To the Graduate Council:

I am submitting herewith a thesis written by Jack Merritt Williams entitled "Natural Killer Cell Activity and Natural Antibody to K-562 Cells." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Microbiology.

Carl J. Wust, Major Professor

We have read this thesis and recommend its acceptance:

Robert N Moore

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

NATURAL KILLER CELL ACTIVITY AND NATURAL ANTIBODY TO K-562 CELLS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Jack Merritt Williams

December 1981

DEDICATION

This work is lovingly dedicated to my parents,

Mr. and Mrs. Jack M. Williams Sr.

ACKNOWLEDGEMENTS

I sincerely appreciate the support, patience, and interest shown me by Dr. Carl J. Wust, Dr. Robert Moore, and Dr. Eugene Perkins. A special heartfelt thanks goes to Dr. Wust for his guidance, friendship, and encouragement given during the course of my graduate work. Much credit goes to Sharon Byrd for her help in the preparation of this manuscript.

ABSTRACT

Natural killer (NK) cell cytotoxicity is initiated by recognition of an appropriate target cell with subsequent killing. The K-562 cell line has been used in many laboratories as a target cell for NK activity but the molecular site(s) of recognition is not known. We find that human NK cells from peripheral blood competitively reduce a specific antibody-dependent, complement-mediated cytotoxicity (ADCMC). The antibody used was present in the gamma globulin fraction of goat antiserum to K-562 cells that had been extensively absorbed with normal human peripheral blood leukocytes and normal bone marrow cells. Furthermore, extracts of K-562 cells, from which a specific antigen can be isolated, specifically and competitively inhibited NK-lysis of K-562 cells as well as the ADCMC. These findings suggest that NK cells recognize the same antigen as does the antibody. Lymphocyte populations from human donors, containing NK cells, were fractionated by their adherence to plastic, nylon wool, and K-562 monolayers. Cells non-adherent to plastic or nylon wool but adherent to K-562 monolayers and eluted gave NK cytolysis of K-562 cells. Goat immune globulin added to unfractionated human lymphocytes gave antibody-dependent, cellular cytotoxicity (ADCC) and it was possible that NK activity could be interpreted as antibody-dependent. However, this ADCC activity was

associated with Fc receptor positive (sensitized E-rosettes) cells that showed partial, non-selective adherence to K-562 monolayers. Furthermore, trypsin treatment of the lymphocytes removed the Fc receptor so that no E-rosettes formed or ADCC occurred, but trypsin treatment did not affect NK activity. From 80 normal humans, 52 (65%) showed NK cell activity and a level of natural antibody (ADCMC) to K-562, 24 additional showed antibody only, and 4 had neither activity. The natural antibody from several donors did not bind to protein A-Sepharose, migrated as 19S in sucrose gradients, and was rendered inactive by 2-mercaptoethanol. These criteria identified it as IgM.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Natural killer (NK) cells have been described as null cells in that they do not express B or T-cell specific surface markers (1,2). It has been shown that NK cells do have receptors which bind sheep erythrocytes, which is characteristic of but not specific for T-cells (5,6). Although NK cells do not express typical T-cell markers, Herberman et al (1) reported that following the incubation of NK cells with a thymic hormone preparation, the cells lose their natural killer function and express Thy antigen. The findings that NK cells can bind erythrocytes in a rosetting technique and can be converted to Thy-bearing cells suggest that NK cells may be of T-cell lineage.

Although the molecular recognition of target cells by NK cells has not been resolved, some workers have proposed that antibody may be involved. West et al.,(5) demonstrated that NK cells express receptors for the Fc portion of IgG, suggesting that NK cell activity may be an antibody-dependent cellular cytotoxicity phenomenon. However, Kiessling et al.,(6)showed that no detectable surface immunoglobulin could be found on active NK cells. One cannot conclude from this that antibody is not involved in NK cell activity since this later study was carried out

in vitro and does not necessarily rule out an ADCC reaction by the same cells *in vivo*.

Various other characteristics of NK cells have been examined. It was demonstrated that NK cells are non-adherent to plastic or nylon wool (3,11,12,13). Another feature is that about one-third of the NK cell population bears receptors for complement (10).

NK cells can lyse various target cells *in vitro*, most notably the K-562 multipotential cell line (34). K-562 cells are also sensitive to antibody-dependent cellular cytotoxicity (ADCMC) (3,8), making them an ideal cell line for study. This project has several aims. The first is to determine whether peripheral blood lymphocytes and serum from normal individuals can be cytolytic for K-562 cells. Second, lymphocyte subpopulations will be examined to determine which can participate in ADCC reactions. A third focus of this work will be to determine by means of competitive inhibition assays with antigen preparations and lymphocyte subpopulations whether the antigen on the K-562 cells recognized by the NK cell is the same as that recognized by Ab in the ADCC and ADCMC reactions.

CHAPTER II

SURVEY OF THE LITERATURE

I. CHARACTERISTICS OF NATURAL KILLER CELLS

Natural killer (NK) cells are defined as a subpopulation of spleen cells in mice or peripheral blood leukocytes in humans that can kill appropriate target cells in the absence of any immune stimulation. Morphologically, these cells resemble lymphocytes. They are described as null cells in that they do not express B or T-cell surface markers, including surface immunoglobulin (1,2,3). Murine NK cells express H-2 K and D alloantigens, but not Lyt-1, Lyt-2, or Qa-1 T-cell associated antigens (4). A subset of either human or murine NK cells does possess receptors for binding sheep erythrocytes, which is a property characteristic of, but not specific for, T-cells (5,6). Although the NK cells do not express the typical T-cell markers, they can be stimulated to undergo blastogenesis by incubation with thymic hormone preparations (7). After stimulation, the NK cells express Thy alloantigens and are not functional in NK assays (7). These findings suggest that the NK cells are normally of the pre T-cell lineage.

There appear to be fractions of both mouse and human NK cells which express receptors for the Fc portion of

IgG (8,9) and the C3b component of complement (10). Neither human nor murine NK cells adhere to plastic or glass and do not bind to nylon wool (3,11,12,13).

Human NK cell activity may be associated with large granular lymphocytes, which have azurophilic granules in their cytoplasm and a high cytoplasm to nucleus ratio (11,13). These large granular cells were found to adhere to target cells and appear to cause cytolysis. The cells were separated on a discontinuous density gradient of human peripheral blood lymphocytes (14). The gradient consisted of seven layers, ranging from 40% to 57% Percoll, with each consecutive fraction increasing by 2.5%. Of the cytolytic activity recovered from these fractions, 81% was detected in two low density fractions (42.5% and 45.0%), which were enriched for large granular lymphocytes (15).

II. NATURAL KILLER CELL CYTOTOXICITY

Natural killer cell cytotoxicity has been proposed to play an important role in immunosurveillance of transformed cells that could become malignant. A prime example of this mechanism is in the nude mouse model. Although these mice are athymic and lack mature T lymphocytes, they do not show a high incidence of tumors and they reject tumor transplants. This has been attributed to a relatively high level of NK activity (16,17). On the other hand, beige mice, which have very low NK activity, have a high

incidence of spontaneous tumors and tumor transplants grow rapidly with metastases (17).

Of particular interest in the study of NK cells is the observation that interferon is capable of enhancing murine and human NK activity both *in vivo* and *in vitro* (18,19). This was first evident when Oehler *et al.*,(20) showed that rat NK activity could be elevated by treatment of the animals with poly I:C, a potent interferon inducer. Various studies have shown this effect to be most pronounced in mice that are greater than 12 weeks of age, a time when NK activity declines to low levels (18). The enhancement of NK activity with interferon inducers can be reversed by the injection of specific anti-interferon serum (21).

It seems probable that the enhancement of NK activity by some agents is mediated by the production of interferon. For example, NK activity of spleen cells is increased after mice are treated with *Corynebacterium parvum* (22). *C. parvum* induces the synthesis of interferon which has the characteristics of IFN . NK activity is elevated after the injection of NK-sensitive target cells, which also will induce the production of interferon (1). The augmentation of depressed NK activity has led to the use of interferon in the treatment of human cancer, since a low level of NK activity, analogous to the beige mouse model, has been suggested as predisposing individuals to cancer growth.

Although the mechanisms of recognition and destruction of target cells by NK cells have not been resolved, some evidence suggests that antibodies may be involved in the recognition phase. Since some cells in the NK population express Fc receptors, it has been proposed that low levels of antibody (IgG) could bind to these cells and NK activity was actually antibody-dependent cellular cytotoxicity (ADCC) (8,2). The antibody was suggested to have been produced in response to heterologous antigens and be cross reactive with the tumor antigens. Thus, the target cell antigens would be recognized by the bound antibody (8,2,3,24). In support of this possibility, the trypsinization of effector lymphocytes, under conditions which remove surface-bound immunoglobulins, eliminates NK cell cytotoxicity against cultured cells (25). Furthermore, ADCC does occur when target cells coated with antibody are incubated with trypsinized NK cells, which does not remove the Fc receptor (26).

There exists, however, an opposing school of thought in regard to recognition of target cells by NK cells. For example, some investigators have observed cytotoxicity against tumor cells by NK cells which do not bear Fc receptors for IgG (27). Also, the elution of cytophilic IgG has no effect on human NK activity (28). Therefore, it cannot be concluded from these <u>in vitro</u> studies to what extent NK activity is actually an ADCC reaction <u>in vivo</u>.

However, significant levels of NK activity can be demonstrated in some patients with agammaglobulinemia, suggesting the lack of ADCC to explain NK activity.

NK cells are capable of lysing many different types of target cells. Rat and mouse NK cells can lyse murine and non-murine targets, including certain human cell lines (29). Lymphoma cell lines such as the mouse YAC cell line and human leukemia cell lines, such as K-562, are used primarily as targets for in vitro assays. Some of these cells when grown as ascites in vivo decrease in number. This decrease has been attributed to NK activity (30). Recognition of the target cells does not necessarily lead to cytolysis, as normal cells will bind to NK cells and/or compete with NK-sensitive targets in lytic assays (31). For example, Collins et al.,(32)showed that a normal fibroblastic cell line not susceptible to NK cytolysis would compete with susceptible transformed cells derived from the original cell line.

Studies on NK activity can yield information concerning immune regulation in the body. The cytolysis of tumor cells by NK cells and augmentation of this cytolysis by production of interferon ensures that NK cell activity will be further examined to determine possible therapeutic benefits.

