5	10 ± 1
2.5:1	26 ± 4	11 ± 0
1.25:1	13 ± 1	5 ± 1
0.6:1	4 ± 1	1 ± 0
Expt. 2		
10:1	27 ± 1	0 ± 2
5:1	17 ± 1	5 ± 4
2.5:1	9 ± 0	3 ± 1
1.25:1	5 ± 1	1 ± 3
0.6:1	4 ± 0	2 ± 1
Expt. 3		
10:1	36 ± 2	5 ± 2
5:1	31 ± 2	6 ± 3
2.5:1	22 ± 2	7 ± 2
1.25:1	15 ± 1	11 ± 4
0.6:1	8 ± 0	10 ± 3
Expt. 4	Whole Spleen Cell Targets	Mononuclear Cell Targets[c]
20:1	-13 ± 4	2 ± 4
10:1	-1 ± 2	1 ± 3
5:1	7 ± 4	2 ± 2
2.5:1	15 ± 8	5 ± 5
1.25:1	18 ± 5	10 ± 2
0.6:1	18 ± 5	10 ± 6

[a]All cultures were supplemented with 10% AB serum.

[b]Heparinised rabbit blood was centrifuged, the buffy coat layer removed, then washed before labelling.

[c]Heparinised rabbit blood or rabbit spleen cells were layered on Ficoll Hypaque (sp. gr. 1.1) with 6% methyl cellulose and centrifuged at 900xg. The interface cells were collected, washed and labelled. The differential count of the mononuclear cell fraction was 94% lymphocytes, 5% monocytes, 1% heterophils.

3. TARGET CELL SPECIFICITY:

3.1 The lytic-susceptibility of erythrocytes from different species:

Comparison of erythrocytes from several species showed that rabbit, horse, sheep and bovine erythrocytes are susceptible targets in descending order of sensitivity whereas chicken and human erythrocytes are resistant (Table 9).

3.2 The lytic-susceptibility of human erythrocytes with different major blood group antigens:

Various effector-target combinations crossing ABO antigen barriers revealed no cytotoxicity (Table 10).

4. IDENTIFICATION OF THE EFFECTOR CELLS:

4.1 The role of T lymphocytes:

Depletion of T lymphocytes from the mononuclear cell population resulted in either no change or a slight increase in cytotoxicity (Table 11). The T lymphocyte enriched fraction had no significant lytic activity. The lack of activity of the T lymphocyte enriched fraction was not related to the use of Boyle's solution (buffered ammonium chloride solution) to lyse the adherent erythrocytes in the E-rosette (T lymphocyte) enriched fraction since incubation of unfractionated mononuclear cells with Boyle's solution resulted in only a small decrease in cytotoxicity. When T lymphocytes were incubated for 48 or 72 hours in 20% FBS in RPMI 1640, they became capable of lysing rabbit erythrocytes.

4.2 The role of Fc -receptor-bearing cells:

TABLE 9.
THE LYTIC-SUSCEPTIBILITY OF ERYTHROCYTES OF DIFFERENT SPECIES IN NOCC[a]

	Percent Corrected ^{51}Cr Release with the Following Targets					
	Rabbit RBC	Sheep RBC	Ox RBC	Horse RBC	Chicken RBC	Human RBC
Expt. 1	61[c]	28	24	49	5	4
Expt. 2	77	22	16	60	1	0
Expt. 3	63	32	11	61	2	0
Expt. 4	64	26	17	53	3	ND[b]
Expt. 5	51	53	21	100	5	ND

[a]Effector:target ratio 5:1, with 10% AB serum.

[b]Not done.

[c]Standard deviations not shown (usually 0-5%)

TABLE 10.
LYTIC SUSCEPTIBILITY OF HUMAN ERYTHROCYTES WITH DIFFERENT MAJOR BLOOD GROUP ANTIGENS

Blood Group of Effector Cell Donor	Percent ^{51}Cr Release				
	Targets[a]				
	Rabbit RBC	Human Erythrocytes of Type Indicated			
		O	A	AB	B
O	69 ± 4[b]	0 ± 0 -1 ± 0	-1 ± 0 -1 ± 0	0 ± 0	-1 ± 0 0 ± 0
A	62 ± 3	1 ± 5 -3 ± 0	-1 ± 0	0 ± 1	0 ± 0 -1 ± 0
AB	70 ± 2	0 ± 0 0 ± 0	-1 ± 1 -1 ± 1	-1 ± 1	0 ± 0 0 ± 0

[a]Each effector cell preparation was tested against ^{51}Cr labelled rabbit erythrocytes, autologous erythrocytes (data not shown - no cytotoxicity was seen in any experiment), human erythrocytes (O, A, AB, B), at a ratio of 5:1 in 10% AB serum.

[b]Mean corrected ^{51}release ± standard deviaton from triplicates.

TABLE 11.
EFFECT OF DEPLETION OF T LYMPHOCYTES FROM THE EFFECTOR CELL PREPARATION, ON NOCC

	Percent Corrected ^{51}Cr Release by the Following Effector Cells						
					E-RFC[e] Preincub. in FBS for		
Expt. No.	MC[a]	MC Control[b]	E-RFC Depleted[c]	E-RFC[d] Enriched	24 hrs.	48 hrs.	72 hrs.
1	61	55	53	0	ND	ND	ND
2	55	42	54	13	ND	ND	ND
3	54	49	53	1	ND	ND	ND
4	78	73	75	2	ND	ND	ND
5	53	ND	46	5	-3	11	8
6	43	ND	42	4	8	15	26

[a]Peripheral blood mononuclear cells obtained by Ficoll Hypaque centrifugation.

[b]Mononuclear cells incubated in Boyle's solution for 10 minutes and washed x3.

[c]Mononuclear cells depleted of T lymphocytes by centrifugation of E-rosette forming cells on Ficoll Hypaque gradients.

[d]T lymphocyte pellet obtained from Ficoll Hypaque gradient used for T lymphocyte depletion, treated with Boyle's solution to remove adherent red cells.

[e]T lymphocyte enriched fraction incubated in RPMI 1640 + 20% RBS for 24, 48 and 72 hours prior to assay.

Depletion of Fc -receptor positive cells resulted in a marked decrease in cytotoxic activity of the mononuclear cell fractions whereas enriched fractions of Fc -receptor positive cells were highly cytotoxic (Table 12). The cytotoxicity curve showed a slight shift to the left when Fc -receptor positive cells were used as effector cells (Fig. 6).

4.3 The role of C3-receptor-bearing cells:

Depletion of C3 receptor positive cells resulted in a loss of cytotoxicity whereas C3 receptor positive cell fractions showed enhanced cytotoxic activity (Table 13). The cytotoxicity was increased when C3 receptor positive cell fractions were used as effector cells (Fig. 7).

4.4 The role of Fc -receptor positive (FcR+), C3 receptor positive (C3R+)cells:

Cytotoxic activity was found to be associated particularly with FcR+/C3R- cells rather than FcR-/C3R+ or FcR-/C3R- cells (Fig. 8). FcR+/C3R+ cells were less cytotoxic than unfractionated cells or FcR+/C3R- cells.

4.5 The role of phagocytic cells:

Depletion of phagocytic cells using the carbonyl iron method resulted in marked reduction in cytotoxic activity (Table 14). The method, however, was found to result in selective loss of 19-40% of the lymphocytes as well (Table 15). Phagocyte depletion was effective by this method with usually only 1% contamination by non-specific esterase positive cells.

TABLE 12.
EFFECT OF DEPLETION OF Fc RECEPTOR POSITIVE CELLS FROM THE EFFECTOR CELL FRACTION

	Percent Corrected ^{51}Cr Release (± S.D.) by the Following Cell Fractions[a]		
	Unfractionated[b] Mononuclear Cells	Fc Receptor[c] Negative Cells	Fc Receptor[d] Positive Cells
Expt. 1	77 ± 2	6 ± 1	74 ± 2
Expt. 2	66 ± 1	4 ± 1	64 ± 2
Expt. 3	68 ± 1	4 ± 1	79 ± 1
Expt. 4	69 ± 1	12 ± 1	74 ± 2

[a]Effector cells were cultured with 10^5 ^{51}Cr labelled rabbit erythrocytes at an effector:target ratio of 5:1, in Medl99 with 10% AB serum.

[b]Ficoll Hypaque purified peripheral blood mononuclear cells.

[c]Mononuclear cells depleted of EA-RFC by Ficoll Hypaque centrifugation of EA-rosetted cells.

[d]EA-RFC enriched pellets from centrifugation tubes in c.

[e]All cell fractions were incubated with Boyle's solution prior to assay.

FIG. 6 THE ROLE OF Fc RECEPTOR BEARING CELLS

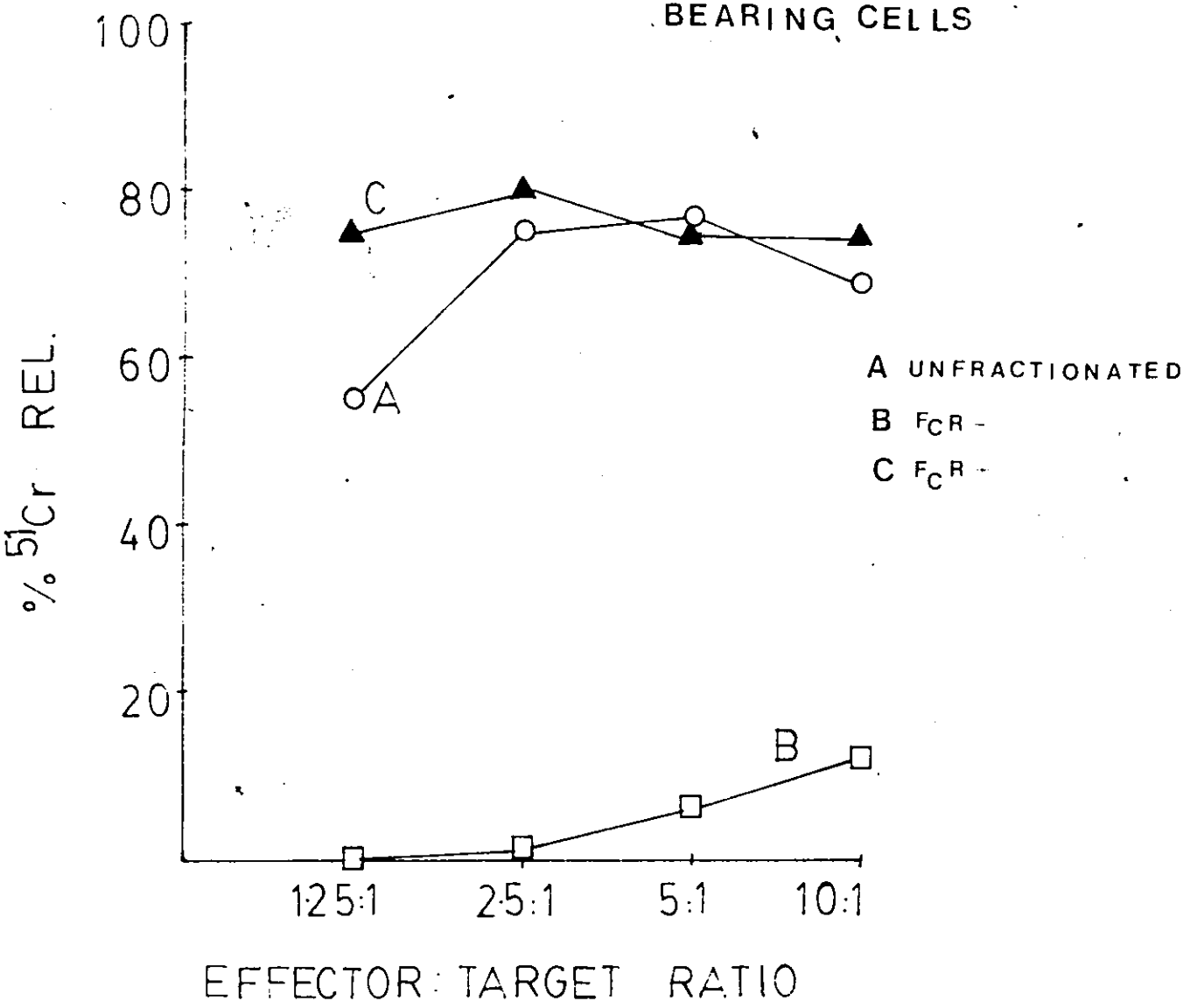


TABLE 13.
EFFECT OF DEPLETION OF C3 RECEPTOR POSITIVE CELLS FROM THE EFFECTOR CELL FRACTION

	Percent Corrected ^{51}Cr Release (± S.D.) by the Following Cell Fractions[a]		
	Unfractionated Cells[be]	C3 Receptor[ce] Negative Cells	C3 Receptor[de] Positive Cells
Expt. 1	58 ± 1	8 ± 1	68 ± 1
Expt. 2	62 ± 1	26 ± 2	67 ± 1
Expt. 3	55 ± 1	2 ± 1	57 ± 1
Expt. 4	68 ± 4	23 ± 2	73 ± 3

[a] Effector cell fractions were incubated with ^{51}Cr labelled rabbit erythrocytes at an effector:target ratio of 5:1, in Medl99 with 10% AB serum.

[b] Ficoll Hypaque purified peripheral blood mononuclear cells.

[c] Mononuclear cells depleted of EAC-RFC by centrifugation of EAC-rosetted cells on Ficoll Hypaque.

[d] EAC-RFC enriched cell fractions collected from the pellets following the Ficoll Hypaque centrifugation in c.

[e] All effector cells were incubated with Boyle's solution prior to the assay.