III. THE K-562 LEUKEMIA CELL LINE

The K-562 leukemia cell line was isolated in 1970 by Dr. Carmen B. Lozzio from a patient with chronic myelogenous leukemia (CML) in blastic crisis. This cell line is the first reported to have a positive Philadelphia (Ph'+) chromosome (33). The K-562 cells are multipotential stem cells which, upon cultivation for ten to eleven days in conditioned medium, are able to differentiate into early progenitors of the erythrocytic, granulocytic, and monocytic series (34). Differentiation into the various cell types was determined by the appearance of enzymes that are characteristic for cells of the granulocytic and monocytic series, and by the production of hemoglobin in the erythrocytic series.

A specific leukemia-associated antigen has been isolated and characterized from the K-562 cell (35). Furthermore, antisera to the cell antigen produced in rabbits, monkeys, and goats have been studied (36). The antisera injected into nude or lasat (Lozzio asplenic athymic) mice that have been transplanted with K-562 cells will abrogate the growth of the cells, indicating the continued presence of the antigen (37).

K-562 cells are used in many laboratories worldwide, particularly as targets in NK cytotoxicity assays (38). Vose et al.,(39)have shown that cultured K-562 cells are more sensitive to NK lysis than cells freshly isolated from

lung tumors in patients. NK activity to K-562 cells can be enhanced by pretreatment of effectors with interferon, which appears to recruit new NK cells and increases the activity of committed NK cells. Thus, K-562 cells are of great value in the study to understand the role of NK in immunosurveillance.

CHAPTER III

MATERIALS AND METHODS

I. CELL CULTURE

The human leukemia cell line K-562, extensively described elsewhere (33,34), was used in this investigation. The cell line has been maintained in the laboratory of Dr. Bismarck Lozzio at the University of Tennessee Memorial Research Center in Knoxville and was used at passage 275. Passage 275 was recovered from liquid nitrogen storage and examined for karyotype and morphology. The cells were grown as a suspension in Eagle's Minimum Essential Medium (MEM) with Earle's salts (Flow Laboratories, Rockville, Maryland) supplemented with L-glutamine (292 mg/L) and 15% fetal calf serum (heat inactivated at 56°C for thirty minutes) to a density not exceeding 2×10^5 cells/ml.

II. GOAT ANTISERUM TO THE K-562 CELL LINE

Antiserum to K-562 cells was raised in goats according to the following procedure (37). K-562 cells were grown in medium containing 15% normal goat serum for four passages. They were then injected intramuscularly into nine to twelve month old goats at a concentration of 10^6 cells/kg in complete Freund's adjuvant. After four weekly

muscular injections of cells in complete Freund's adjuvant, a final injection of cells without adjuvant was given intravenously. Blood was collected on day 35, the serum separated and heat inactivated at 56°C for 90 minutes. The inactivated serum was absorbed with normal, washed human AB positive blood cells until agglutination and cytotoxic activity against normal human leukocytes was not microscopically detectable. This first absorption was carried out at 25°C and was repeated five to seven times at 4°C. The serum was then absorbed with normal human bone marrow (5.7×10^7 cells/ml serum) for 2 hours at 4°C.

Gamma globulins were isolated from preimmune and immune absorbed sera by three precipitations with ammonium sulfate at a concentration of 33%. The resulting precipitate was pooled and left as a slurry at 4°C until used. To prepare for use, the slurry was centrifuged and the precipitate dissolved in phosphate buffered saline (PBS, 0.1 M phosphate, 0.145 M NaCl, pH 7.2). This solution was dialyzed against PBS (1 liter for 8 hours x 3) and filter-sterilized. The globulins prepared and used averaged 13 mg/ml at final concentration.

III. SEPARATION OF HUMAN LYMPHOCYTE SUBPOPULATIONS

Human peripheral lymphocytes subpopulations were separated according to the following procedure (40). Whole heparinized peripheral blood from normal human donors was mixed with a solution of 10% dextran (Dextran

500, Pharmacia) to yield a final concentration of 1%. Erythrocytes were sedimented within 30 minutes at room temperature. The leukocyte rich plasma was removed and a sample (50 µl) was taken to determine the cell concentration and viability by the trypan blue dye exclusion test. The plasma was diluted 1:3 (plasma:medium) with Eagle's Minimum Essential Medium and layered on Ficoll-Hypaque (Pharmacia) in a polypropylene centrifuge tube (Corning). The tube was centrifuged at 400 xg for 45 minutes. The lymphocyte-containing layer was removed from the interface between the plasma and Ficoll-Hypaque with a Pasteur pipette and suspended in MEM. A sample (50 µl) was taken for a cell count. The cell concentration was adjusted to 1 x 10^6 cells/ml with MEM and three 50 µl samples were placed in separate wells of a microtiter plate (IS-MRC 96 Linbro) to assay for cytotoxicity against K-562 cells. The remaining suspension was incubated in a plastic tissue culture flask (Falcon) at room temperature for one hour to remove adherent cells. After incubation, a sample (50 µl) was taken to determine the cell number. After adjusting the concentration of the non-adherent cell population to 1 x 10^6 cells/ml, three 50 µl samples were placed in separate wells in a microtiter plate to assay for cytotoxicity against K-562 cells. The remaining suspension was divided into two parts, one for passage over nylon wool and the other for incubation on K-562 cell monolayers.

Nylon wool columns were prepared by packing washed nylon wool to a 5 ml bed volume in a 10 ml syringe. The concentration of one part of the non-adherent cell suspension was adjusted to 2 x 10^6 cells/ml and 1 ml of the suspension was added to the nylon wool column with 4 ml of MEM. The column was closed, incubated at 37°C for 45 minutes, and then the nylon non-adherent cells were washed from the column with 20 ml of MEM at a flow rate of 1 ml/minute. The nylon adherent cells were eluted by adding 5 ml MEM and vigorously shaking the column. The fluid was collected and the nylon wool was washed two more times. Samples were taken to determine the cell number. After adjusting the concentration to 1 x 10^6 cells/ml, three 50 µl samples of the nylon adherent cell population were placed in separate wells of a microtiter plate to assay for cytotoxicity against K-562 cells. The same was done for the nylon non-adherent cell population. The remaining nylon non-adherent cell suspension was incubated with a 1:15 dilution of anti-human thymocyte serum and Low Tox-H Rabbit Complement (Cedar Lane) diluted 1:4 with MEM (41). After 1 hour incubation at 4°C, the cells were centrifuged at 1500 xg for 5 minutes, and the pellet was suspended in MEM. A sample (50 µl) was taken to determine cell number and the concentration of the cell suspension was adjusted to 1 x 10^6 cells/ml. Three 50 µl samples were placed in separate wells of a microtiter plate to assay for cytotoxicity against K-562 cells.

IV. ISOLATION OF CELLS ADHERENT TO K-562 MONOLAYERS

To isolate cells that are positive for NK activity, the T-cell depleted and the plastic non-adherent cell suspensions were incubated on K-562 monolayers (42). One ml of poly-L-lysine [M.W. 80,000 (SIGMA) 50 ug/ml in PBS] was added to a 60 mm polystyrene tissue culture dishes (Falcon) for one hour. K-562 cells were washed twice with PBS and adjusted to a concentration to 9×10^6 cells/ml. The K-562 cells were added to the tissue culture dishes and incubated at room temperature for 30 minutes. The fluid was gently decanted and 2 ml of cold 0.2% formaldehyde in PBS was added to increase the stability of the monolayers. The plates were then incubated at 4°C for 1 hour, washed 5 times with PBS (pH 7.2), and left to stand at room temperature for 20 minutes. The monolayers were then ready for use.

The different cell suspensions were adjusted to a concentration of 6×10^6 cells/ml and two ml of each were added to separate monolayers and incubated for 60 minutes at 37°C. After incubation, the cells non-adherent to K-562 were removed. Cells adherent to K-562 were eluted by adding 1 ml of PBS containing EDTA (5 mM EDTA in PBS, pH 7.2), rocking the plates for 10 minutes at 37°C, and removing the medium with a pipette (43). The concentration of each suspension was adjusted to 1×10^6 cells/ml, and three 50 µl samples of each were added to separate

wells of a microtiter plate to assay for cytotoxicity against K-562 cells.

V. TRYPSINIZATION OF EFFECTOR CELLS

For removal of antibodies bound to effector cells, lymphocytes which were non-adherent to plastic and nylon wool, depleted of T-cells, and either adherent or non-adherent to K-562 monolayers were washed with PBS and centrifuged. The cells were suspended to a concentration of 1 x 10^6 cells/ml in MEM containing 0.025% trypsin (25) (Reheis Chemical Co.) with EDTA (0.2g disodium salt/liter). The suspension was incubated for 30 minutes at 37°C with shaking. The cells were collected and washed 3 times with PBS, and the cell concentration adjusted to 1 x 10^6 cells/ml. Three 50 µl samples were removed and added to separate wells of a microtiter plate to assay for cytotoxicity against K-562 cells.

VI. SEPARATION OF Fc-RECEPTOR BEARING CELLS

Effector cell subpopulations which were non-adherent to plastic or nylon wool and were depleted of T-cells were subjected to standard E rosetting techniques (40). This included the cells which were either adherent or non-adherent to the K-562 monolayer. Neuraminidase-treated sheep RBC were prepared by incubating 50 units of neuraminidase (Calbiochem) with 1 ml of a 5% sheep

RBC suspension for 30 minutes at 37°C. The treated cells were then centrifuged at 700 xg for 7 minutes and suspended in PBS to a final concentration of 1%. Equal volumes of effector cells (4×10^6 cells/ml) and treated sheep RBC were mixed and incubated for 10 minutes at 37°C. The cell suspensions were then centrifuged for 5 minutes at 150 xg and incubated at 4°C for 60 minutes. Rosette forming cells, those to which 3 or more sheep RBC were attached, were microscopically observed and counted with a hemocytometer. The suspension was gently layered on a Ficoll-Hypaque solution in a polystyrene centrifuge tube and centrifuged for 15 minutes at 400 xg. The non-rosette forming cells at the interface were removed with a Pasteur pipette and washed. The pellet was removed and suspended in one part PBS to which three parts H_2O was added for 30 seconds, followed by the rapid addition of one part NaCl (3.5%). This hypotonic shock treatment lysed the sheep RBC. The rosette-forming lymphocytes were then washed with MEM and incubated for 18 hours in MEM-10% FCS to allow membrane restoration. A portion of the non-rosette forming cells was subjected to the hypotonic lysis and 18 hour incubation as a control. The remaining non-rosette forming cell suspension was incubated on a K-562 monolayer as previously described. After removal with EDTA, the K-562 adherent cell concentration was adjusted to 1×10^6 cells/ml and three 50 µl portions were removed and placed

in separate wells of a microtiter plate to assay for cytotoxicity against K-562 cells. After the 18 hour incubation, the rosette forming cell concentration was adjusted to 1 x 10^6 cells/ml and three 50 µl portions were removed for the cytotoxicity assay. The separation procedures are summarized in Figure 1.