FIG. 7 THE ROLE OF C3 RECEPTOR BEARING CELLS

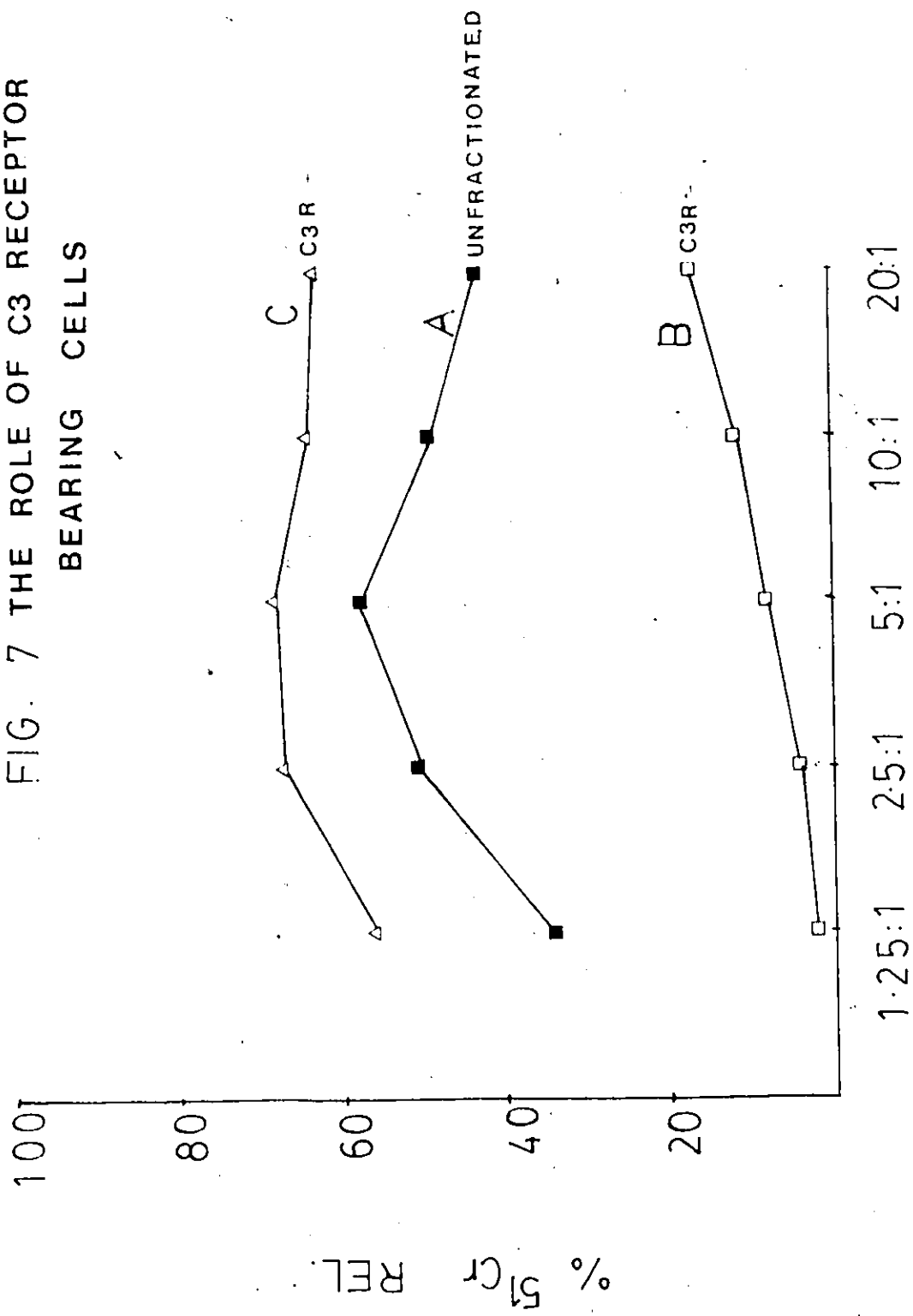


FIG. 8 THE ROLE OF FcR - C3R POSITIVE CELLS

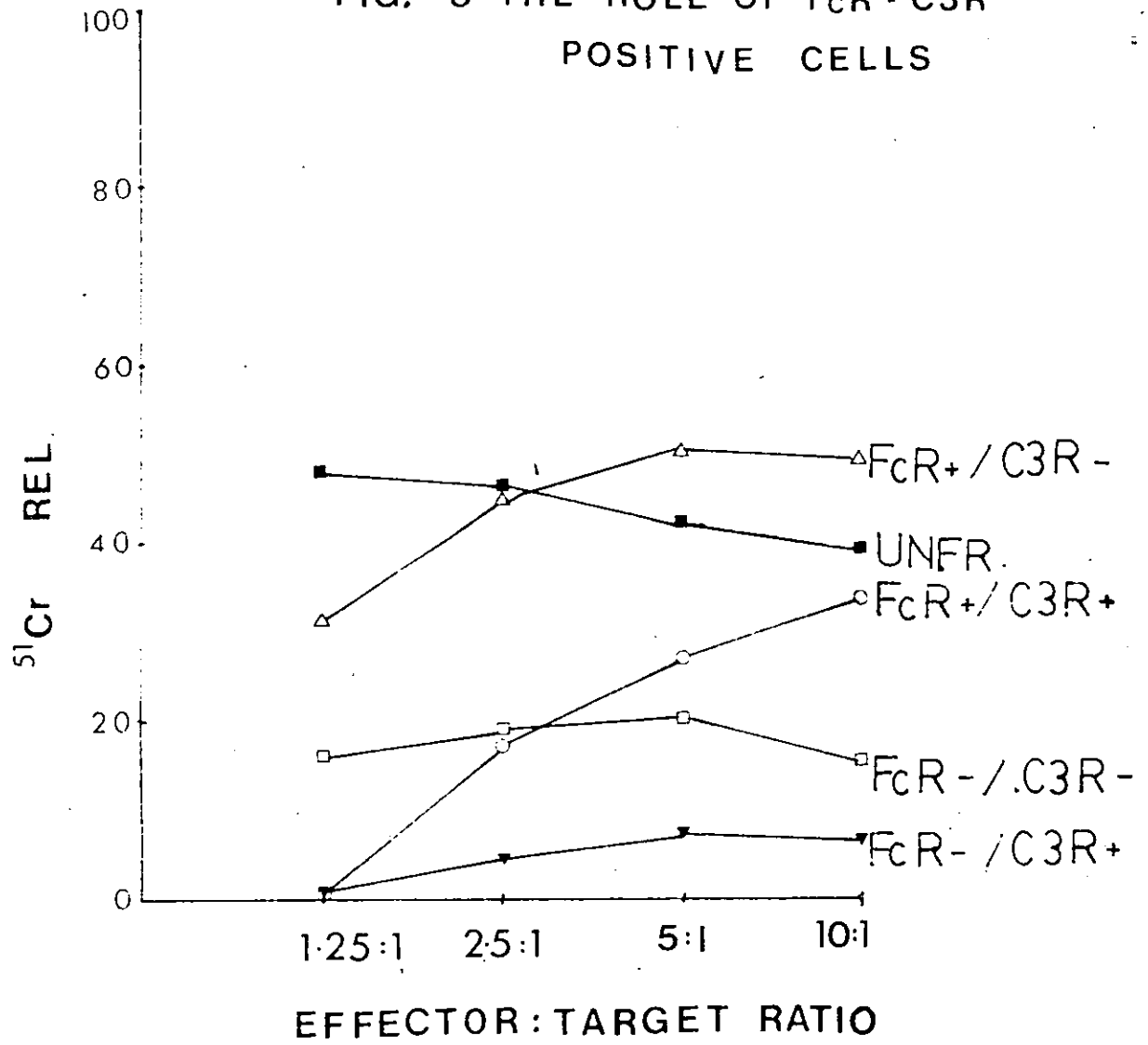


TABLE 14.
THE ROLE OF PHAGOCYTIC CELLS IN NOCC

	Percent Corrected ^{51}Cr Release (±S.D.) by the Following Cell Fractions[a]	
	Unfractionated[b] Mononuclear Cells	Phagocyte Depleted[c] Mononuclear Cells
Expt. 1	43 ± 1	3 ± 1
Expt. 2	57 ± 1	7 ± 1
Expt. 3	58 ± 1	11 ± 1
Expt. 4	43 ± 1	3 ± 1

[a]Effector cell fractions were incubated with ^{51}Cr labelled rabbit erythrocytes at an effector:target ratio of 5:1 in Medl99 with 10% AB serum.

[b]Ficoll-hypaque purified peripheral blood mononuclear cells.

[c]Mononuclear cells depleted of phagocytic cells by incubation with carbonyl iron particles followed by separation of particle containing cells by placing over a magnet.

TABLE 15.
EFFECT OF PHAGOCYTE DEPLETION BY CARBONYL IRON METHOD ON LYMPHOCYTE RECOVERY

	Differential Count (%)			% Recovery of Lymphocytes
	Lymphocytes	Monocytes	Polymorphs	
Expt. 1				
Unfractionated cells[a]	86	13	1	
Phagocyte-depleted cells[b]	98	1	1	60
Expt. 2				
Unfractionated cells[a]	83	15	2	
Phagocyte-depleted cells[b]	98	1	1	70
Expt. 3				
Unfractionated cells[a]	94	6	1	
Phagocyte-depleted cells[b]	98	1	1	72
Expt. 4				
Unfractionated cells[a]	82	16	2	
Phagocyte-depleted cells[b]	98	1	1	81
Expt. 5				
Unfractionated cells[a]	92	8	1	
Phagocyte-depleted cells[b]	99	1	0	80

[a] Ficoll-hypaque purified peripheral blood mononuclear cells.

[b] Mononuclear cells depleted of phagocytes by incubation with carbonyl iron particles followed by magnetic separation.

4.6 The role of plastic adherent cells:

Plastic adherent cells were more cytotoxic than non-adherent cells (Table 16). However, significant cytotoxicity was detectable in the non-adherent fraction. This was probably due to incomplete separation of adherent-non-adherent fractions, although the role of non-adherent cells could not be completely excluded.

4.7 The role of Concanavalin A (Con A) binding cells:

Con A-binding mononuclear cells showed greater cytotoxicity than non-binding cells (Fig. 9). The cytotoxic activity, however, was not equal to that of unfractionated mononuclear cells.

4.8 The role of polymorphonuclear neutrophils (PMN):

PMN were highly cytotoxic, with greater cytotoxic activity on a cell per cell basis than mononuclear cells (Table 17) and the addition of small numbers of granulocytes to phagocyte-depleted mononuclear cell fractions resulted in enhancement of cytotoxicity (Table 18).

5. COMPARISON OF MONONUCLEAR-CELL AND PMN-MEDIATED NOCC:

5.1 Target cell selectivity:

Erythrocytes from different species showed the same pattern of susceptibility to lysis by both types of effector cells (Table 19).

5.2 Kinetics:

PMN cytotoxicity peaked by 2 hours whereas mononuclear cells showed cytotoxic activity only after 4-6 hours of incubation with peak cytotoxicity occurring at 18-22 hours (Fig. 10).

TABLE 16.
THE ROLE OF PLASTIC-ADHERENT CELLS IN NOCC.

	Percent Corrected ^{51}Cr Release (±S.D.) by the Following Fractions:		
	Unfractionated Mononuclear Cells	Non-Adherent Cells	Adherent Cells
Expt. 1[a]	62 ± 1	35 ± 2	60 ± 2
Expt. 2[b]	62 ± 0	31 ± 1	62 ± 2

[a]Differential counts: Unfractionated — 85% lymphocytes, 13% monocytes and 2% polymorphs
Non-adherent — 90% lymphocytes, 9% monocytes and 1% polymorphs
Adherent — 43% lymphocytes, 46% monocytes and 11% polymorphs

[b]Differential counts: Unfractionated — 86.5% lymphocytes, 12.9% monocytes and 0.6% polymorphs
Non-adherent — 97.9% lymphocytes, 2.1% monocytes and 0% polymorphs
Adherent — 67% lymphocytes, 32% monocytes and 1% polymorphs

FIG. 9 EFFECT OF CON A RECEPTOR BEARING CELLS

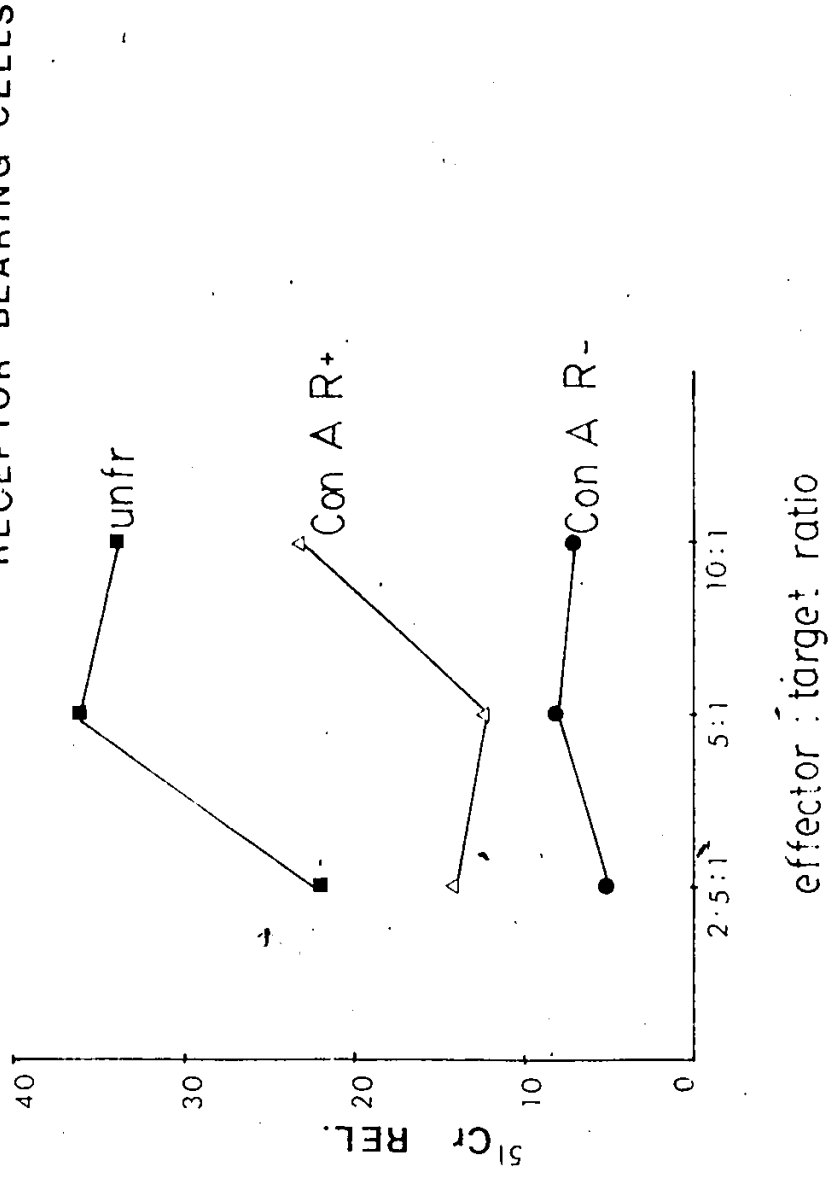


TABLE 17.
CYTOLYTIC ABILITY OF POLYMORPHONUCLEAR NEUTROPHILS

	Effector: Target Ratio	Percent Corrected ^{51}Cr Release (±S.D.) by the Following Cell Fractions[a]: Buffy Coat Cells[b]	Mononuclear Cells[c]	PMN[d]
Expt. 1	20:1	73 ± 3	43 ± 2	85 ± 1
	10:1	73 ± 3	47 ± 1	82 ± 2
	5:1	77 ± 6	52 ± 4	85 ± 2
	2.5:1	73 ± 6	42 ± 1	77 ± 3
	1.25:1	73 ± 3	22 ± 6	71 ± 4
Expt. 2	20:1	80 ± 2	55 ± 3	86 ± 1
	10:1	75 ± 3	50 ± 1	83 ± 3
	5:1	80 ± 3	39 ± 2	84 ± 3
	2.5:1	83 ± 2	26 ± 2	84 ± 3
	1.25:1	78 ± 10	23 ± 2	82 ± 3
Expt. 3	10:1	57 ± 4	40 ± 3	48 ± 6
	5:1	61 ± 1	40 ± 2	60 ± 1
	2.5:1	59 ± 3	25 ± 1	66 ± 2
	1.25:1	48 ± 3	14 ± 0	68 ± 1
	0.6:1	32 ± 3	7 ± 2	65 ± 5

[a] Target cells were ^{51}Cr labelled rabbit erythrocytes. All cultures were supplemented with 10% AB serum.

[b] Heparinized blood was allowed to sediment at unit gravity for 1 hour. The plasma and buffy coat layer were collected, washed and used for further fractionation and as effector cells. (Mean differential count: 5% lymphocytes, 38% monocytes and 57% polymorphs.)

[c] Buffy coat cells from 6 were layered on Ficoll-hypaque gradients and following centrifugation, the interface cells were collected, washed and used for the assay. (Mean differential count: 82% lymphocytes, 14% monocytes and 4% polymorphs.)

[d] Pellets from the Ficoll-hypaque gradients in C were collected, washed and used for the assay. (Mean differential count: 2% lymphocytes, 1% monocytes and 97% polymorphs.)

TABLE 18.
EFFECT OF ADDING PMN TO PHAGOCYTE-DEPLETED MONONUCLEAR EFFECTOR CELLS

	Effector: Target Ratio	Percent Corrected ^{51}Cr Release (±S.D.) by the Following Cell Preparations: PMN[a]	Mc[b]	Phagocyte depleted Mc with the following percentage of added PMN[c]: 0%	1%	3%	10%	20%
Expt. 1	10:1	95 ± 3	42 ± 1	6 ± 1	15 ± 1	33 ± 1	ND	ND
	5:1	93 ± 4	43 ± 1	3 ± 1	8 ± 1	18 ± 1	ND	ND
	2.5:1	94 ± 3	38 ± 1	2 ± 1	5 ± 1	10 ± 2	ND	ND
	1.25:1	86 ± 2	27 ± 1	0 ± 1	2 ± 0	5 ± 2	ND	ND
	0.6:1	66 ± 1	18 ± 1	0 ± 1	1 ± 1	2 ± 1	ND	ND
Expt. 2	10:1	86 ± 5	53 ± 2	6 ± 1	13 ± 2	21 ± 1	60 ± 2	75 ± 5
	5:1	84 ± 6	57 ± 3	7 ± 1	9 ± 1	13 ± 3	50 ± 1	67 ± 5
	2.5:1	87 ± 3	48 ± 1	5 ± 1	6 ± 1	10 ± 4	27 ± 2	44 ± 3
	1.25:1	86 ± 3	28 ± 1	5 ± 2	3 ± 1	7 ± 1	16 ± 1	25 ± 2
	0.6:1	81 ± 4	17 ± 0	7 ± 2	6 ± 2	8 ± 1	12 ± 1	16 ± 1
Expt. 3	10:1	83 ± 6	52 ± 3	18 ± 1	42 ± 2	68 ± 3	86 ± 5	89 ± 5
	5:1	83 ± 6	58 ± 2	11 ± 1	27 ± 2	55 ± 5	83 ± 6	84 ± 3
	2.5:1	87 ± 5	53 ± 5	6 ± 0	14 ± 1	29 ± 3	72 ± 5	82 ± 4
	1.25:1	87 ± 5	38 ± 4	3 ± 1	6 ± 1	15 ± 1	40 ± 3	67 ± 3
	0.6:1	89 ± 6	18 ± 3	1 ± 1	0 ± 1	3 ± 1	16 ± 2	41 ± 3

[a]Polymorphonuclear neutrophils obtained by centrifugation of buffy coat cells on Ficoll-hypaque and collecting the pellet.

[b]Mononuclear cells collected from the interface of the Ficoll-hypaque gradients in a.

[c]Mononuclear cells depleted of phagocytes by carbonyl iron phagocytosis and magnetism, to which pure PMN (from a) were added to the required final proportions (expressed as percentages).

TABLE 19.
NOCC ACTIVITY OF MONONUCLEAR CELLS AND POLYMORPHONUCLEAR LEUKOCYTES AGAINST DIFFERENT ERYTHROCYTE TARGETS

Percent ^{51}Cr Release (±S.D.)*

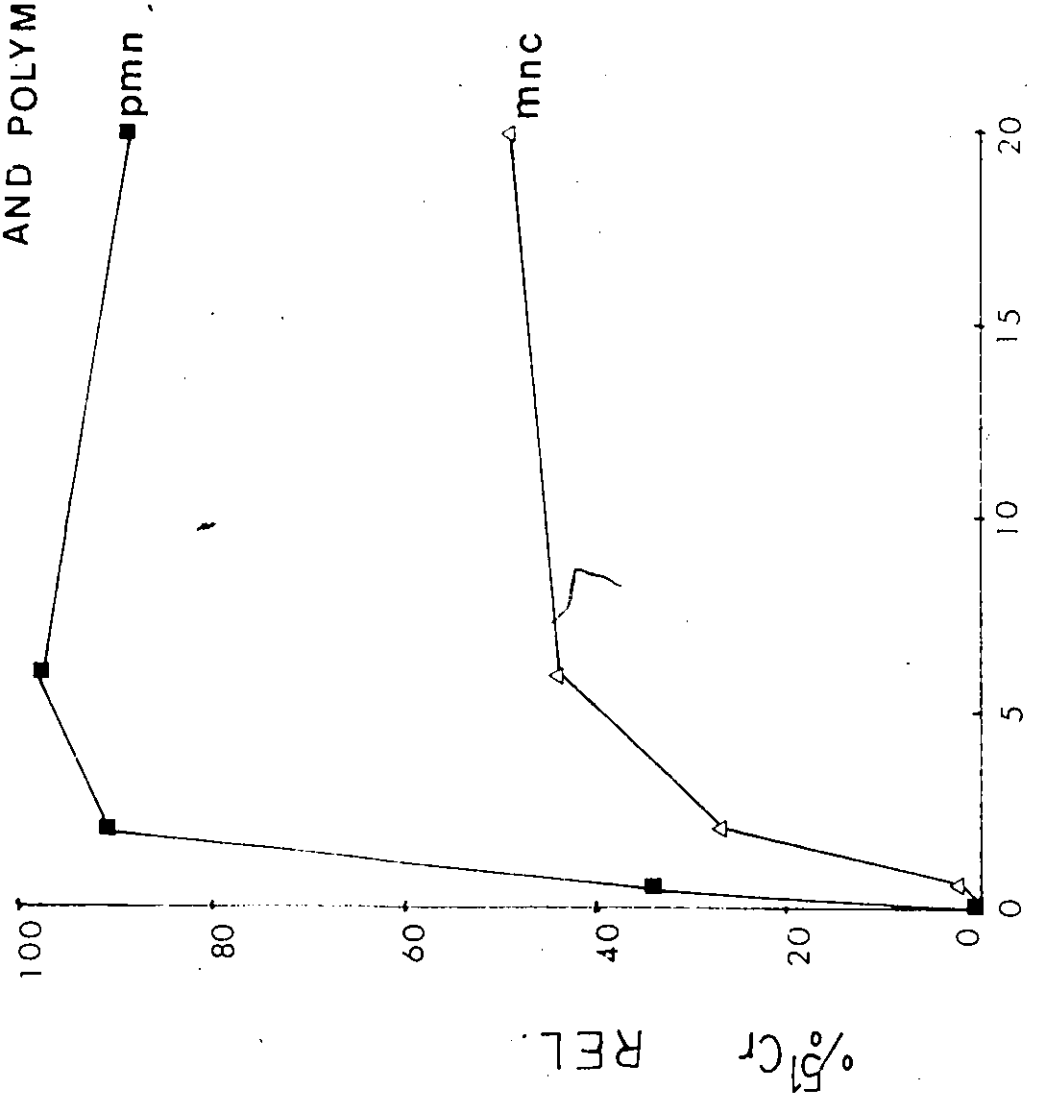
	Rabbit RBC		Sheep RBC		Ox RBC		Horse RBC		Chick.RBC		Human RBC	
	MC[a]	PMN[b]	MC	PMN	MC	PMN	MC	PMN	MC	PMN	MC	PMN
Expt.												
1	61±5	103±10	28±3	61±4	24±2	17±1	49±3	92±8	5±1	9±1	0±1	0±1
2	77±3	100±11	22±1	70±5	16±1	34±2	60±3	91±6	1±1	28±2	0±1	2±1
3	62±4	98± 9	32±3	11±1	11±1	21±2	61±2	93±4	2±1	3±1	0±1	1±1
4	64±3	91± 6	26±2	61±6	17±2	62±4	53±3	83±7	3±1	34±1		
5	51±5	93± 7	53±4	30±3	21±5	12±1	100±8	98±6	5±1	8±1		

[a] Mc = Mononuclear cells.

[b] PMN = Polymorphonuclear leukocytes.

* Effector:Target Ratio: 5:1

FIG. 10 TIME COURSE OF MONONUCLEAR AND POLYMORPHONUCLEAR CELL MEDIATED NOCC



6. THE NATURE OF THE CYTOTOXIC REACTION:

6.1 Lack of evidence for the release of soluble toxic factors:

Supernatants from effector-target cell cultures were not found to have lytic activity (Table 20). Resistant target cells co-cultured with effector-susceptible target mixtures were not lysed (Table 21).

6.2 Are Fc -receptors and/or C3 receptors involved in the effector-target cell interaction?

Blockade of Fc -receptors by heat-aggregated human gammaglobulin failed to inhibit NOCC (Table 22). Blockade of Fc -receptors by IgG sensitized bovine erythrocytes (EA indicator cells) resulted in some inhibition of cytotoxic activity, as did blockade of C3 receptors with IgM-C3 coated bovine erythrocytes (Fig. 11). On the other hand, blockade of both Fc- and C3 receptors simultaneously, resulted in enhancement of cytotoxicity.

7. THE NATURE OF THE ENHANCING FACTOR IN HUMAN SERUM:

7.1 The role of serum gammaglobulins:

Whole AB serum or commercial gammaglobulin-free serum had similar enhancing activity (Table 23). Since the gammaglobulin free serum was found to have detectable amounts of IgA (54 mg%) and IgG (100 mg%), further fractionation was carried out on a Sephadex G200 column. The albumin peak was collected and rechromatographed; the resulting fraction was found to have no detectable immunoglobulins by radial immunodiffusion, but still contained enhancing activity (Table 23).

7.2 Effect of absorption of serum by different erythrocytes:

The enhancing effect was completely abolished by prior absorption of the agamma serum with rabbit, horse or bovine erythrocytes, partially decreased by absorption with sheep erythrocytes but unaffected by absorption

TABLE 20.
FAILURE OF CULTURE SUPERNATANTS TO LYSE ^{51}Cr LABELLED RABBIT RBC

	Percent ^{51}Cr Release (±S.D.)		
	RRBC + Med 199[a]	RRBC + Culture Supernatants[b]	RRBC + MC[c]
Expt. 1	0 ± 1	0 ± 1	73 ± 5
Expt. 2	0 ± 1	0 ± 2	66 ± 3
Expt. 3	0 ± 1	0 ± 1	76 ± 4

[a] ^{51}Cr labelled RRBC incubated with 0.2 ml Medium 199 for 18 hours.

[b] ^{51}Cr labelled RRBC incubated for 18 hours with 0.1 ml Medium 199 + 0.1 ml of supernatants from 18 hour cultures of mononuclear cells + RRBC (Ratio 5:1).

[c] ^{51}Cr labelled RRBC incubated with mononuclear cells (Effector:target ratio 5:1) for 18 hours.

TABLE 21.
FAILURE OF BYSTANDER ERYTHROCYTES TO BE LYSED IN NOCC REACTION

Expt.	Effector:Target Ratio	Percent ^{51}Cr Release ± S.D. from the Following Targets		
		^{51}Cr-RRBC	^{51}Cr-HuRBC	^{51}CrHuRBC + RRBC[a]
1	20:1	40 ± 2	0 ± 1	0 ± 1
	10:1	40 ± 2	0 ± 1	0 ± 0
	5:1	50 ± 3	0 ± 0	0 ± 1
	2.5:1	58 ± 3	0 ± 1	1 ± 1
	1.25:1	57 ± 3	1 ± 1	0 ± 1
2	20:1	41 ± 2	0 ± 1	1 ± 1
	10:1	48 ± 1	0 ± 1	1 ± 1
	5:1	58 ± 3	0 ± 1	0 ± 1
	2.5:1	64 ± 3	0 ± 1	0 ± 1
	1.25:1	55 ± 2	0 ± 1	0 ± 1
3	20:1	51 ± 1	0 ± 1	0 ± 1
	10:1	55 ± 3	0 ± 1	0 ± 1
	5:1	68 ± 2	0 ± 1	0 ± 1
	2.5:1	74 ± 3	1 ± 1	1 ± 1
	1.25:1	68 ± 3	0 ± 1	0 ± 1

[a]Unlabelled rabbit RBC.