VII. ASSAY FOR NK-SPECIFIC ^{51}Cr RELEASE FROM K-562 CELLS

The cell suspensions obtained by the lymphocyte separation procedure previously described were assayed for cytotoxicity against ^{51}Cr-labeled K-562 cells by the following procedure. K-562 cells were washed 3 times with PBS and the concentration adjusted to 4 x 10^6 cells/ml. One hundred µCi of $Na^{51}CrO_4$ (New England Nuclear) were added to the cell suspension and incubated for 45 minutes at 37°C on a rocker plate (Labquake). The cells were then washed 3 times with PBS, and adjusted to varying concentrations. Fifty µl of the ^{51}Cr-labeled cells were added to separate wells in a microtiter plate with the various lymphocyte subpopulations, giving varying effector:target ratios. Labeled cells were placed in additional wells with MEM to provide a control for spontaneous release. The plate was covered, incubated for 4 hours at 37°C, and centrifuged at 300 xg for 5 minutes at 4°C. Fifty µl of the supernatant fluid was removed from the wells and

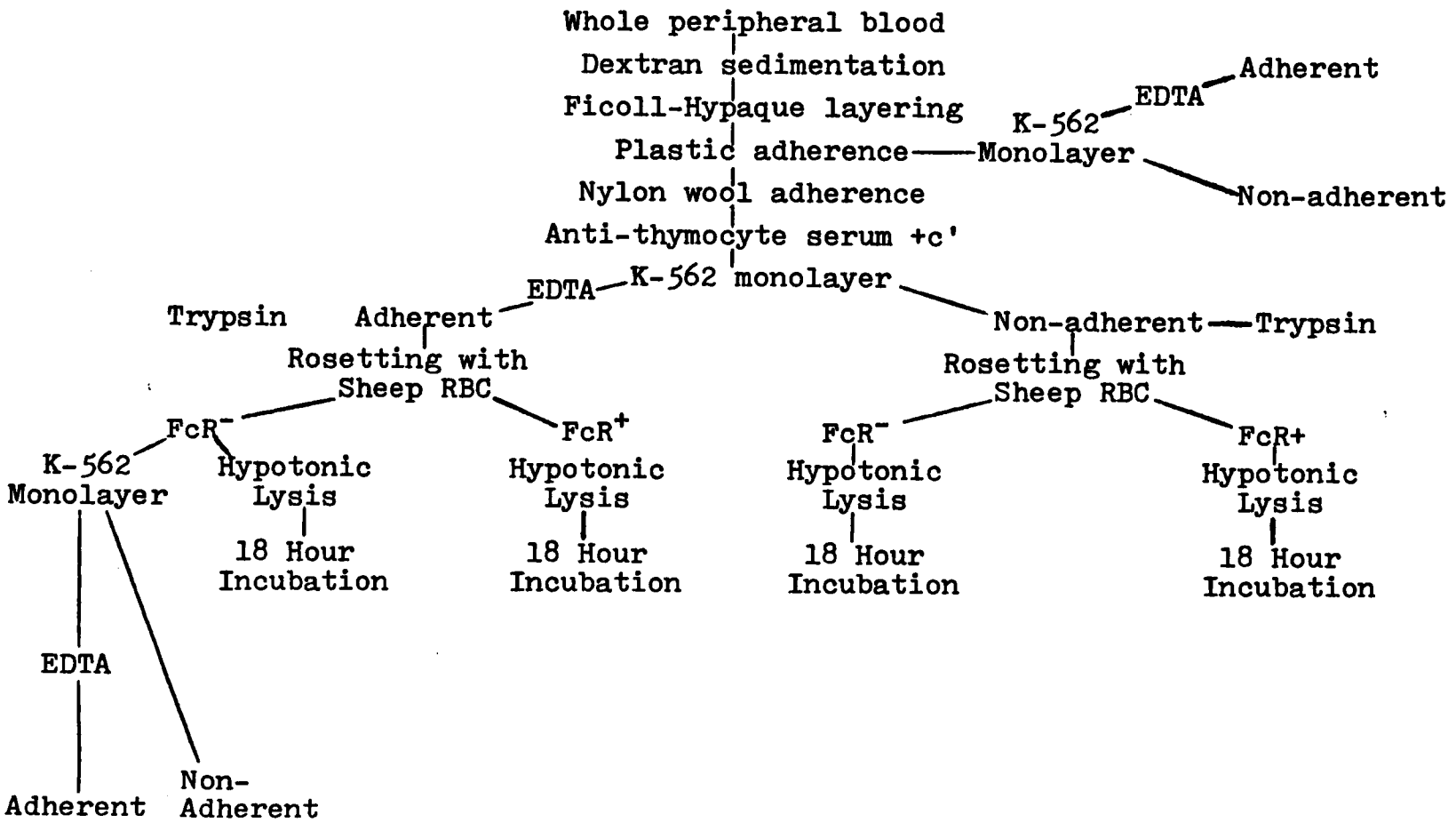

Figure 1. Flow diagram for separation of human lymphocyte subpopulations

placed in separate test tubes (12 x 75 mm, Fischer) for measurement of radioactivity. Fifty µl of 0.1% Triton X-100 was added to the wells to solubilize the cells and the solution was removed by absorption to the wicks of a Titertek Collection System (Flow) from which the cell retaining pads were removed. The wicks were also placed in tubes for measurement of ^{51}Cr content in the pellets. All measurement of radioactivity was done in a Beckman 4000 Gamma Spectrometer. Specific ^{51}Cr-release due to lysis by the lymphocytes is calculated as:

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental Sup} \times 2)-(\text{Control Sup} \times 2)}{(\text{Experimental Sup+Wick})-(\text{Control Sup} \times 2)} \times 100$$

VIII. ASSAY FOR ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC)

Anti-K-562 serum raised in goats was added to additional wells of microtiter plates with 50 µl samples of the various lymphocyte subpopulations to determine antibody-dependent cellular cytotoxicity. Cytotoxicity against K-562 cells by plastic adherent cells, both with and without anti-K-562 serum, was determined. Anti-K-562 serum was also added to wells containing trypsinized effectors to examine the effects of trypsin on ADCC. The ADCC was determined after a four hour incubation by subtracting the amount of cytotoxicity measured in the wells with lymphocytes and labeled K-562 cells from the cytotoxicity

found in wells with lymphocytes, anti-K-562 serum, and labeled K-562 cells. Additional wells containing anti-K-562 serum and labeled K-562 cells served as antibody controls. All assays were performed in triplicate.

IX. ASSAY FOR ANTIBODY-DEPENDENT COMPLEMENT-MEDIATED CYTOTOXICITY (ADCMC)

Fifty µl samples of ^{51}Cr-labeled K-562 cells were placed in separate wells of a microtiter plate with 50 µl of anti-K-562 serum and 50 µl of Low Tox-H Rabbit Complement (Cedar Lane) diluted 1:4 with MEM. The anti-serum dilutions varied from 1:100 to 1:1000. When demonstrating the presence of natural antibody to K-562 cells, normal human serum was used in place of goat anti-serum in 1:4, 1:8, 1:16, 1:32, and 1:64 dilutions. All assays were done in triplicate. Complement was added to additional wells with labeled K-562 cells to serve as complement controls. After a 2 hour incubation at 37°C, the supernatant fluids and pellets were removed as previously described and ADCMC measured as:

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental Sup} \times 2) - (\text{Complement Sup} \times 2)}{(\text{Experimental Sup+Wick}) - (\text{Cell Control Sup} \times 2)} \times 100$$

X. ISOLATION AND ASSAY OF IgG AND IgM FROM NORMAL HUMAN SERUM

Normal globulin was isolated from human serum samples

by ammonium sulfate precipitation according to the following procedure (44). The pH of a 5 ml aliquot of saturated ammonium sulfate was adjusted to 7.8 with 2 N NaOH. With constant stirring, the ammonium sulfate solution was slowly added to a serum sample. Stirring was continued for 2 to 3 hours to avoid trapping other serum components in the precipitate. The precipitate was then centrifuged at 1500 xg for 30 minutes at room temperature and the pellet dissolved in borate-buffered saline to a final volume equal to that of the original serum sample. This precipitate contains primarily gamma globulin and second and third precipiration was done. The ammonium sulfate was removed by dialysis at 4°C against several changes of borate-buffered saline. After dialysis, the solution was centrifuged at 4°C for 30 minutes at 1500 xg. The final product was assayed for cytotoxicity against K-562 cells as previously described in an ADCMC reaction.

For isolation of IgG and IgM from the normal serum, the product of the first ammonium sulfate precipitation was passed over a Protein A-Sepharose column according to the following procedure (45). An immunosorbent column was prepared dissolving 1.5 g of dry Protein A-Sepharose CL-4B (Pharmacia) in distilled water. The column was attached to a Peristaltic Pump P-3 (Pharmacia) to control the flow rate. The product of the ammonium sulfate

precipitation was loaded onto the column with 0.1 M phosphate buffer, pH 7.5, and washed with this buffer to remove non-absorbing proteins. The buffer for elution of bound proteins was glycine-HCl buffer 0.1 M, pH 218. The elution profile was monitored on an Isco Model UA-2 Ultraviolet Analyzer. Fractions passing the column contained IgM and a minute amount of IgG_3, a subclass of IgG. The fraction eluted with the glycine-HCl buffer contained IgG_1, IgG_2, and IgG_4. Most of the IgG_3 was included in this fraction (46).

An ADCMC assay was carried out with the bound and unbound fractions by methods previously described. Portions of each fraction were added to microtiter wells with 0.015 M 2-mercaptoethanol to observe for elimination of natural ADCMC. Whole normal serum was also assayed as well as samples treated with 2-mercaptoethanol.

As a third parameter for determining the entity involved in the natural ADCMC reaction, sucrose gradient centrifugation of normal serum was carried out to separate IgG (7S) and IgM (19S). Sucrose solutions were prepared which were 40% and 10% secrose in 0.15 M NaCl. A Model V 5 gradient maker (Fischer) was used in preparing linear gradients. With the stopcock between the 2 compartments of the gradient maker closed, 6.7 ml of the 10% sucrose solution was pipetted into compartment 2. The stopcock was opened to allow the passage between the compartments

to fill with the sucrose solution and was then closed. To compartment 1, 6.4 ml of 40% sucrose was added. The spin bar was then placed in compartment 1 and magnetic stirring begun. The stopcock between the compartments was opened and the flow of the gradient solution into the tubing was begun. The end of the tubing was placed against the wall of a plastic 9/16 inch tube (Beckman). The serum sample, diluted 1:2 with 0.15 M NaCl, was layered on top of the gradient with a syringe and bent needle to avoid disturbing the gradient. The tubes were placed in rotor buckets and the buckets were attached to the rotor (SW 27, Beckman) in proper orientation. The rotor was placed in the precooled chamber of the ultracentrifuge (Model L 3-50, Beckman). The gradients were centrifuged at 138,000 xg for 16.5 hours. After centrifugation, the gradient tubes were removed, the tops sealed, and placed in a tube holder in an elevated condition. A 21 gauge needle with clamped tubing attached was used to pierce the bottom of the gradient. The other end of the tubing was attached to a flowmeter which was attached to a fraction collector (Ultrorac, LKB). Sequential fractions were collected from the bottom of the gradient to the top. The percentage of sucrose in the fractions was read on a refractometer. Fractions were assayed for cytotoxicity against K-562 cells in an ADCMC reaction as previously described. Portions of each fraction were also added to

other wells with 2-mercaptoethanol to observe for elimination of natural ADCMC. Optical density readings were made for each fraction in a Zeiss PM 6 Spectrophotometer and refractive indices were measured in an Abbe Refractometer (American Optical Corporation).

XI. ASSAY FOR COMPETITIVE INHIBITION OF ADCMC BY NK CELLS AND NON-NK CELLS

The various lymphocyte subpopulations obtained by the procedures previously described were assayed in competition with ADCMC. ^{51}Cr-labeled K-562 cells were incubated with anti-K-562 serum and complement and lymphocytes in varying effector:target ratios for 2 hours. Since natural killing requires a minimum of 4 hours, cytotoxicity by ADCMC was observed at 90 minutes. Cytotoxicity was measured as previously described. All assays were done in triplicate.

XII. PREPARATION OF A 3M KCl EXTRACT FROM K-562 CELLS AND USE IN INHIBITION ASSAYS

A 3M KCl antigen extract was prepared from K-562 cells by the following procedure (35). K-562 cells stored in glycerol at -80°C were rapidly thawed in a 37°C water bath. The cells were washed twice in PBS (7.2) and suspended in 20 ml of 3M KCl. After 20 hours of incubation at 4°C, the suspension was centrifuged at 100,000 xg

for 1 hour. The supernatant fluid was removed and dialyzed against 4L PBS, 2L times 2, for 20 hours. Finally the fluid was removed and stored at $4^{o}C$.

The 3M KCl extract was assayed for inhibitory activity against the NK, ADCC, and ADCMC reactions. This was done by adding varying amounts of the extract in competition with cells or antibody and observing for a decrease in the % of ^{51}Cr-release compared to antigen-free controls.

CHAPTER IV

RESULTS

I. NK AND ADCMC AGAINST K-562 CELLS FOUND WITH NORMAL PERIPHERAL BLOOD AND SERUM SAMPLES

Peripheral blood lymphocytes and serum samples from 80 normal human donors were assayed for NK cell activity and ADCMC, respectively, against K-562 cells. The NK cells used in the assay were isolated and designated as non-adherent to nylon wool or plastic, not sensitive to anti-thymocyte serum plus C', and adherent to K-562 monolayers. The normal serum samples were used in place of anti-K-562 serum in the ADCMC assays. As shown in Table 1, the results allowed the sera to be placed into three distinct groups. Group 1 consisted of PBL samples showing NK activity of greater than 2.5% ^{51}Cr-release and ADCMC of greater than 5.0% from the same donor. This group comprised 52 of the 80 samples tested with a mean value of NK activity of 12.2% and a range of $\pm$ 9.8%. The mean value for ADCMC was 18.3% $\pm$ 10.2.

The second group consisted of donors demonstrating NK activity less than 2.5% ^{51}Cr-release, designated as NK(-), and ADCMC of greater than 5.0%. This group comprised 24 of the 80 samples tested with a NK mean value of 1.1% $\pm$ 1.2%. The ADCMC mean was 7.7% $\pm$ 2.7%.

Table 1. Percent of ^{51}Cr released from K-562 cells due to natural killer cell cytotoxicity (NK) and antibody-dependent complement-mediated cytotoxicity (ADCMC) with normal serum

	Student Number	NK	Natural ADCMC
Group 1*	0716	6.6	15.7
	7493	6.2	11.9
	9333	7.0	12.0
	4186	13.6	17.3
	9094	13.5	21.4
	1987	18.1	22.5
	8840	7.2	11.1
	3185	13.2	23.4
	5827	14.5	22.0
	7860	13.0	14.0
	1216	14.0	24.5
	1399	7.6	13.4
	5005	8.2	16.0
	8382	15.0	23.0
	1286	10.0	19.0
	1961	10.0	16.0
	3471	10.0	11.0
	7595	23.0	26.0
	7397	9.0	11.4
	6563	14.0	22.3
	0102	11.0	17.0
	6183	13.0	21.4
	7692	13.0	21.0
	0776	10.0	22.0
	1805	10.0	25.0
	7100	14.5	23.2
	6686	15.0	26.9
	6314	10.4	18.0
	6513	15.9	21.0
	1102	19.0	27.0
	9274	25.9	28.0
	8582	13.9	13.7
	5470	11.0	11.8
	5741	12.1	10.0
	5884	13.1	21.6
	8089	14.2	21.8
	7768	12.0	17.6
	6350	8.1	6.5

Table 1. (Continued)

	Student Number	NK	Natural ADCMC
	8657	15.0	25.8
	5039	13.7	18.0
	6156	13.0	16.3
	0891	8.7	8.0
	1162	14.0	18.6
	6747	17.8	17.6
	5257	11.8	20.8
	7860	8.0	7.7
	8721	14.0	26.7
	4281	14.0	21.0
	9328	8.9	10.8
	1087	9.2	9.3
	6629	8.8	8.7
	0373	12.4	18.6
Group 2**	3283	1.7	8.9
	2781	1.8	7.6
	8785	1.4	6.2
	3176	1.8	7.5
	7595	1.5	6.4
	9412	2.4	8.5
	4458	0.0	8.4
	3971	0.0	6.8
	5208	0.0	6.4
	9165	1.0	8.2
	3562	1.0	6.2
	0560	1.0	9.7
	3347	1.8	8.4
	6980	0.0	8.3
	1814	0.0	6.4
	7887	0.0	6.7
	5952	1.2	8.9
	7815	0.0	6.7
	6406	0.0	8.8
	9367	2.2	6.9
	2423	2.1	8.8
	6850	2.1	7.3
	2920	1.8	9.0
	5244	1.2	8.6
Group 3***	0979	0.0	0.0
	3523	0.0	0.0
	4402	0.0	1.2
	9916	1.0	2.8

Table 1. (Continued)

*Group 1. Designated NK(+) with cytotoxicity $\geq$2.5% and ADCMC(+) with cytotoxicity $\geq$ 5.0%. This group contained 52 of 80 samples tested with an NK cytotoxicity mean value of 12.2% and range of $\pm$9.7%. The ADCMC cytotoxicity mean value was 18.2% with a range of $\pm$10.2%.

**Group 2. Designated NK(-) with cytotoxicity $\leq$2.5% and ADCMC(+) with cytotoxicity $\geq$ 5.0%. This group contained 24 of 80 samples tested with an NK cytotoxicity mean value of 1.1% and range of $\pm$1.2%. The ADCMC cytotoxicity mean value was 7.7% with a range of $\pm$1.7%.

***Group 3. Designated NK(-) with cytotoxicity $\leq$2.5% and ADCMC(-) with cytotoxicity $\leq$ 5.0%. This group contained 4 of 80 samples tested with an NK cytotoxicity mean value of 0.3% and range of $\pm$0.5%. The ADCMC cytotoxicity mean value was 1.0% with a range of $\pm$1.4%.

The third group comprised donors with NK activity of less than 2.5% ^{51}Cr-release and ADCMC less than 5.0%, designated NK(-) and ADCMC(-). This group comprised 4 of the 80 donors having an NK activity of 0.3% ± 0.5%. The ADCMC activity was 1.0% ± 1.4%. There were no donors who showed a positive NK activity and negative ADCMC.

II. COMPARISON OF ADCC REACTIONS WITH LYMPHOCYTES ADHERENT ON K-562 MONOLAYERS, NON-ADHERENT ON K-562, AND PLASTIC ADHERENT CELLS

Peripheral blood samples were obtained from donors in each of the three groups described in Table 1 and were tested for ADCC activity. After addition of anti-K-562 serum, three populations of lymphocytes from donors of each group were tested; those adherent to K-562 monolayers, those non-adherent to K-562 cells, and those that were adherent to plastic. As shown in Table 2, twelve PBL samples were tested, five from Group 1, NK(+) and ADCMC(+), five from Group 2, NK(-) and ADCMC(+), and two from Group 3, NK(-) and ADCMC(-). Two of the five samples in Group 1 showed 31-40% ADCC. The other three samples showed 21-30% ADCC. This was true for both K-562 adherent and non-adherent lymphocytes. Each of the five samples in Group 2 gave ADCC in the 11-15% range with K-562 non-adherent lymphocytes and also with K-562 adherent lymphocytes. Of the two samples in Group 3, one showed ADCC in the 11-15%

Table 2. Comparison of ADCC reactions with K-562 adherent lymphocytes, non-adherent lymphocytes, and plastic adherent cells*

Sample	K-562 adherent lymphocyte NK	Serum ADCMC	Goat anti-K-562 serum added to K-562 adherent	Non-adherent	Plastic adherent
1987	+++	++++	++++	++++	++++
7595	++++	++++	+++++	+++++	++++
9274	++++	++++	+++++	+++++	++++
1102	+++	++++	++++	++++	++++
6747	+++	+++	++++	++++	++++
6980	-	++	++	++	+++
0560	-	++	++	++	+++
3283	-	+	++	++	+++
5952	-	+	++	++	+++
6406	-	+	++	++	+++
3523	-	-	++	++	+++
4402	-	-	+	+	+++

*Cytotoxicity level- +=5-10%, ++=11-15%, +++=16-20%, ++++=21-30%, +++++= 31-40%, -+ 5.0% for serum ADCMC and 2.5% for NK and ADCC. All effector cells are from peripheral blood leukocytes. The effector:target ratio is 100:1. The Ab dilutions are 1:4 in normal serum ADCMC and 1:300 in ADCC reactions. Assays are four hours for NK and ADCC and two hours for normal serum ADCMC. Lymphocytes non-adherent to K-562 gave no natural cytotoxicity.

range with K-562 adherent and non-adherent lymphocytes while the other sample gave ADCC in the 5-10% range with both K-562 adherent and non-adherent lymphocytes.