TABLE 22.
EFFECT OF HEAT AGGREGATED GAMMAGLOBULINS (HAGG) ON NOCC

Expt.	Effector: Target Ratio	Percent ^{51}Cr Release (±S.D.) in the Presence of the Following Concentrations of HAGG (in ug per ml)			
		0 ug	10 ug	50 ug	500 ug
1	10:1	61 ± 3	63 ± 3	58 ± 2	61 ± 3
	5:1	65 ± 3	62 ± 2	59 ± 2	59 ± 2
	2.5:1	56 ± 2	52 ± 2	51 ± 2	38 ± 1
2	10:1	52 ± 2	48 ± 2	47 ± 2	42 ± 2
	5:1	54 ± 2	41 ± 2	46 ± 3	42 ± 3
	2.5:1	57 ± 3	52 ± 3	52 ± 3	46 ± 3
3	10:1	48 ± 5	49 ± 5	40 ± 3	38 ± 3
	5:1	52 ± 2	53 ± 2	50 ± 2	51 ± 6
	2.5:1	54 ± 3	55 ± 2	58 ± 4	55 ± 7

FIG. 11

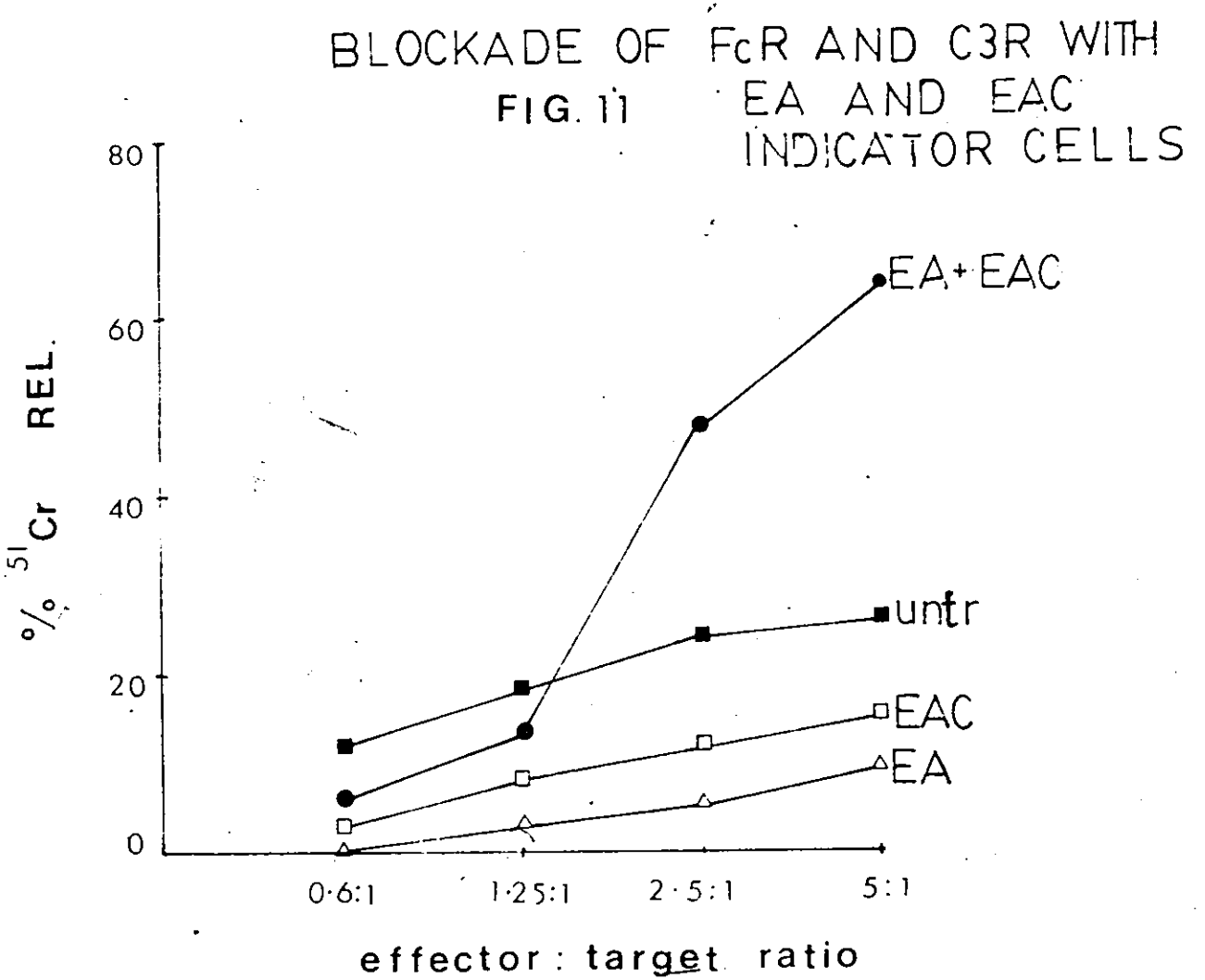

BLOCKADE OF FcR AND C3R WITH EA AND EAC INDICATOR CELLS
80
60
40
20
0
% ^{51}Cr REL.
EA+EAC
untr
EAC
EA
0·6:1
1·25:1
2·5:1
5:1
effector : target ratio

TABLE 23.
NOCC ACTIVITY IN THE PRESENCE OF SERUM FRACTIONS*

	Percent ^{51}Cr Release (± S.D.)	
	Expt. 1	Expt. 2
Normal AB serum[a]	62 ± 6	73 ± 5
Agamma serum[b]	65 ± 5	70 ± 4
Albumin fraction[c]	60 ± 4	72 ± 4
IgG fraction[d]	47 ± 2	51 ± 2
IgM fraction[e]	20 ± 1	27 ± 1

* Protein concentrations in culture were equal in all fractions.

[a] IgA 180 mg%, IgM 140 mg%, IgG 890 mg%

[b] IgA 54 mg%, IgM 0 mg%, IgG 100 mg%

[c] IgA 0 mg%, IgM 0 mg%, IgG 0 mg%

[d] IgA 60 mg%, IgM 0 mg%, IgG 1200 mg%

[e] IgA 34 mg%, IgM 250 mg%, IgG 60 mg%

with chicken or human erythrocytes (Table 24). When cross-over experiments were done, it was clear that the enhancing factor was operative against rabbit, sheep, bovine and horse erythrocytes although there appeared to be specificity for rabbit erythrocytes.

7.3 Temperature sensitivity of the serum factor:

Pre-heating agamma serum to 37°C, 56°C, 60°C and 70°C for 30 minutes followed by cooling to room temperature prior to use in the assay showed that the factor was heat stable up to 60°C but labile at 70°C (Table 25).

8. COMPARISON OF NATURAL KILLING AND NOCC:

8.1 Surface markers of the NK and NOCC effector cells:

Parallel assays with rabbit erythrocytes and K562 cells as targets showed that the effector cells in the two systems are distinct. Macrophage depletion led to a marked decrease in NOCC activity, as previously demonstrated, whereas NK activity was unaffected (Table 26). Depletion of high affinity E-rosette forming cells had no consistent effect on either system (Table 27). Plastic-adherent cells were active against both K562 cells and rabbit erythrocytes but the addition of adherent to non-adherent cells resulted in enhancement of NK activity, although at different adherent:non-adherent cell ratios (Fig. 12).

8.2 Effect of Fc -receptor blockade with heat-aggregated gammaglobulin (HAGG):

In the presence of HAGG, no change in the NK or NOCC cytotoxicity was observed (Table 28).

TABLE 24.
EFFECT OF SERUM PREVIOUSLY ABSORBED WITH DIFFERENT TARGET CELLS, ON NOCC

Target cells used to absorb serum	Percent ^{51}Cr Release from the Following Target Erythrocytes*							
	MC Effectors				PMN Effectors			
	Rabbit	Sheep	Ox	Horse	Rabbit	Sheep	Ox	Horse
Nil	87	53	35	100	93	68	63	100
Rabbit	5	25	11	10	9	2	5	0
Sheep	74	0	18	30	100	0	4	2
Ox	69	14	4	33	80	3	2	2
Horse	100	20	14	2	96	0	2	0
Chicken	100	45	37	95	100	50	58	100
Human	95	46	33	95	99	48	62	100

* Effector:target rato is 5:1. Standard errors not shown (usually less than 6%).

TABLE 25.
TEMPERATURE SENSITIVITY OF THE SERUM FACTOR

Expt.	Effector: Target Ratio	Percent ^{51}Cr Release from Target Cells in the Presence of Serum Previously Subjected to the Following Temperatures			
		37°C	56°C	60°C	70°C
1	20:1	46 ± 3	54 ± 1	48 ± 2	28 ± 3
	10:1	52 ± 2	61 ± 2	52 ± 1	22 ± 1
	5:1	60 ± 3	70 ± 4	60 ± 1	16 ± 4
	2.5:1	67 ± 1	68 ± 0	58 ± 1	9 ± 1
2	20:1	37 ± 1	44 ± 0	36 ± 1	18 ± 9
	10:1	47 ± 3	51 ± 1	41 ± 2	12 ± 4
	5:1	57 ± 1	60 ± 1	51 ± 1	9 ± 2
	2.5:1	62 ± 3	67 ± 2	56 ± 1	8 ± 1

TABLE 26.
EFFECT OF MACROPHAGE DEPLETION ON NOCC AND NK ACTIVITY

		Percent ^{51}Cr Release			
	Effector:	RRBC Targets		K562 Targets	
Expt.	Target Ratio	MC	MDMC	MC	MCMD
1	10:1	48 ± 1	4 ± 1	24 ± 2	31 ± 2
	5:1	54 ± 1	2 ± 1	19 ± 1	26 ± 1
	2.5:1	39 ± 1	1 ± 1	18 ± 3	17 ± 1
2	10:1	37 ± 2	6 ± 3	41 ± 2	50 ± 2
	5:1	52 ± 2	8 ± 2	36 ± 3	42 ± 2
	2.5:1	59 ± 1	5 ± 1	27 ± 1	35 ± 1
3	10:1	51 ± 1	−7 ± 3	34 ± 1	39 ± 1
	5:1	57 ± 2	−3 ± 3	32 ± 1	31 ± 1
	2.5:1	52 ± 1	0 ± 1	30 ± 1	25 ± 1

TABLE 27.
EFFECT OF DEPLETION OF HIGH AND LOW AFFINITY E-ROSETTES

Expt.	Cell Fraction	RRBC Targets	K562 Targets
1	MC	59 ± 2	45 ± 0
	E_{29} depleted[a]	68 ± 1	76 ± 7
	E_4 depleted[b]	47 ± 2	50 ± 0
	E_{29} enriched[c]	8 ± 1	24 ± 1
	E_4 enriched[d]	1 ± 1	30 ± 1
2	MC	52 ± 1	22 ± 3
	E_{29} depleted[a]	58 ± 1	44 ± 3
	E_4 depleted[b]	53 ± 1	34 ± 3
	E_{29} enriched[c]	-4 ± 0	21 ± 6
	E_4 enriched[d]	-4 ± 0	15 ± 1

[a] Lymphocytes were rosetted at 29°C with SRBC (high affinity rosettes) and rosetted cells were removed by Ficoll-hypaque centrifugation.

[b] Same as a but rosetted at 4°C (low affinity rosettes).

[c] Ficoll-hypaque pellet cells from a.

[d] Ficoll-hypaque pellet cells from b.

Rosettes were not lysed with Boyle's solution.

FIG 12

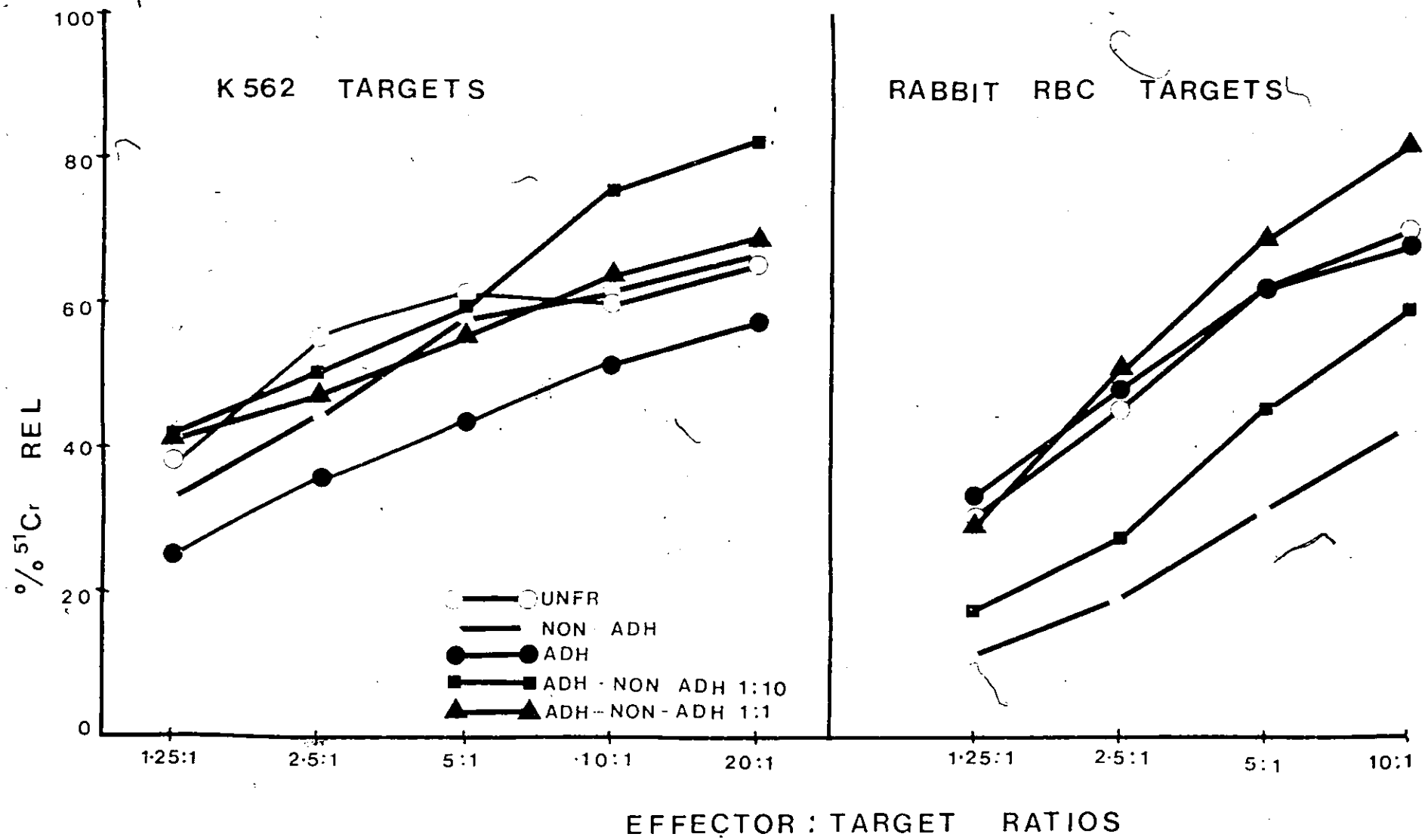

K 562 TARGETS
RABBIT RBC TARGETS
% 51Cr REL
100
80
60
40
20
0
UNFR
NON ADH
ADH
ADH - NON ADH 1:10
ADH - NON - ADH 1:1
1·25:1
2·5:1
5:1
·10:1
20:1
1·25:1
2·5:1
5:1
10:1
EFFECTOR : TARGET RATIOS