Of the 12 samples tested, the five samples in Group 1 demonstrated ADCC with plastic adherent cells in the 21-30% range. The five samples in Group 2 and the two samples in Group 3 showed ADCC in the 16-20% range with plastic adherent cells.

III. NK AND ADCC ACTIVITIES OF PLASTIC NON-ADHERENT AND FICOLL-HYPAQUE SEPARATED LYMPHOCYTES

NK and ADCC activities of various lymphocyte subpopulations are presented in Table 3. The lymphocytes which were adherent to K-562 monolayers demonstrated NK activity of 31.0% and ADCC of 43.0%. Trypsinization of effector cells had no effect on NK but eliminated ADCC. The K-562 adherent lymphocytes which formed rosettes with sheep RBC demonstrated NK activity of 8.0% with an ADCC of 12.0%. The K-562 adherent lymphocytes not forming rosettes showed 24.0% NK activity and no ADCC.

The lymphocyte subpopulation which was not adherent to K-562 cells demonstrated no NK activity but showed 43.0% ADCC. The level of ADCC dropped to 12.0% when B and T-cells were removed. Trypsinization of effector cells, which has been shown to remove Fc receptors, eliminated

Table 3. NK and ADCC activities of plastic non-adherent and Ficoll-Hypaque separated lymphocytes

Subpopulation	% Cytotoxicity by NK	ADCC
Adherent to K-562 monolayers	31.0	43.0
+Trypsinization	32.0	0.0
+Sheep RBC forming rosettes	8.0	12.0
+Sheep RBC not forming rosettes	24.0	0.0
Non-adherent to K-562 monolayers	0.0	43.0
Minus B Cells[1], minus T Cells[2]	0.0	12.0
+Trypsinization	0.0	0.0
+Sheep RBC forming rosettes	0.0	12.0
+Sheep RBC not forming rosettes	0.0	0.0
Adherent on K-562 monolayers, eluted with EDTA, non-rosetting with sheep RBC, adherent on K-562 in second incubation	22.0	0.0
Non-adherent on K-562 monolayers, non-rosetting with sheep RBC, non-adherent on K-562 in second incubation	0.0	0.0

[1]B-cells removed on nylon wool

[2]T-cells removed with anti-human thymocyte serum +C'

ADCC. The K-562 non-adherent lymphocytes (minus B and T-cells) which formed rosettes with sheep RBC gave 12.0% ADCC while the non-rosetting cells showed no ADCC.

The lymphocyte subpopulation adherent to K-562 monolayers was removed from the monolayer with EDTA and tested. Of these cells, those which did not form rosettes with sheep RBC were incubated a second time on K-562 monolayers. The adherent cells were removed with EDTA and gave 22.0% NK activity. The cells which were not adherent in this second incubation showed no NK activity.

IV. ASSAY FOR IgG AND IgM FROM NORMAL HUMAN SERUM

Fractions of donor serum were chromatographed on Protein A-Sepharose and assayed in an ADCMC reaction. Figure 2 shows the cytotoxicity obtained with various serum dilutions. This cytolysis was eliminated by addition of 2-mercaptoethanol. The Protein-A bound fraction showed no cytolysis at the highest dilution and only 2.0% at the lowest dilution.

Normal human serum which was fractionated by centrifugation in a 10 to 40% sucrose gradient was assayed in an ADCMC reaction. In Figure 3, the tenth through fourteenth fractions from the bottom of the gradient comprised the peak ADCMC activity at 22%. Fractions 21 and 22 showed ADCMC at 2.0%. These peaks are identified in

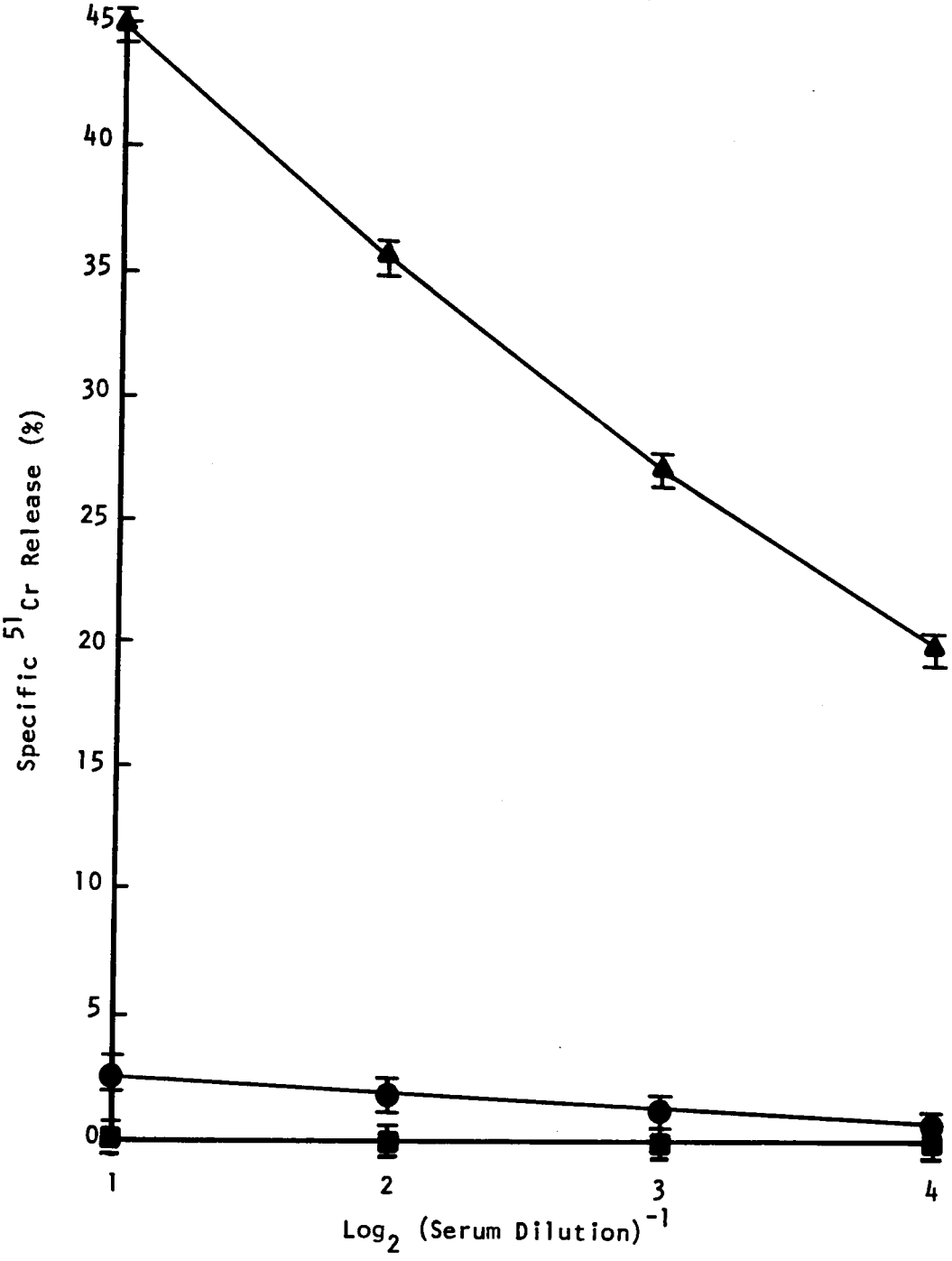

Figure 2. Cytolysis curve for serum ADCMC with both Protein A-bound and unbound fractions and elimination of cytolysis by addition of 2-Mercaptoethanol; ▲=unbound fraction, ■=unbound fraction + 2-Mercaptoethanol, ●=bound fraction.

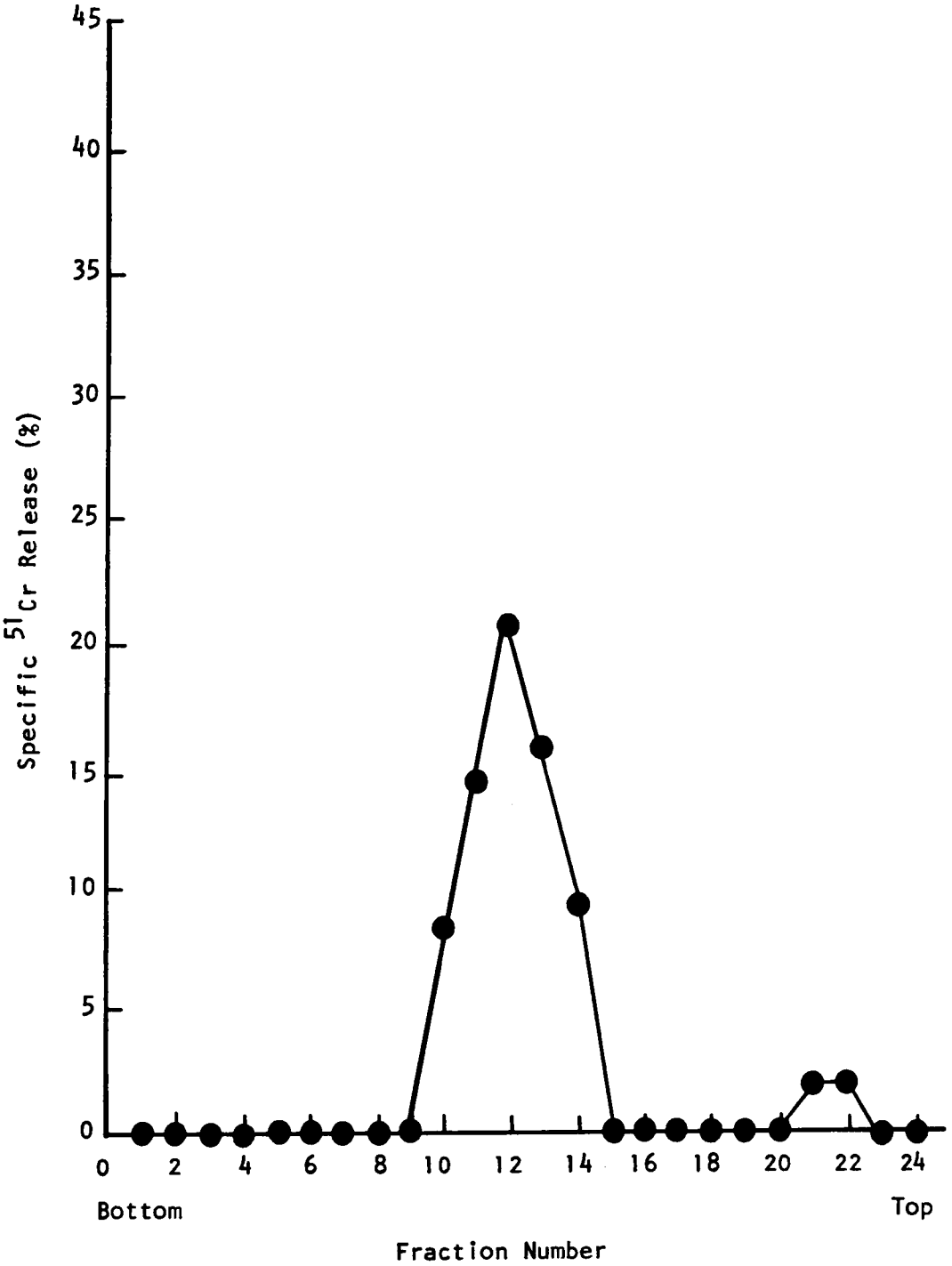

Figure 3. Fractionation of normal human serum by centrifugation in a 10 to 40% sucrose gradient at 27,000 rpm (138,000 xg) for 16.5 hours versus ^{51}Cr-release in a two hour ADCMC assay.

Figure 4 by optical densities and refractive indices. The peak near the top of the gradient contains IgG (A_{280}=6.5) which corresponds to fractions 21 and 22 in Figure 3. The intermediate peak contains IgM (A_{280}=1.5) and corresponds to fractions 10 through 14 in Figure 3.

V. COMPETITIVE INHIBITION OF ADCMC BY LYMPHOCYTES

Lymphocytes subpopulations were added competitively to ADCMC assays. As shown in Figure 5, non-NK lymphocytes, including B, T, and null cells gave no reduction in the level of cytotoxicity when anti-K-562 serum was used as the source of antibody. Similar results were obtained with null NK-depleted lymphocytes, as shown in Figure 6. Null NK-depleted are preparations from which B and T-cells and cells adherent to K-562 were deleted. The null NK cells eluted from K-562 monolayers with EDTA did compete with ADCMC. This is shown in Figure 7. There was a gradual decrease in % ^{51}Cr-release from 55% in the control reaction at a 1:50 Ab dilution to 25% upon addition of NK cells using a 100:1 effector:target ratio. A gradual decrease was shown from 48% in the control reaction at a 1:100 Ab dilution to 21% after adding NK cells at a 100:1 effector:target cell ratio. The control reaction at a 1:300 Ab dilution showed 43% and decreased to 18% after addition of NK cells at 100:1 ratio. The NK controls with

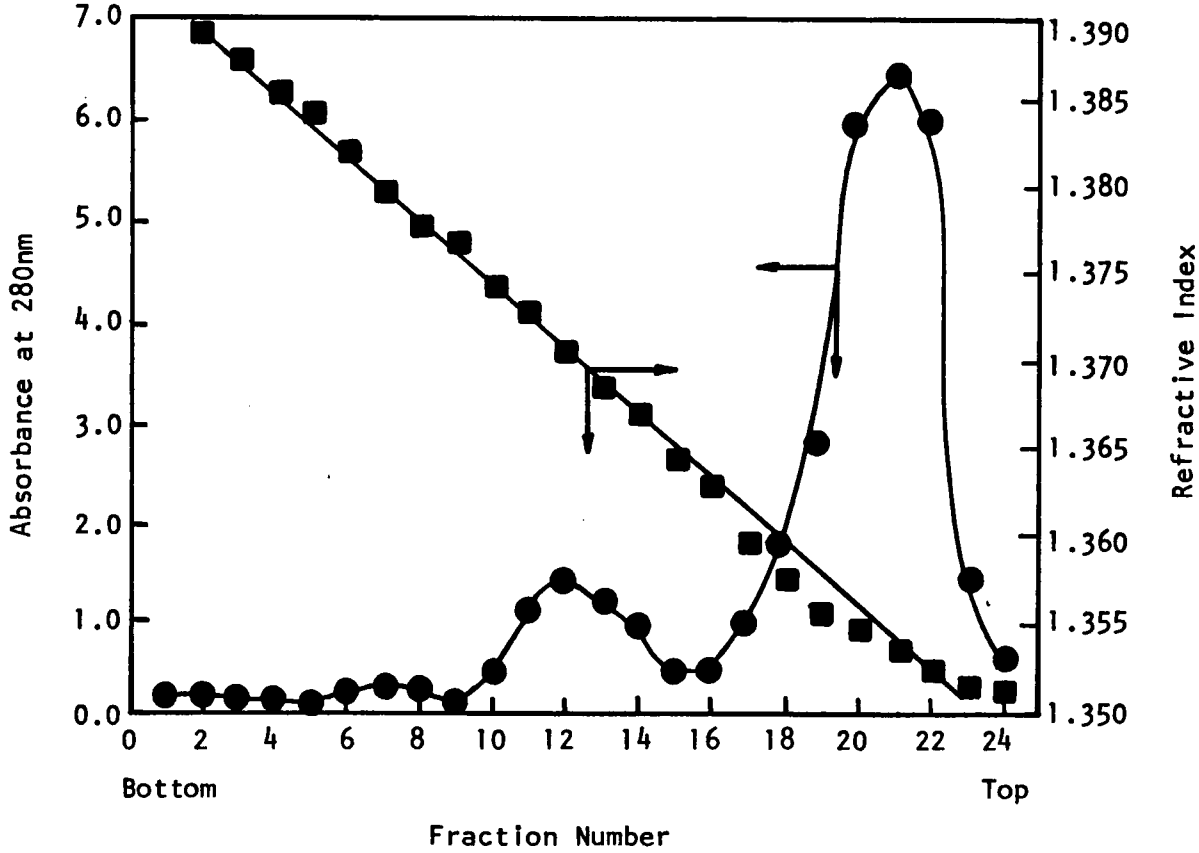

Figure 4. Curve of absorbance (280 nm) and refractive indices for fractionated normal human serum in a 10 to 40% sucrose gradient. The peak at the top of the gradient contains IgG, and the intermediate peak contains IgM.

Figure 5. Competitive inhibition of ADCMC by addition of (A) NK lymphocytes (B) null NK-depleted lymphocytes and (C) non-NK (T, B, and null) lymphocytes in increasing competitor:target ratios versus ADCMC with anti-K-562 serum and complement, antibody dilutions: ▲=1:50 (Control=55%), ■=1:100 (Control=48%), ●=1:300 (Control=43%).

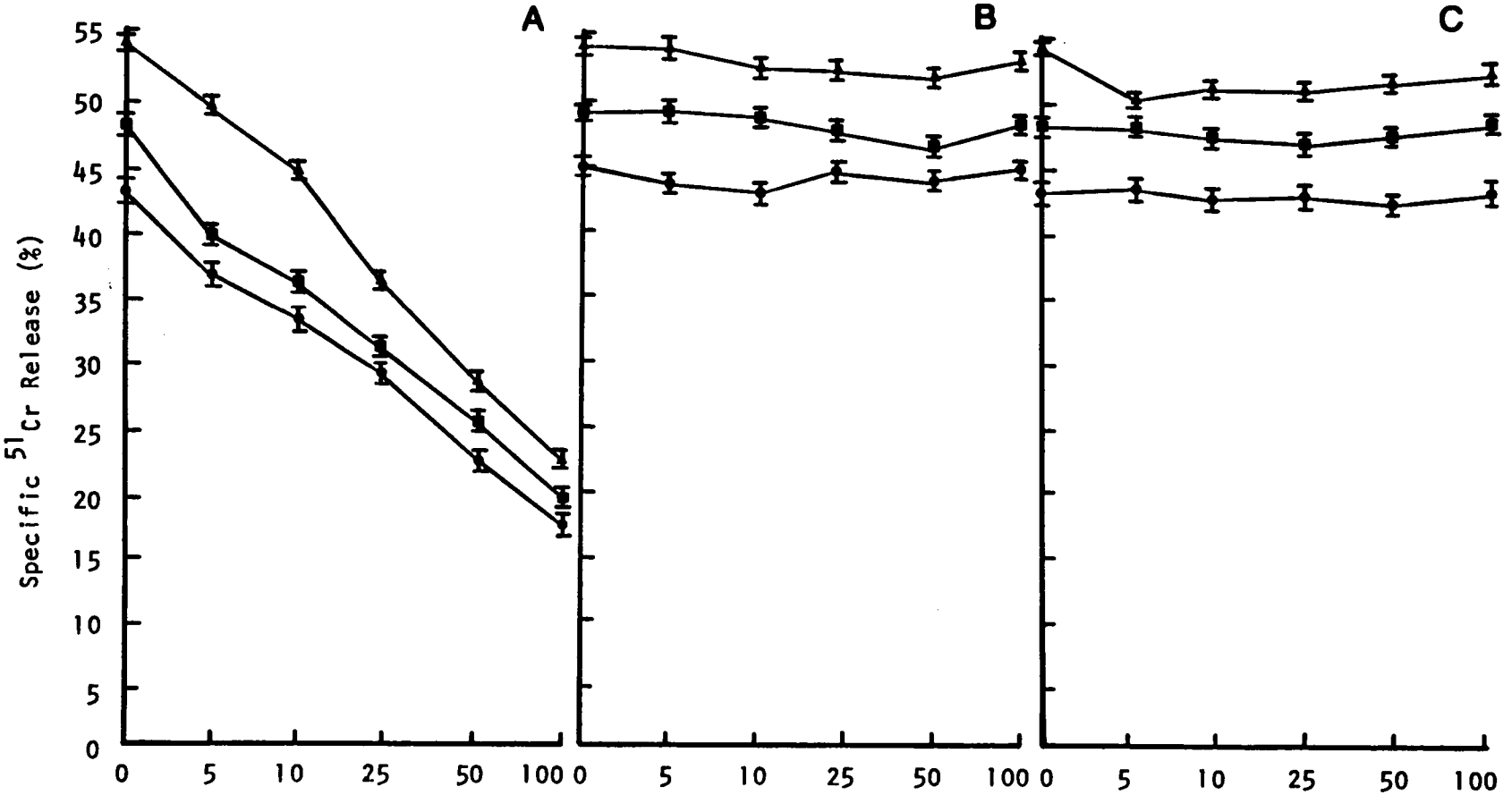
A
B
C
Specific ^{51}Cr Release (%)
55
50
45
40
35
30
25
20
15
10
5
0
0 5 10 25 50 100
Competitor:Target Ratio in ADCMC Reaction

Figure 5.

no Ab or complement showed no ^{51}Cr-release after ninety minutes.