TABLE 28.
EFFECT OF HEAT AGGREGATED GAMMAGLOBULINS (HAGG) ON NOCC AND NK ACTIVITY

	RRBC Targets[a]				K562 Targets			
	Percent ^{51}Cr Release ± S.D. in the Presence of the Following Concentrations of HAGG (ug/ml)							
	0	10	50	500	0	10	50	500
Expt. 1	65 ± 1	63 ± 2	58 ± 1	61 ± 2	29 ± 1	26 ± 1	23 ± 1	26 ± 1
Expt. 2	52 ± 1	48 ± 2	47 ± 1	42 ± 1	26 ± 1	28 ± 1	23 ± 1	21 ± 1
Expt. 3	54 ± 1	49 ± 1	44 ± 1	46 ± 1	22 ± 1	20 ± 1	24 ± 1	29 ± 1

[a]Effector:Target Ratio 5:1.

8.3 Effect of Boyle's Solution:

Pre-treatment of effector cells with 0.83% Tris-buffered ammonium chloride solution (Boyle's Solution) resulted in loss of NK activity whereas little or no change was seen in the NOCC activity (Table 29).

9. COMPARISON OF NOCC AND ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC):

9.1 Effect of HAGG:

HAGG added to the culture at various concentrations could inhibit ADCC in a dose-dependent fashion whereas little change was seen in the NOCC activity (Table 30).

9.2 Effect of Trypsin:

Treatment of effector cells with different concentrations of trypsin resulted in a decrease in NOCC activity and not ADCC activity (Table 31).

10. NOCC IN DISEASE STATES:

10.1 Chronic lymphocytic leukemia:

Patients with chronic lymphocytic leukemia (CLL) were found to have depressed NOCC activity (Table 32). Ficoll-hypaque purified CLL cells had lower NOCC activity than buffy coat cells of CLL patients. When CLL cells were incubated with target cells at higher effector:target ratios, no significant increase in cytotoxicity was found (Fig. 13).

10.2 Patients with breast cancer and mammary dysplasia:

Patients with fibrocystic disease of the breast or carcinoma of the breast had significantly depressed NOCC activity in comparison to controls in the same age range (Fig. 14). When patients were tested over an effector:target range, no shift of the cytotoxicity curve was seen in most instances (Fig. 15). However, in two cases, reduced cytotoxicity at 10:1 was

TABLE 29.
EFFECT OF BOYLE'S SOLUTION ON NOCC AND NK EFFECTOR CELLS

	Percent ^{51}Cr Release	
	RRBC Targets[a]	K562 Targets[b]
Expt. 1		
MC, untreated	59 ± 2	59 ± 3
MC, Boyle's treated	60 ± 1	32 ± 6
Expt. 2		
MC, untreated	52 ± 1	30 ± 2
MC, Boyle's treated	53 ± 1	21 ± 1

[a]Effector:target ratio 5:1.

[b]Effector:target ratio 20:1.

TABLE 30.
EFFECT OF HEAT AGGREGATED GAMMAGLOBULINS (HAGG) ON NOCC[a] AND ADCC[b]

Percent ^{51}Cr Release from the RRBC and Antibody-Sensitized RRBC in the Presence of HAGG at the Following Concentrations

	RRBC Targets HAGG Conc. (ug/ml)				Ab-RRBC Targets HAGG Conc. (ug/ml)			
Expt.	0	10	50	500	0	10	50	500
1	56	52	51	38	70	55	5	0
2	57	52	52	46	65	40	8	1
3	56	55	66	58	60	31	1	1
4	54	55	58	55	38	8	2	0

[a]Target cells RRBC without goat anti-RRBC antibody. 10% AB serum in medium. Effector:Target Ratio 5:1.

[b]Target cells RRBC sensitized with goat anti-RRBC antibodies. 6% FBS in medium. Effector:Target Ratio 5:1.

TABLE 31.
EFFECT OF TRYPSIN TREATMENT OF EFFECTOR CELLS, ON NOCC[a] AND ADCC[b]

	Percent ^{51}Cr Release	
	NOCC	ADCC
Untreated MC[c]	48	53
MC treated with 0.5 mg/ml Trypsin	6	50
MC treated with 2 mg/ml Trypsin	8	48

[a]Target cells RRBC without anti-RRBC antibody. 10% AB serum in medium. Effector:Target Ratio 10:1.

[b]Target cells RRBC with goat anti-RRBC antibody. 6% FBS in medium. Effector:target ratio 10:1.

[c]MC = Mononuclear cells.

TABLE 32.
NOCC* IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Percent ^{51}Cr Release

	Normals		CLL Patients	
	Buffy coat cells	FH	Buffy coat cells	FH
1.	65	29	10	0
2.	69	35	19	2
3.	55	30	14	3
4.	65	31	11	2
5.	55	27	13	4
6.	58	32	5	ND
7.	62	29	11	ND
8.	71	28	10	ND
9.	49	32	19	ND
10.	53	37	6	ND
11.	47	ND		
12.	61	ND		
13.	75	ND		
14.	68	ND		
15.	57	ND		
16.	43	ND		
17.	45	ND		
18.	52	ND		
Mean ± S.E.M.	58 ± 2	30 ± 1	12 ± 1[a]	2 ± 1[b]

[a] $p < .0005$

[b] $p < .0005$

Effector:target Ratio = 10:1

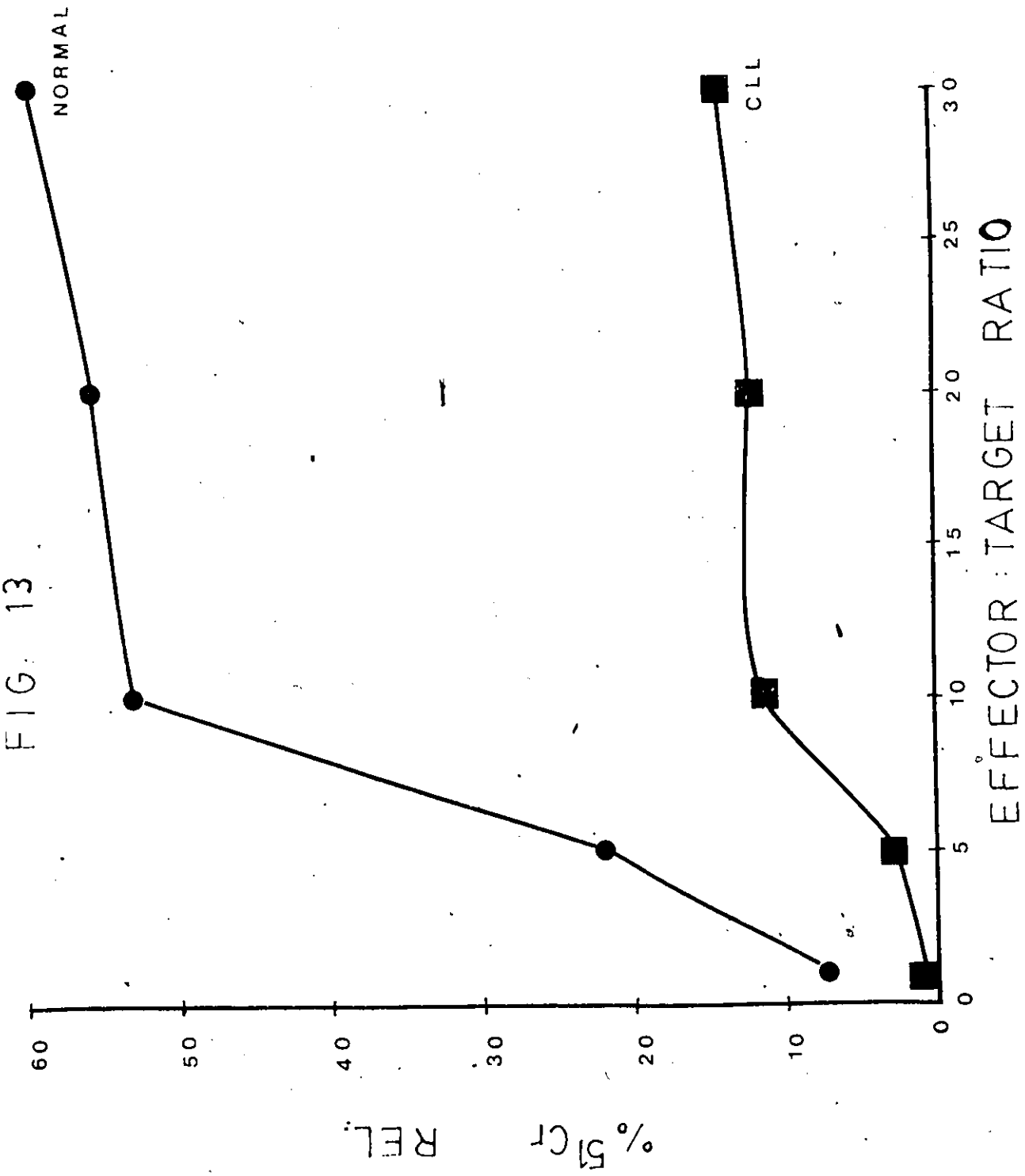

NORMAL
CLL
% ^{51}Cr REL.
60
50
40
30
20
10
0
0
5
10
15
20
25
30
EFFECTOR : TARGET RATIO

FIG. 13

Legend

Figure 14

Mononuclear cell-mediated lysis of ^{51}Chromium-labelled rabbit spleen cells. Effector: target cell ratio 10:1. Each dot represents the mean value obtained for one individual. The horizontal bars indicate the mean corrected ^{51}Chromium release in each group.

\+ = Marked plasma cell infiltrate in stroma of breast

++ = Marked perivenous infiltration and moderate stromal infiltration by lymphocytes

* = Marked tumor necrosis

** = Regional nodes showed marked paracortical hyperplasia

*** = Metaplastic carcinoma (squamous)

**** = Advanced skin involvement

1 All patients

2 All patients excluding the ones marked, +, ++, * and **

N Number studied

SD = Standard deviation

N.S. = Difference not statistically significant (T test)

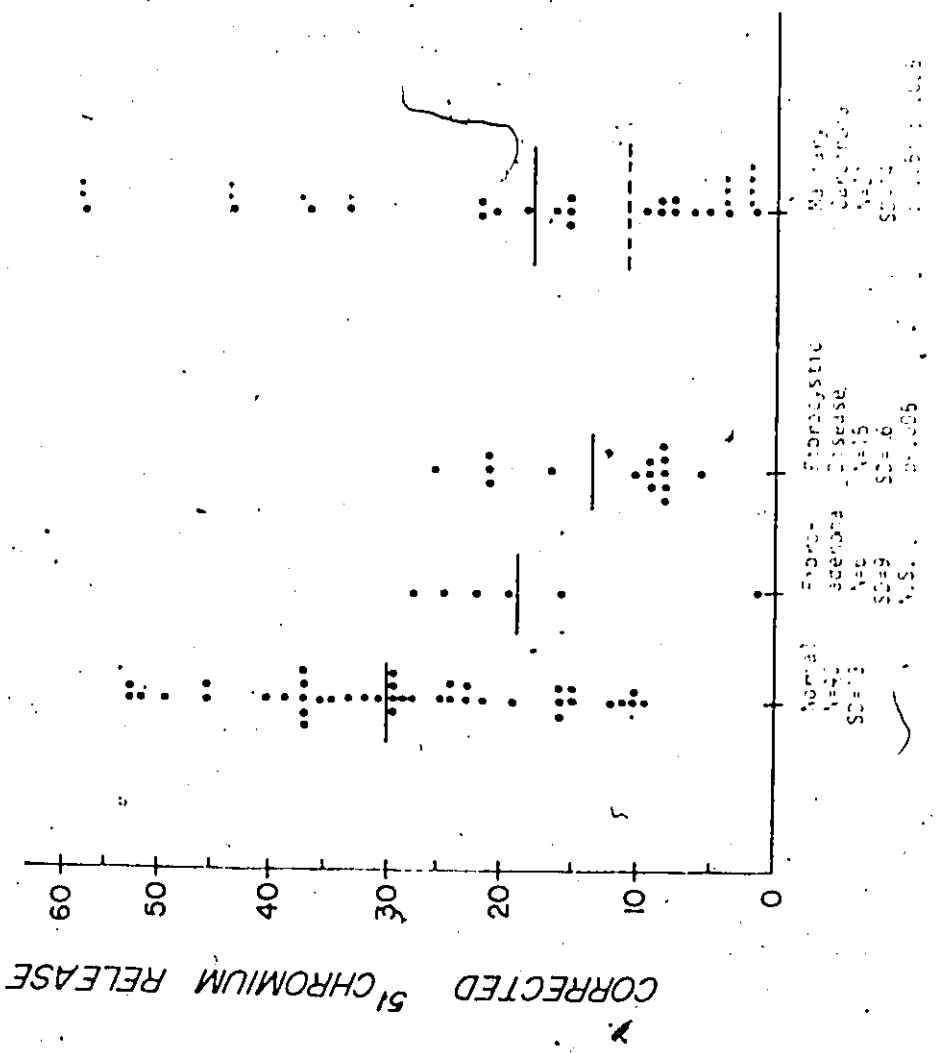


FIG 14

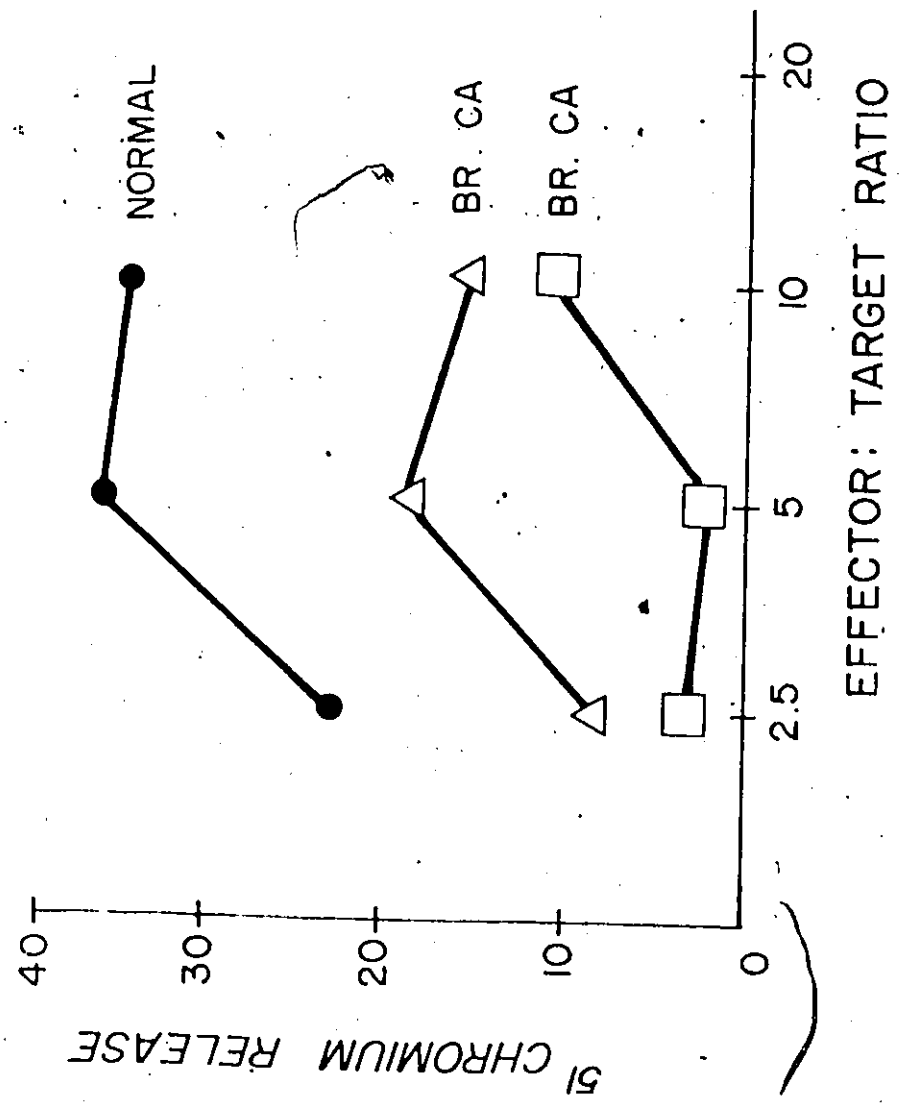
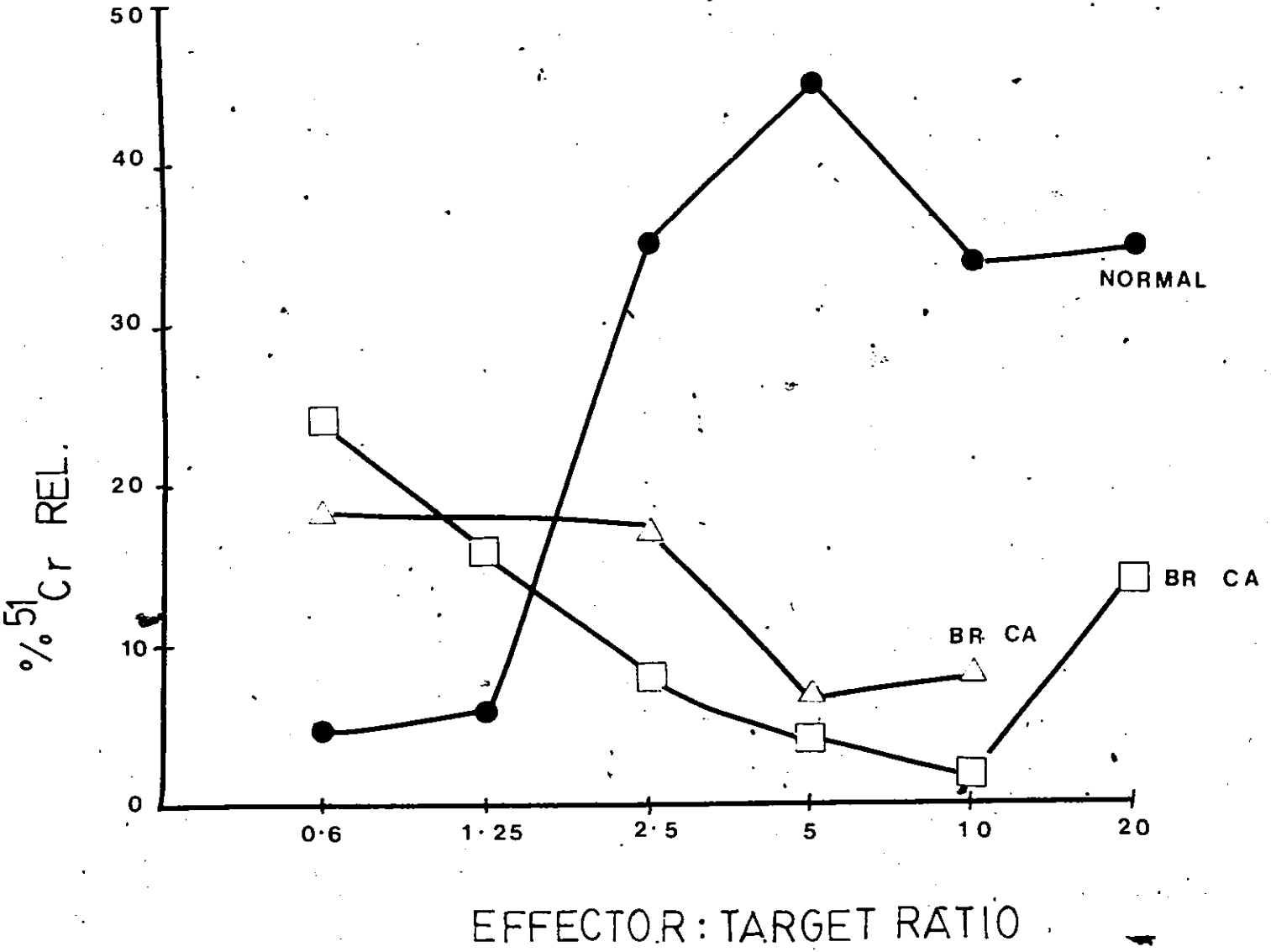

NORMAL
BR. CA
BR. CA
40
30
20
10
0
2.5
5
10
20
^{51} CHROMIUM RELEASE
EFFECTOR: TARGET RATIO

FIG 15

found to be due to a shift of the cytotoxicity curve to the left (Fig. 16). Review of the histopathology of these two cases showed rather unusual patterns: one had a metaplastic (squamous) carcinoma of the breast while the second showed sarcoid-like granulomata in the draining nodes, with advanced skin involvement over the affected breast. Some patients showed normal or increased levels of cytotoxicity. Such patients showed marked tumor necrosis, stromal infiltration by lymphocytes or plasma cells or T-zone hyperplasia in the regional lymph nodes, features not seen in the remainder of the cases.

FIG. 16


50
40
30
20
10
0
% ^{51}Cr REL.
0·6
1·25
2·5
5
10
20
NORMAL
BR CA
BR CA
EFFECTOR : TARGET RATIO

CHAPTER V

DISCUSSION

This work shows that "natural killing" is definable not only in terms of the identity of the effector cell but also by the nature of the target cell, and is a heterogenous system with certain distinctions as well as common denominators. In the fifteen years since the original report by Holm (1967) on cytolytic activity by normal, unsensitized lymphocytes, an incredibly massive amount of data has been generated on natural killing.

Normally circulating cells exhibiting naturally-occurring cytotoxic activity were detected in the circulation of the normal rabbit (Behelak and Richter, 1972; Behelak et al., 1973; Behelak and Richter, 1974) and later in the normal human (Behelak and Richter, 1975; Kay, Bonnard, West and Herberman, 1977; West, Cannon, Kay, Bonnard and Herberman, 1977; Takasugi, Koide, Akira and Ramseyer, 1977; Peter, Eife and Kalder, 1976), mouse and rat (Pross and Baines, 1977; Lotzova and McCredie, 1978; Herberman and Holden, 1978). These cytotoxic cells may be distinguished from the ADCC cytotoxic (or K) cells as their activity is not dependent on the known presence of anti-target cell antibodies (Behelak and Richter, 1972; Behelak et al., 1973; Behelak and Richter, 1974; Timonen, 1979). A number of non-randomly selected allogeneic tumour cells, which bear no apparent antigenic relationship to each other, can function as targets to varying degrees for these normally-occurring cytotoxic cells *in vitro* (Koren and Williams, 1978; Ortaldo et al., 1977; Callewaert et al., 1977; Jondal and Pross, 1975). In view of this apparent anti-tumour cell activity exhibited by these normally-circulating cytotoxic cells *in vitro*, it

has been proposed that these cells confer anti-tumour immunity to the normal host, an activity which is referred to euphemistically as immunological surveillance (Haller, Kiessling, Orn, Karré, Nilsson and Wigzell, 1977)

The results presented here demonstrate that both human neutrophils and monocytes, but not lymphocytes, exhibit cytotoxic activity against the RRBC target cell. Neutrophils attain cytotoxic activity much faster than do monocytes. Neutrophils express almost optimal cytotoxic activity by 2 hours in culture whereas monocytes require more than 6 hours to attain maximum cytotoxic activity.

The cytotoxic reaction described here appears to be dependent upon direct effector cell-target contact of a transient nature and does not involve a soluble cytotoxic factor secreted into the culture medium since (i) only the RRBC target cells were lysed by the effector cells whereas bystander erythrocytes (HuRBC and CRBC) were not affected (Table 21) and (ii) supernatants of NOCC-lysed RRBC were unable to lyse fresh RRBC. Findings of a similar nature have been reported previously by other investigators (Jondal and Pross, 1975; Stott, Probert and Thomas, 1975; Kiessling, Klein and Wigzell, 1975). It is also obvious from the results presented in Fig. 10 that the duration of cell-cell contact must be short (minutes, rather than hours) as significant neutrophil-mediated lysis is detected within 30 minutes of incubation and maximum lysis is evident within 2 hours of incubation.

The NOCC cytotoxic reaction can be compared and contrasted with the SLMC cytotoxic reaction originally described by Pross and Jondal (1975). In both of these reactions, cytotoxicity is carried out by normally-circulating cells of individuals not previously intentionally or knowingly exposed to the target cells. These cytotoxic cells are referred to as natural killer or NK cells. Pross and Jondal (1975) detected cytotoxic activity only among the lymphocytes

utilizing the K562 target cells and coined the term spontaneous lymphocyte-mediated cytotoxicity (SLMC) on the assumption that only lymphocytes are endowed with this form of cytotoxic activity. The demonstration of cytotoxic monocytes is supported by the findings of Melsom and Seljelid (1973), Stott et al. (1975) and Muchmore, Decker and Blaese (1979) who observed that macrophages are capable of mediating lysis of xenogeneic non-malignant target cells, and of Horwitz, Kight, Temple and Allison (1979) and Mantovani, Jerrells, Dean and Herberman (1979) who demonstrated that monocytes are capable of killing tumour cells. Therefore, since monocytes and neutrophils can exhibit natural killing activity as well as lymphocytes, the less restrictive term NOCC should be used to generally define the reaction as it allows for the consideration of cytotoxic cells other than lymphocytes. The SLMC reaction constitutes only one form of the NOCC reaction, that in which cytotoxicity is mediated by lymphocytes. It may be that distinct subclasses of NOCC cytotoxic cells have evolved in the normal unimmunized animal, under the influence of as yet undefined stimuli, characterized by differences in their morphology, cell-surface receptors and target-cell specificity.