VI. INHIBITION OF NK AND ADCC WITH A 3M KCl ANTIGEN PREPARATION

A 3M KCl antigen preparation was made and added to NK and ADCC reactions to observe for inhibition of cytolysis. As shown in Figure 6, the % ^{51}Cr-release of 43% in the ADCC reaction decreased gradually to 16% when increasing amounts of the Ag preparation were added. The % ^{51}Cr-release of 31% in the NK reaction gradually decreased to 15% with addition of increasing amounts of the antigen preparation.

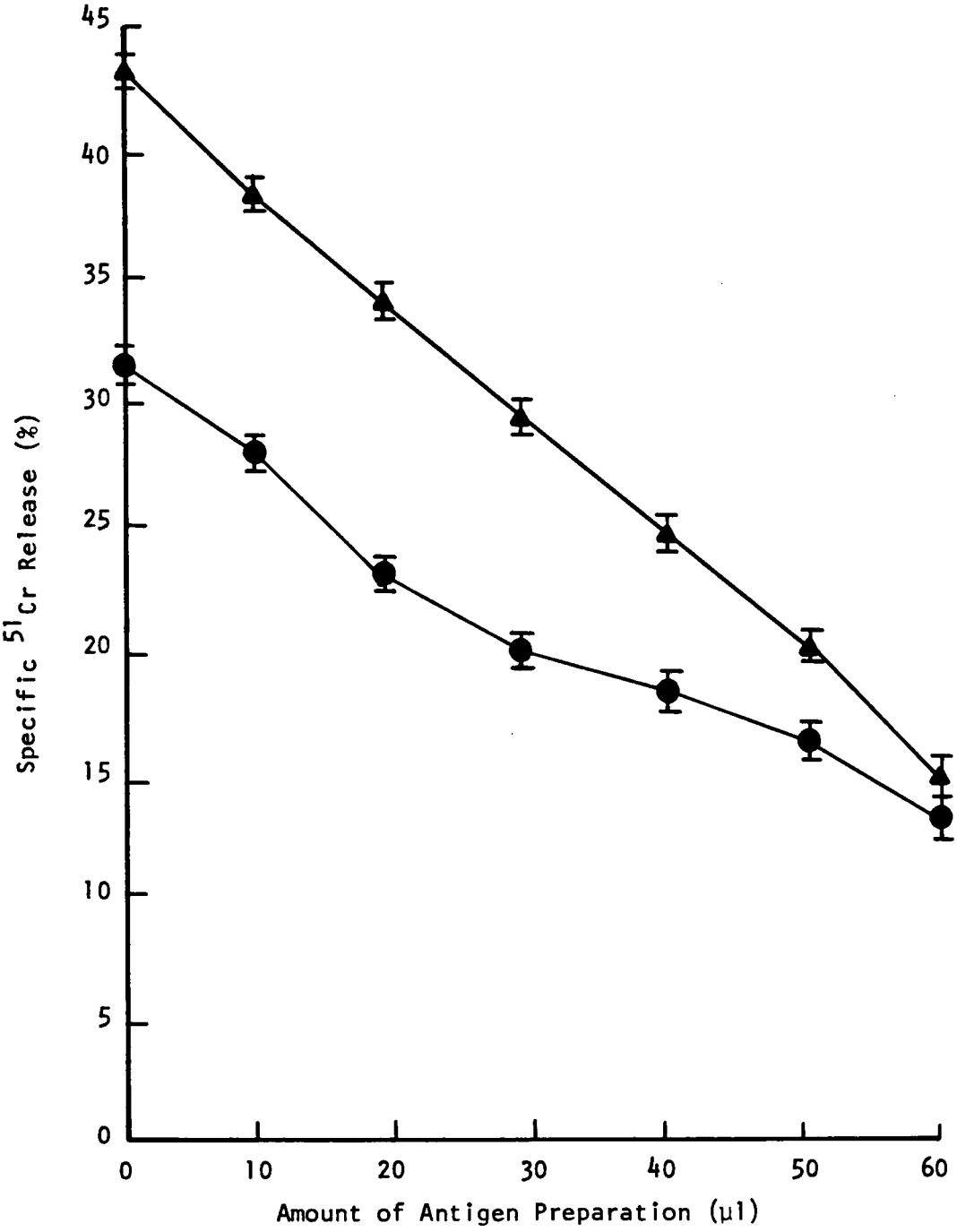

Figure 6. Inhibition of NK and ADCC to K-562 cells with 3M KCl antigen preparation; ▲=NK + anti-K-562 serum (Control=43%), ●=NK (Control=31%).

CHAPTER V

DISCUSSION AND CONCLUSIONS

In this report I initially asked whether the antigen on the K-562 cell recognized by human NK cells is the same as that recognized by specific antibody. Human NK cells from peripheral blood did competitively inhibit ADCMC. The ninety minute assay provides a period of time which is adequate for ADCMC but is not sufficient for NK cell activity, which requires at least 4 hours of incubation. In the same type of inhibition assay non-NK cells, specifically those that do not bind to K-562 cells, showed no competition. In addition, K-562 cell extracts competitively inhibited NK cell lysis of K-562 cells as well as ADCC. These findings taken together suggest that the NK cell and the antibody recognize the same antigen on the K-562 cell.

This interpretation is supported by investigators using other systems (47,48,49,50) who demonstrated inhibition of lymphocyte-mediated cytolysis to evaluate cell surface antigens on tumor cells in vitro. Huber et al.,(47) found with soluble tumor antigen from the supernatant fluid of 13762A mammary tumor cultures that blocking of cell-mediated cytolysis is linearly related to antigen concentration.

Recognition may be directed to the same antigenic

determinants or to different determinants (or antigens per se) in close proximity to each other. The inhibition observed in this case would be then consistent with the steric hindrance of one effector by another. Another possibility to explain apparent inhibition is that the K-562 antigen preparation may be injurious to the effector cell, rather than specifically inhibiting cytolysis. Further investigation of this problem would require use of another NK-sensitive human cell line to observe for inhibition of cytolysis by the K-562 extract to determine definitive inhibition.

These experiments suggest, as other workers have suggested (32), that NK cell activity is a two-step process. The first step involves the recognition of and binding to the target antigen, which occurs apparently within the first ninety minutes during the time when antibody competes. The second step or killing process requires at least 4 hours. Collins et al.,(32) have shown that normal cells resistant to NK cell lysis will competitively inhibit NK cytolysis of sensitive tumor cells, thus sensitivity or resistance results in later steps upon activation of the lytic mechanism after a minimum lag period of 4 hours.

The second question addressed in this thesis was whether NK and/or non-NK cells manifest an ADCC response. The results indicated that some NK cells, defined as cells which were adherent to K-562 monolayers (42), participated

to a limited extent in ADCC reactions. The non-NK cells gave similar ADCC activity. The non-NK cells were those lymphocytes which did not adhere to K-562 cell monolayers. Cells responsible for ADCC are identified by the presence of Fc receptors, which was noted by the ability of the cells to form rosettes with sensitized sheep erythrocytes. The ADCC activity with NK or non-NK cells was eliminated by trypsin treatment, which removed the Fc receptor. On the other hand, trypsin treatment had no effect on NK cell activity. The conclusion that NK cell activity is independent of Fc receptors and ADCC is supported by the work of Kay et al.,(27)who showed that Fc receptor negative cells from humans were responsible for NK cell activity against K-562 cells. In contrast, Takasugi et al.,(29)showed that trypsin treatment of NK cells eliminated their cytolytic activity. However, in their assay the effector cells consisted of a population of lymphocytes possessing antibodies attached to Fc receptors on their surfaces. The effector cells in my NK assays did not include this group and they were separated by rosetting from Fc receptor negative cells, which were used in my assays. Also considered is the possibility that heterogeneous NK cell subpopulations exist, some which possess trypsin-resistant receptors and others which do not. The effector cells in my assay lyse K-562 cells and this particular subpopulation may possess trypsin-resistant receptors. The effectors in

Takasugi's assay may possess receptors for his particular target cell lines, other than K-562, and these receptors may not be resistant to trypsin.

Another focus of this project was to investigate in vitro cytolytic activity against K-562 by peripheral blood lymphocytes and serum from normal individuals. The results showed that three groups of individuals could be identified: one group showing both NK cell activity and a natural Ab level to K-562 cells in serum, a second group having only natural Ab and no NK cell activity, and a third group having neither activity. Although 5.0% of the population studied was in the last group, no apparent defect in immunosurveillance was indicated since these individuals had no increased familial levels of malignancies.

The natural antibody activity found in the normal serum was identified as IgM by several criteria. Namely, it did not adhere to Protein A-Sepharose, it migrated as IgM in centrifugation through sucrose gradients, and it was sensitive to 2-merceptoethanol. The origin of this antibody to K-562 cell antigens remains unclear. It is possible that it is a cross-reactive antibody made in response to an undefined antigen. The fact that it was present in such a high percentage of individuals suggests an analogy to blood group isoantibodies. If this were the case, one might expect to find cells with surface antigens

reactive with the antibody in those individuals who were antibody-negative. Another possibility, related to the proposed role of this antigen on K-562 as a differentiation receptor (51), is that the antibody is a feedback control mechanism for hematopoietic development. These possibilities, or others, would have important implications for the well-being of an individual and are suggested areas for future study.

Although human NK cell activity has been reported in numerous studies, to my knowledge, the finding that many individuals (35%) of a random, normal human population did not show NK cell activity, is unique. It is not apparent why such a large percentage of NK(-) individuals would not have been observed in other studies.

LIST OF REFERENCES

LIST OF REFERENCES

1. Herberman, R. B., M. E. Nunn, H. T. Holden, S. Stahl, and J. Y. Djeu. 1977. Augmentation of cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic target cells. Int. J. Cancer. 19: 555-564.

2. Herberman, R. B., and T. H. Holden. 1978. Natural cell-mediated immunity. Adv. Cancer Research. 271: 305-377.

3. Jondal, M., and H. Pross. 1975. Surface markers on Human B and T lymphocytes. VI. Cytotoxicity against cell lines as a functional marker for lymphocyte subpopulation. Int. J. Cancer. 15: 596-605.

4. Pollack, S. B., M. R. Tam, R. C. Nowinski, and S. L. Emmons. 1979. Presence of T-cell associated antigens on murine NK cells. J. Immunol. 123: 1818-1821.