The results of this investigation indicate that the NOCC cytotoxic reaction can be distinguished from the antibody and Fc receptor-dependent ADCC cytotoxic reaction on the basis of the following criteria: (i) soluble aggregates of human gammaglobulin in high concentration (500 ug per ml) do not inhibit the NOCC reaction whereas these identical aggregates in a much lower concentration (50 ug per ml) can totally inhibit the ADCC reaction (Table 30); (ii) fetal calf serum inhibits the NOCC cytotoxic reaction (Table 3) whereas FCS is required for the optimal expression of the ADCC cytotoxic reaction (Keaney et al., 1979); (iii) the NOCC reaction can take place in the total absence of serum although the cytotoxicity is considerably lower than that

observed in the presence of even small quantities of serum. Furthermore, agamma serum and immunoglobulin-free human serum albumin are just as supportive of the NOCC reaction as is normal serum (Table 23); (iv) the absence of a stochiometric or dose-response relationship between the cytotoxic activity of the effector cells and the concentration of the normal human serum used to supplement the culture medium which precludes participation by antibodies; (v) the failure of cell-free supernatants to mediate lysis in the presence of the effector and target cells; and (vi) exposure to trypsin resulted in the total loss of NOCC cytotoxic activity without any accompanying deleterious effect on the ADCC cytotoxic activity. Thus, trypsin has no deleterious effect on the Fc receptor.

In order to define the cell surface markers of the cytotoxic cell in the NOCC assay system, depletion or enrichment of specific subpopulations bearing definable cell surface receptors was carried out. It is clear that freshly isolated T lymphocytes have no significant NOCC activity (Table 11). Unlike the NK system using K562 targets, the lack of cytotoxic activity by T lymphocytes could not be attributed to the use of Boyle's solution to lyse rosetted erythrocytes in the T cell enriched fractions (West et al., 1977), since Boyle's solution had no effect on the NOCC activity of unfractionated mononuclear cells. When T lymphocytes were incubated for 48 to 72 hours in 20% FBS in RPMI 1640, they did become cytotoxic although not to the same extent as unfractionated cells. There are two possible explanations for the appearance of T cell mediated NOCC after prolonged in vitro culture. Firstly, there may be a loss of a suppressor cell during the in vitro culture, as shown by Muchmore et al. (1977b). Secondly, culture of T cells in RPMI 1640 with FBS is known to "unmask" or lead to the expression of IgG Fc receptors (Moretta et al., 1976). It is possible that the acquisition or expression of

IgG Fc receptors is directly related to or a parallel occurrence during the induction of T cell mediated NOCC. Certainly, Fc receptors are present on NOCC effector cells (Table 12 and Fig. 6). The effector cells were also found to express C3 receptors (Table 13, Fig. 7). However, when the question of dual expression of FcR/C3R was addressed, unexpectedly, the highest cytotoxic activity was found in the FcR+/C3R- subset, with the FcR+/C3R+ subset less cytotoxic on a cell per cell basis (Fig. 8). It may be argued that simultaneous rosetting of the FcR+/C3R+ population may have led to some suppression of its NOCC activity through an unknown mechanism, possibly through steric hindrance of an effector site closely situated to both FcR and C3R. This is not likely to be the explanation since blocking of FcR and C3R receptors simultaneously resulted in enhanced cytotoxicity rather than inhibition (Fig. 11). A more probable explanation may be that the NOCC cell may modulate its surface receptors more rapidly than other FcR/C3R cells. In this situation, the first rosetting procedure (in this study, EA rosetting) may lead to modulation (loss) of both Fc and C3 receptors rapidly so that in the second (EAC) rosetting procedure, the effector cell would remain in the Ficoll-hypaque interface while non-modulating cells would go down to the pellet fraction.

Although the NOCC effector cell clearly expresses IgG Fc receptors, it appears unlikely that the Fc receptors have any direct role in the NOCC reaction, unlike the ADCC reaction, since HAGG fails to inhibit NOCC. HAGG binds to Fc receptors, thus resulting in blockade of Fc receptors (Keaney et al., 1979). Blockade of Fc receptors by immune complexes such as EA indicator cells resulted in some inhibition of NOCC, but this may be due to cold competition by ORBC which are NOCC-sensitive targets although less susceptible to lysis than RRBC (Table 9). Similarly, blockade of C3R by EAC indicator

cells may be related to cold competition by ORBC. The enhancement of NOCC by simultaneous blockade of Fc and C3 receptors is an interesting phenomenon which may indicate that occupation of FcR and C3R simultaneously may be capable of activating NOCC effector cells, or may recruit precursor effector cells by activation.

The separation of mononuclear cells by plastic adherence showed that adherent cells were more cytotoxic than non-adherent cells (Table 16) but for optimal cytotoxicity, both adherent and non-adherent cells were required (Fig. 12). This was also noted in the NK system against K562 cells. It is possible that the adherence method may activate lytic ability in both types of effector cells against both target cell types. Fischer et al. (1981) have also noted that monocytes isolated by adherence techniques are cytotoxic to K562 cells, particularly if autologous serum rather than FBS is used during incubation in petri dishes. The NOCC effector cell also possesses membrane receptors for Concanavalin A (Fig. 9). Concanavalin A (Con A) receptor positive cells were more cytotoxic than Con A receptor negative cells, although unfractionated cells were more cytotoxic on a cell per cell basis. The NOCC effector cell may be inhibited by Con A or Con A may activate a suppressor cell capable of inhibitng NOCC activity. Mitogen induced suppression of spontaneous monocyte mediated lysis of erythrocyte targets has been reported by Muchmore et al. (1979), using Pokeweed mitogen. A similar effect by Con A cannot be ruled out yet.

Depletion of phagocytic cells by the carbonyl iron-magnetism method results in a marked reduction of NOCC activity (Table 14). This, taken with the evidence of FcR expression and plastic adherence would strongly be in favour of the NOCC effector cell being a monocyte. However, since some loss

of lymphocytes also occurs, the possibility of selective loss of FcR+, adherent lymphocytes, during phagocyte depletion cannot be excluded, without further study.

The role of serum in the NOCC reaction is an interesting question. It was found that whole pooled AB serum or gammaglobulin free serum could support the reaction. In fact, NOCC enhancing activity was found in the protein peak close to albumin (Table 23). This finding is identical to that by Koide and Takasugi (1980) of the N cell activating factor (NAF). Like NAF, the NOCC enhancing factor was found to be heat sensitive (Table 25) to temperatures above 60°C. NAF, and probably NOCC enhancing factor, may be related to interferon if not identical (Koide and Takasugi, 1980). A similar factor has been described by Muchmore et al. (1979b and 1979c) which induces cytotoxicity by both monocytes and T lymphocytes. If this factor is, indeed, identical to interferon, it would indicate that a variety of effector cells, ranging from monocytes, T lymphocytes and NK cells (Einhorn et al., 1978a) can be activated by interferon to become spontaneously cytotoxic.

Although the NOCC enhancing factor appeared to be a non-immunoglobulin molecule, it was absorbable by rabbit, horse or bovine erythrocytes, and to a lesser extent by sheep erythrocytes but not chicken or human erythrocytes. Furthermore, the absorbed sera did not facilitate or augment the NOCC cytotoxic activity of the monocytes and polymorphonuclear cells to the same degree. With respect to the rabbit RBC targets and the monocyte effector cells, only the rabbit RBC could absorb out the cytotoxic enhancing factor; absorption of the serum with any of the other RBC did not adversely affect the capacity of the serum to sustain optimal monocyte-mediated NOCC lysis of the rabbit RBC. With respect to the horse, sheep and ox RBC targets and monocyte effector cells, such specificity associated with absorption with the RRBC was

lacking. Serum absorbed with any of these three RBC displayed a markedly diminished capacity to enhance the monocyte-mediated NOCC lysis with respect to any of these three RBC targets, but more striking was the total loss of the capacity to enhance the NOCC lysis with respect to the absorbing erythrocyte (Table 3). Furthermore, even serum absorbed with rabbit RBC displayed minimal capacity to sustain NOCC lysis of the horse, sheep or ox RBC target cells. The results with the polymorphonuclear cytotoxic cells were in some respects similar but in other respects more extreme than those obtained with the monocytes. With respect to the rabbit RBC targets, only the serum absorbed with rabbit RBC lost its ability to sustain the NOCC cytotoxic activity. However, with respect to the horse, sheep and ox RBC targets, absorption of the serum with any of the four RBC (rabbit, horse, sheep or ox) resulted in total absorption of the cytotoxic enhancing factor. The RBC-absorbed sera could not sustain the NOCC lysis of any of the RBC targets by the polymorphonuclear effector cells. Therefore, as with the monocyte effector cells, the absorption of the serum with the rabbit RBC imparts a degree of specificity to the assay system since only the rabbit RBC could absorb out the cytotoxic enhancing factor with respect to rabbit RBC targets. The non-specific absorptive capacities of the horse, sheep and ox RBC were most pronounced when the polymorphonuclear leucocytes were assessed for cytotoxic activity with respect to the horse, sheep or ox RBC targets.

The results with the monocyte effector cells suggest that the NOCC cytotoxic-enhancing factors in normal human serum are heterogeneous, that there are at least two in number, one specific for the target cell and one present on all erythrocyte target cells with the possible exception of the rabbit RBC. It may be necessary to postulate a third, non-specific,

cytotoxic-enhancing factor to account for the loss of neutrophil-mediated NOCC activity in the presence of absorbed serum under conditions where the effector monocytes exhibit significant cytotoxic activity.

There is therefore a general but not an absolute relationship between an erythrocyte which can function as a target cell and its ability to absorb the cytotoxic enhancing factors in normal serum which are required for the expression of maximum NOCC cytotoxic activity by the effector monocytes and neutrophils. These factors cannot be considered to be antibodies in view of the fact that serum albumin and agamma serum can substitute for serum in the NOCC assay and the non-specific manner whereby they are absorbed.

Is the lysis of the erythrocytes related to Forssman heterophile antigens? This appears to be unlikely since sheep and chicken erythrocytes, which are known to possess Forssman antigens (Boyd, 1966) are only weakly susceptible or resistant to NOCC lysis whereas rabbit and horse erythrocytes, which are Forssman antigen negative, are highly susceptible to lysis.

Patients with chronic lymphocytic leukemia (CLL) were found to have reduced NOCC activity in comparison to normal subjects assayed simultaneously (Table 31). This was true with buffy coat cells as well as Ficoll-hypaque purified mononuclear cells. There was no shift of the cytotoxicity curve when the assay was performed at various effector:target ratios, indicating that there was a true lack of NOCC activity in CLL patients. This was also reported by Pross and Baines (1976) using peripheral blood mononuclear cells against K562 targets. Zeigler et al. (1981) showed that this lack of NK activity in CLL was not due to dilution of the effector cells by large numbers of leukemic B lymphocytes since B cell depleted preparations also lacked NK activity. When they treated CLL lymphocytes depleted of B cells with interferon, only early stage CLL patients' NK activity was enhanced. Although

T_G cells were numerically greater in CLL patients than in normals, the NK activity was 6 times lower. Since the incidence of second malignancies (skin cancers) in CLL patients is eight times higher than in control subjects (Manusow and Weinerman, 1975) the lack of NOCC or NK activity may be implicated, resulting in diminished immunosurveillance.

Patients with Chediak-Higashi syndrome have been found to lack NK activity and also develop lymphoproliferative diseases (Haliotis et al., 1980; Klein et al., 1980; Roder et al., 1980). It is tempting to speculate that the NK cell defect may predispose such patients to develop lymphoproliferative disorders. However, whether NK defects pre-exist before the development of malignancy, such as in CLL, or are secondary effects of malignancy, cannot yet be clarified. It is particularly important to assay NK activity in patients with non-haematopoietic malignancies, in particular, patients with pre-malignant conditions. When patients with mammary diseases such as fibrocystic disease, fibroadenoma and mammary carcinoma were surveyed, it was clear that NOCC was depressed not only in carcinoma patients but also those with fibrocystic disease. Patients with fibroadenoma had a lower mean NOCC value than controls but this was not statistically significant because of the small sample size, so that no conclusion can be made regarding NOCC in patients with fibroadenoma. Patients with mammary carcinoma are thus the second group that we have identified with depressed NOCC activity.

The association of depressed NOCC activity in patients with biopsy proven fibrocystic disease raises some interesting questions which need to be answered by further investigation. The significance of fibrocystic disease as a condition associated with a higher risk of developing mammary carcinoma has been a much debated issue. In prospective studies of mammary carcinoma in patients with benign breast lesions, a relative risk of between 1.4 to 2.7 has

been arrived at (Donnelly et aL., 1975; Monson et al., 1976; Kodlin et al., 1977; Page et al., 1978). In one of the recent reports, Hutchinson et al. (1980) report a relative risk of 2.1 and conclude that the higher risk is associated with such benign lesions as epithelial hyperplasia and papillomatosis especially with calcification, but not with cysts, apocrine metaplasia, adenosis, sclerosing adenosis or fibroadenoma without fibrocystic disease. Clearly not all lesions classified as "fibrocystic disease" by pathologists are associated with an increased risk of future malignancy. If certain forms of fibrocystic disease are indeed associated with a higher risk of future breast cancer and NOCC is depressed in patients with fibrocystic disease, it is possible that NOCC activity is defective prior to the development of carcinoma and is not a secondary phenomenon. This again supports the possibility for a surveillance role for the NOCC effector cell.

The effector cell in the NOCC system has many features characteristic of a monocyte. No decrease in the proportion of monocytes in the isolated mononuclear cell fraction was noted in the patient groups; a functional defect rather than numerical deficiency may thus be responsible for the depressed NOCC activity. Although the NOCC assay does not directly measure tumoricidal capabilities, it does measure the ability of monocytes to mediate a cytolytic reaction against a standard target cell. Tumoricidal activity of macrophages has been reported by several investigators and is a non-phagocytic process (Evans, 1973; Hibbs, 1976). In some experimental systems tumoricidal macrophages have been found within regressing tumors whereas progressively growing tumors contain macrophages which are poorly tumoricidal (Russel and McIntosh, 1977).

Multiple factors may influence the level of NOCC activity and need to be investigated further. Patients with breast cancer showing tumor necrosis, plasma cell or lymphocytic infiltration of breast stroma or paracortical (T-zone) hyperplasia of regional nodes had higher NOCC levels than patients without these features. Whether these differences are statistically significant is not known since the numbers in our study were small. It is known that NK activity can be enhanced by interferon (Saksela et al., 1979; Mantovani et al., 1980). Tumor necrosis serum is thought to contain interferon and also stimulates NK activity (Chun et al., 1979). It is possible, therefore, that in patients with necrotic mammary carcinomas, there may be interferon-induced activation of natural cytotoxicity. Interleukin 2 has been reported to enhance NK activity. Paracortical lymph node hyperplasia indicates T cell activation. It is possible that such activated T cells may release interleukin-2 to stimulate NK activity or macrophage activating factor (MAF) which may augment NOCC.

In vitro treatment of effector cells with cis-diamminedichloroplatinum (cisplatin) has been found to augment NOCC (Kleinerman et al., 1980a). Furthermore, treatment of cancer patients with cisplatin leads to increase in NOCC activity (Kleinerman et al., 1980b). Thus agents which enhance monocyte function may be effective in cancer therapy.

Other monocyte/macrophage defects have been shown in cancer patients, including defective maturation (Krishnan et al., 1980) and decreased Fc receptor expression (Rhodes et al., 1981). Although monocyte mediated natural killing appears to be distinct from NK cell-mediated killing, there may be interaction between the two cell types and this may be detrimental to NK lysis. Seaman et al. (1982) have shown that monocytes and neutrophils can be induced by phorbol diesters or zymosan to inhibit NK activity by the release

of superoxide anion and hydrogen peroxide. If such *in vivo* interaction occurs, it is possible that activation of monocytes may be antagonistic to NK cell activity. Results such as Seaman's and his co-investigators indicate that NK and NOCC immunosurveillance systems may be far more complex than currently appreciated because of unknown interactions.

Further work in examining the relationship of monocytes and NK cells is clearly indicated.

CHAPTER VI

CONTRIBUTION TO KNOWLEDGE

1. The candidate has determined the nature of the cells mediating naturally occurring cytotoxicity *in vitro* in man.
2. The candidate has determined the nature of the target cells in the NOCC reaction.
3. The candidate has shown that NOCC is distinct from the antibody dependent cellular cytotoxicity (ADCC) reaction.
4. The candidate has shown that NOCC is similar to natural killing as defined by lysis of K562 cells.
5. The candidate has shown that more than one type of leukocyte can mediate NOCC under appropriate conditions.
6. The candidate has shown that the NOCC reaction can occur in the absence of serum but is augmentable by the addition of human serum but not fetal bovine serum.
7. The candidate has shown that the serum NOCC enhancing factor is a non-immunoglobulin, heat sensitive protein.
8. The candidate has shown that the serum NOCC enhancing factor can be absorbed onto erythrocytes from different species to variable degrees.
9. The candidate has shown that patients with chronic lymphocytic leukemia have depressed NOCC activity.
10. The candidate has shown that NOCC activity is depressed not only in patients with breast cancer but also in patients with fibrocystic disease of the breast.

CHAPTER VII

BIBLIOGRAPHY

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Post-graduate Training University of Minnesota Hospitals
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Degrees and Honors (name of conferring institution and date received)

M.B., Ch.B. Makerere University, 1971

F.R.C.P.(C) Royal College of Physicians of Canada, 1977

PROFESSIONAL INFORMATION

Date and Rank of U.W.O. Appointment

(position)	(department)	(date)
Assistant Professor	Pathology	July 1, 1978

Change of Status at U.W.O. (Dean's Office Use Only)

(position)	(department)	(date)

Date When Tenure Conferred _____

University and Faculty Committee Appointments, Indicating Positions Held on Such Committees:

From: (date)	To: (date)	(Name of Committee)	(Position)
1978	Present	A.P.T.	Member
1980	Present	Tissue Committee, U.H.	Member
1979	Present	Laboratory Services	Member
1979	1981	Programme Advisory Cttee, Meidcal Technology Tng. Prog.	Member
1981	Present	Epics V Cell Sorter Management Committee	Member
1981	Present	Director, Anatomic Pathology Training Program	

Previous Academic/Professional Employment (Chronological Order)

(Employer)	(Position or Rank)	(Dates)
Ottawa Civic Hospital	Chief Resident, Lab. Medicine	July 1977 to June 1978
Medical Research Council	Fellow	July 1975 to June 1977
Ottawa Civic Hospital	Resident	July 1974 to June 1975
University of Minnesota Hospitals	Resident	July 1973 to June 1974
Ministry of health, Uganda	Medical Officer	May 1972 to October 197

Continuing Education Teaching at U.W.O. and Elsewhere

1. Workshop in Immunopathology - Canadian Association of Pathologists - June, 1980 St. John's, Newfoundland; June 1981, Toronto. June 1982, Vancouver.

2. Invited lecture - Department of Microbiology and Immunology, U.W.O. "Macrophage Mediated Natural Killing" May 14, 1980.

3. Invited lecture - London Cytotechnologists Association - "The use of immunological markers in the diagnosis of lymphoma. 1980

4. Invited lecture: CSLT - Lymphoproliferative disorders - Current Concepts. 1981

5. Pathology of the prostate and urinary bladder. Invited lecture, Dept. of Urology. 1981

6. ~~Invited lecture: London Cancer Clinic, Ont. Cancer Res. & Treat. Fdn. January 1982.~~

Memberships in Academic/Professional Societies.

1. I.A.P.

2. American Association for the Advancement of Sciences (AAAS)

3. British Society for Immunology
4. Royal Society of Medicine
5. New York Academy of Sciences
6. ~~Society for Hematopathology (U.S.)~~

Service to the Community (Local, Provinical, National) By Date and Position Held

1. Consultant, Canadian Tumor Reference Centre for Tumors of the Reticuloendothelial System 1979 to present date

2. Member, Medical Advisory Board and Grants Review Committee, Canadian Foundation for ~~Ileitis and Colitis. 1980 to present date.~~

3. Director, Medical Technology Training Program, U.H. 1979 to June 1981.

4. Director, Immunopathology Laboratory, U.H. 1980 to present date.

5. ~~Extra-departmental examiner, Ph.D. candidates, Department of Immunology and Microbiology~~ 1980, 1981.

6. Director, Residency Training Program, Anatomical Pathology, U.W.O. July/81 to present date

7. Co-ordinator, Second Year Dental Students, General and Systemic Pathology Course - 1980 to December 1981. (See 8 at bottom of page)

PUBLICATIONS: Please submit in the same style as in the following examples:

BOOKS:
MCLACHLIN, A.D., Venous Disease of the Lower Extremities. Current problems in Surgery (Chicago: Year Book Medical Publishers, 1967), 44p

ARTICLE:
PARKER, J.M., "Human Testing of Drugs", Applied Therapeutics, Vol. 9 (1967), p 612-614

ABSTRACT:
SERGOVICH, F.R., "Chromosome abnormalities and criminal behavior", (Abstract). International Meeting of Forensic Sciences. 5th. Program. (1969), p.73

SEE ATTACHED SHEET

8. Regional Reference Pathologist for National Cancer Institute Non-Hodgkin's Lymphoma Clinical Trial. March 1982 to present date.

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Publications

1. McKhann, C.F., Hendrickson, C.G., Spitler, L.E., Gunnarsson, A., Banerjee, D. and Nelson, W.R.
Immunotherapy of Melanoma with BCG: Two fatalities following intralesional injection. Cancer 35: 514-520, 1975.

2. Behelak, Y.B., Banerjee, D. and Richter, M.
Immunocompetent cells in Man. I: The lack of naturally occurring killer cell activity in patients with chronic lymphatic leukemia. Cancer 38: 2274-2277, 1976.

3. Banerjee, D., Hamdy, H., Walker, T., Bormanis, J. and Richter, M.
Leukemic reticuloendotheliosis: Polyclonal surface immunoglobulin on hairy cells. Cancer 41: 1804-1810, 1978.

4. Banerjee, D. and Silva, E.
Mediastinal mass with acute leukemia: Myeloblastoma masquerading as lymphoblastic lymphoma. Arch. Path. & Lab. Med. 105 (3): 125-129, 1981.

5. Richter, M., Banerjee, D., Fernando, L. and Sklar, S.
The identity of the naturally-occurring cytotoxic cells in Man. In: The Molecular Basis of Immune Cell Function. J. Gordin Kaplan, Ed. Elsevier/North Holland Biomedical Press, 1979.

6. Banerjee, D., Fernando, L. and Richter, M.
The antibody-independent naturally-occurring cellular cytotoxic (NOCC) activity of normal circulating human mononuclear cells. I. The role of serum and the nature of the target cells in the NOCC assay. Immunology 44: 97-108, 1981.

7. Richter, M., Banerjee, D. and Sklar, S.
The antibody-independent naturally-occurring cellular cytotoxic (NOCC) activity of normal circulating human leukocytes. II. Failure to detect effector cell-target cell interaction and target specificity of the circulating cytotoxic-enhancing factor. Immunology 44: 109-118, 1981.

8. Wright, B.A., Wysocki, G.P. and Banerjee, D.
The diagnostic use of immunoperoxidase techniques in plasma cell lesions of the jaws. Oral Surg. 52: 615-622, 1981.

9. Banerjee, D. and Ahmad, D.
Malignant lymphoma complicating lymphocytic interstitial pneumonia. Human Pathol. In press.

10. Banerjee, D.
Metastatic carcinoma expressing monocyte-histiocyte cytochemical markers. Identification of the true nature of the malignant cells by immunohistochemistry and electron microscopy. In: The Proceedings of the International Congress of Lymphology. Sept. 20-25, 1981, Montreal. In press.

Publications (Continued)

11. Banerjee, D.
Extra-medullary myeloblastoma simulating non-Hodgkin's lymphoma: The role of cytochemistry, immunohistochemistry and electron microscopy in diagnosis. In: The Proceedings of the 8th Int. Congr. Lymphology. Sept. 20-25, 1981, Montreal, In press.

12. Banerjee, D., Onosaka, S. and Cherian, M.G.
Immunohistochemical localization of metallothionein in cell nucleus and cytoplasm of rat liver and kidney. Toxicology. In press.

13. Cherian, M.G., Panemangalore, M. and Banerjee, D.
The cellular accumulation and subcellular localization of metallothionein in rat liver during postnatal development. In: Developmental and Reproductive Toxicity of Metals. Plenum Press. In press.

14. Banerjee, D. and Richter, M.
Depressed naturally-occurring cellular cytotoxicity (NOCC) activity in patients with mammary carcinoma and fibrocystic disease of breast. Submitted.

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2. Banerjee, D. and Ahmad, D.
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3. Walley, V., Banerjee, D. and Gilbert, J.J.
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4. Banerjee, D., Walley, V. and Anderson, C.
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7. Banerjee, D.
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Second National Congress of the Canadian Society of Laboratory Technologists, June 21-26, London, Ontario.

8. Banerjee, D.
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Abstracts (Continued)

10. Wright, B.A., Wysocki, G.P. and Banerjee, D.
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14. Banerjee, D.
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15. Banerjee D.
Extra-medullary myeloblastoma simulating non-Hodgkin's lymphoma. 8th Int. Congr. Lymphology, Sept. 20-25, 1981, Montreal.

16. Banerjee, D., Silver, M.M., McClintock, J. and Hudson, A.J.
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18. Banerjee, D., McClintock, J., Silver, M.M. and Hudson, A.J.
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19. Silver, M.M., Banerjee, D. and Hudson, A.J.
Segmental myofibre necrosis in myotonic dystrophy. 6th National Scientific Workshop of the Muscular Dystrophy Association of Canada, Banff, Alberta, Feb. 5-7, 1982.

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Morphologic and immunologic characterization of immunoblastic sarcoma of T cell origin. Can. Rev. of Lab. Med. 4: 26, 1982.

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22. Silver, M.M., Banerjee, D., Hudson, A.J.
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23. Banerjee, D.
Localisation of T lymphocyte subsets in human tonsillar tissue using monoclonal antibodies. Comparison of immunofluorescence and immunoenzymatic systems.
Can. Rev. Lab. Med. 4: 13, 1982.

24. Banerjee, D.
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Can. Rev. Lab. Med. 4: 18, 1982.

25. Banerjee, D.
T lymphocytes in human intestinal mucosa. In-situ demonstration of T-helper and T-suppressor subsets with monoclonal antibodies.
Accepted for presentation Royal College of Physicians and Surgeons, Annual Meeting, Quebec City, September, 1982.

26. Ko, H.S., Banerjee, D.
T lymphocyte subset levels in immunodeficiency and cancer.
Accepted for presention Royal College of Physicians and Surgeons, Annual Meeting, Quebec City, September, 1982.

RESEARCH INTERESTS

Brief Summary (please give a general description of past and present research interests)

1. Naturally occurring cytotoxic cells in man: The nature of the cells, their target specificities and mechanisms of target cell lysis.
2. Lymphocyte differentiation in normal individuals and patients with lympho-proliferative disorders.
3. Tumor-host interaction in patients with malignancies. The role of tumor-specific suppressor cell activity in patients with progressive tumors.
4. Pathogenesis of ulcerative colitis.
5. Mechanisms of IgG hypercatabolism in Myotonia Dystrophica
6. Isolation and characterization of human intestinal lymphoid cells.

Key Words (useful for classification of research interests)

Suppressor cells

Autoimmune Disease

Lymphocyte differentiation

Granting Agency Support (Agency, years supported, present amount)

M.R.C. Fellowship grant - 2 years (1975-1977)

Canadian Foundation for Colitis & Ileitis - 2 years - 1979-81 $40,000 ; 1981-82 $27,400.00

Muscular Dystrophy Association, Canada - 2 years - 1980-82 $62,000 ; 1982-84 $70,000.00

U.H. Pooled Research Funds: 1981- $12,942.14 ; 1982- $12,400.00

TEACHING INTERESTS

Brief Summary (please give subjects taught by course offering, either total or partial)

Pathology 240a - Disorders of Immunity

Meds I - Immunopathology and Connective Tissue Diseases

Dents II - Disorders of Immunity

Meds II - Lymphoreticular Diseases

Meds III - Immunopathology & Electron Microscopy

Meds IV - Lymphomas

- Cancer an an Immunologic Disease Clin. Immunology Selectives
- Immunology of the gut.

Residents: - Pathology of Lymph Nodes

- Diseases of the Lymphoreticular System

- Immunohistochemistry

Clinical Interests (please list specific areas for which you have particular interest)

Nephropathology

Lymphoreticular Neoplasia

Immunopathology (Immunohistochemistry)

Other Academic or Intellectual Interests