5. West, W. H., G. B. Cannon, H. D. Kay, G. D. Bonnard, and R. B. Herberman. 1977. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. J. Immunol. 118: 355-361.

6. Kiessling, R., E. Klein, H. Pross, and H. Wigzell. 1975. Natural killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Maloney leukemia cells: characteristics of the killer cell. Europ. J. Immunol. 5: 117-121.

7. Shoham, J., and I. Eshel. 1980. Thymic hormonal effect on human peripheral blood lymphocytes in vitro III. Conditions for mixed lymphocyte-tumor culture assay. J. Immunol. Methods. 37: 261-273.

8. Kay, H. D., G. D. Bonnard, W. H. West, and R. B. Herberman. 1977. A functional comparison of human Fc-receptor bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. J. Immunol. 118: 2058-2066.

9. Kalden, J. R., H. H. Peter, R. Roubin, and J-P. Cesarini. 1977. Human peripheral null lymphocytes. I. Isolation, immunological and functional characteristics. Europ. J. Immunol. 7: 537-543.

10. Pross, H. F., M. G. Baines, and M. Jondal. 1977. Spontaneous human lymphocyte-mediated cytotoxicity against tumor cells. Is the complement receptor necessarily present on the killer cells? Int. J. Cancer. 20: 353-358.

11. Timonen, T., A. Ranki, E. Saksela, and P. Hayry. 1979. Human natural cell-mediated cytotoxicity against fetal fibroblasts. III. Morphological and functional characterization of the effector cells. Cellular Immunol. 48: 121-132.

12. Nunn, M. E., J. Y. Djeu, M. Glaser, D. H. Lavrin, and R. B. Herberman. 1976. Natural cytotoxic reactivity of rat lymphocytes against syngeneic Gross virus-induced lymphoma. J. Natl. Cancer Inst. 56: 393-399.

13. Timonen, T., E. Saksela, A. Ranki, and P. Hayry. 1979. Fractionation, morphological, and functional characterization of effector cells responsible for human natural killer activity against cell-line targets. Cellular Immunology. 48: 133-148.

14. Timonen, T., and E. Saksela. 1980. Isolation of human natural killer cells by density gradient centrifugation. J. Immunol. Methods. 36: 285.

15. Timonen, T., J. R. Ortaldo, and R. B. Herberman. 1981. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. J. Exp. Med. 153: 569-582.

16. Haller, O., R. Kiessling, M. Gidlund, and H. Wigzell. 1979. In vivo tumor-associated antigens and their specific immune response. (eds. F. Spreafico and P. Arnon). Academic Press, New York.

17. Herberman, R. B., H. T. Holden, W. H. West, G. D. Bonnard, A. Santoni, M. E. Nunn, H. D. Kay, and J. R. Ortaldo. 1979. In vivo tumor-associated antigens and their specific immune response. Proceedings of the Serono Symposia. 16: 129 (eds. F. Spreafico and Arnon). Academic Press, London.

18. Djeu, J. Y., J. Heinbaugh, H. Holden, and R. B. Herberman. 1979. Augmentation of mouse natural killer cell activity by interferon and interferon inducers. J. Immunol. 122: 175-181.

19. Attalah, A., and T. Folks. 1979. Interferon enhanced human natural killer and antibody-dependent cell-mediated cytotoxic activity. Int. Archs. Allergy and Applied Immunol. 60: 377-382.

20. Oehler, J. R., L. R. Lindsay, M. E. Nunn, H. T. Holden, and R. B. Herberman. 1978b. Natural cell-mediated cytotoxicity in rats. II. In vivo augmentation of NK cell activity. Int. J. Cancer. 21: 210-220.

21. Gidlund, M., A. Orn, H. Wigzell, A. Senik, and I. Gresser. 1978. Enhanced NK cell activity in mice infected with interferon and interferon inducers. Nature. 273: 259.

22. Kirchner, H., H. M. Hirt, H. Becker, and K. Munk. 1977. Production of an antiviral factor by murine spleen cells after treatment with Corynebacterium parvum. Cellular Immunology. 31: 172-176.

23. Oehler, J. R., L. R. Lindsay, M. E. Nunn, and R. B. Herberman. 1978a. Natural cell-mediated cytotoxicity in rats. I. Tissue and strain distribution, and demonstration of a membrane receptor for the Fc portion of IgG. Int. J. Cancer. 21: 204-209.

24. Koide, Y., and M. Takasugi. 1977. Determination of specificity in natural cell-mediated cytotoxicity by natural antibodies. J. Natl. Cancer Inst. 59: 1099-1105.

25. Takasugi, M., and D. Akira. 1979. Role of antibodies in the specificity of natural cell-mediated cytotoxicity. J. Natl. Cancer Inst. 62(6): 1361-1365.

26. Scheiner, O., G. Weidermann, H. Rumpold, R. Steiner, D. Kraft, and H. Steinberger. 1980. Modulation of NK and K cell activity by trypsin treatment of effector cells. Immunobiology. 157: 343-357.

27. Kay, H. D., R. Fagnani, and G. D. Bonnard. 1979. Cytotoxicity against the K-562 erythro-leukemia cell line by human natural killer (NK) cells which do not bear free Fc receptors for IgG. Int. J. Cancer. 24: 141-150.

28. Herberman, R. B., J. Y. Djeu, H. D. Kay, J. R. Ortaldo, C. Riccardi, G. D. Bonnard, H. T. Holden, R. Fagnani, A. Santoni, and P. Puccetti. 1979. Natural killer cells: characteristics and regulation of activity. Immunol. Rev. 44: 43-70.

29. Haller, O., R. Kiessling, A. Orn, K. Karre, K. Nilsson, and H. Wigzell. 1977. Natural cytotoxicity to human leukemia mediated by mouse non-T cells. Int. J. Cancer. 20: 93-103.

30. Nunn, M. E., R. B. Herberman, and H. T. Holden. 1977. Natural cell-mediated cytotoxicity in mice against non-lymphoid tumor cells and some normal cells. Int. J. Cancer. 20: 381-387.

31. Galili. U., E. Klein, G. Klein, and P. Biberfield. 1980. The natural attachment of thymocytes and activated T-lymphocytes to normal and malignant cells: an interspecies study. Dev. and Comp. Immunol. 4: 367-374.

32. Collins, J. L., P. Q. Patek, and M. Cohn. 1981. Tumorigenicity and lysis by natural killer cells. J. Exp. Med. 153: 89-106.

33. Lozzio, C. B., and B. B. Lozzio. 1975. Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. Blood. 45(3): 321-334.

34. Lozzio, B. B., C. B. Lozzio, E. G. Bamberger, and A. S. Feliu. 1981. A multi-potential cell line (K-562) of human origin. Proc. of the Soc. for Exp. Biol. and Med. 166: 546-550.

35. Collins, J. L., C. J. Wust, B. B. Lozzio, and C. B. Lozzio. 1977. Characterization of an antigen from the myelogenous cell line K-562. J. Natl. Cancer Inst. 59(6): 1667-1673.

36. Lozzio, B. B., C. B. Lozzio, C. J. Wust, and A. Girardi. 1977. Leukemia-associated antigens detected by a nonhuman primate antiserum to a Ph'+ myelogenous leukemia cell line. Blood. 50: 115-124.

37. Latif, Z. A., B. B. Lozzio, C. B. Lozzio, R. B. Herberman, and C. J. Wust. 1979. Abrogation of the proliferation of human leukemia cells in nude mice by a xenoantiserum. Leukemia Research. 3(6): 371-378.

38. Ortaldo, J. R., R. K. Oldham, G. C. Cannon, and R. B. Herberman. 1977. Specificity of natural cytotoxic reactivity of normal human lymphocytes against a myeloid leukemia cell line. J. Natl. Cancer Inst. 59: 77-82.

39. Vose, B., and M. Moore. 1980. Natural cytotoxicity in humans: susceptibility of freshly isolated tumor cells to lysis. J. Natl. Cancer Inst. 65(2): 257-263.

40. Kall, M. A., and H. S. Koren. 1978. Heterogeneity of human natural killer cell populations. Cellular Immunology. 40: 58-68.

41. Peck, R., A. Brown, and C. J. Wust. 1979. In vitro heterologous cytotoxicity by T effector cells from mice immunized with Sindbis virus. J. Immunol. 123(4): 1763-1766.

42. Jensen, P. J., and H. S. Koren. 1979. Depletion of NK by cellular immunoabsorption. J. Immunol. 123(3): 1127-1132.

43. Stulting, R. D., and G. Berke. 1973. Nature of lymphocyte-tumor interaction: a general method for cellular immunoabsorption. J. Exp. Med. 137: 932-942.

44. Garvey, J. S., N. E. Cremer, and G. H. Susdorf. 1977. Methods in Immunology. W. A. Benjamin, Inc., Reading, Massachusetts.

45. Skvaril, F. 1976. The question of specificity in binding human IgG subclasses to Protein A-Sepharose. Immunochemistry. 13: 871-872.

46. Kronvall, G., and R. C. Williams. 1969. Differences in anti-Protein A activity among IgG subgroups. J. Immunol. 103: 828-833.

47. Huber, S., and Z. J. Lucas. 1979. A quantitative assay for tumor antigen based on inhibition of cytotoxicity of sensitized lymphocytes. Int. J. Cancer. 23: 224-232.

48. Zoller, M., M. R. Price, and R. W. Baldwin. 1976. Inhibition of cell-mediated cytotoxicity to chemically induced rat tumors by soluble tumor and embryo cell extracts. Int. J. Cancer. 17: 129-137.

49. Prather, S. O., and R. N. Lausch. 1976. Membrane-associated antigen from the SV-40 induced hamster fibrosarcoma PARA-7. I. Role in immune complex formation and effector cell blockade. Int. J. Cancer. 18: 820-828.

50. Baldwin, R. W., M. R. Price, and A. Robins. 1973. Significance of serum factors modifying cellular immune responses to growing tumors. Brit. J. Cancer. 28: 37-47.

51. Wust, C. J., M. E. Green, C. B. Lozzio, and B. B. Lozzio. 1981. Antibody-dependent cytolysis of K-562 cells. Blood, in press.

VITA

Jack M. Williams was born in Knoxville, Tennessee on December 22, 1955. He attended primary and secondary schools in Knoxville and was graduated from Holston High School in June, 1973. Mr. Williams entered the University of Tennessee in June, 1973, and was graduated with honors in December, 1976, with a major in biology. He entered graduate school at the same university in September, 1978, and received the M.S. degree in December, 1981.