5-1983

Natural Killer Activity in Gardner's Syndrome

Ann Marie Stembridge
Utah State University

Follow this and additional works at: https://digitalcommons.usu.edu/etd

Part of the Biology Commons, Genetics Commons, and the Immunology and Infectious Disease Commons

Recommended Citation
https://digitalcommons.usu.edu/etd/4623

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact dylan.burns@usu.edu.
NATURAL KILLER ACTIVITY IN
GARDNER'S SYNDROME

by

Ann Marie Stembridge

A thesis submitted in partial fulfillment
of the requirement for the degree
of
MASTER OF SCIENCE
in
Biology
(Immunology/Genetics)

Approved:

UTAH STATE UNIVERSITY
Logan, Utah
1983
ACKNOWLEDGEMENTS

I want to express my gratitude to my thesis advisor, Dr. Reed Warren for guiding me through a trying year of research with such patience and kindness. To my committee members, Dr. John Simmons, Dr. Wilbur Thain, and Dr. Reed Warren, I thank them for their persistence in assisting me to achieve my best work.

I also wish to extend my gratitude to my parents, Mr. and Mrs. Sidney Stembridge and my boyfriend, Daniel Dulaney for their undying support, love and belief in me.

Ann M. Stembridge
TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................ ii
LIST OF TABLES ........................................ iv
LIST OF FIGURES ........................................ iv
ABSTRACT ....................................................... v
INTRODUCTION ................................................ 1
REVIEW OF LITERATURE ...................................... 3
  Gardner's Syndrome ........................................ 3
  Review of the Immune System .............................. 8
  Origin of NK Cells ......................................... 10
  Mechanisms of NK .......................................... 14
  NK Activity in the Gut ..................................... 18
  Lymphoid Tissue ........................................... 18
  Age, Sex and Environment ................................ 19
  Affecting NK Levels in Man ............................... 19
MATERIALS AND METHODS ................................... 22
  Preparation of Lymphocytes ............................... 22
  Target Cell Preparation ................................... 23
  Effector Cell Preparation .................................. 24
RESULTS AND DISCUSSION .................................. 26
  Results ..................................................... 26
  Discussion .................................................. 31
  Conclusion ................................................ 33
REFERENCES .................................................. 35
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Clinical Follow-up and Status of Patients and Internal Controls</td>
<td>6</td>
</tr>
<tr>
<td>2. Review of the Immune System</td>
<td>11</td>
</tr>
<tr>
<td>3. Analysis of Variance for Gardner's Syndrome vs. Controls</td>
<td>28</td>
</tr>
<tr>
<td>4. Analysis of Natural Killer Activity in Males vs. Females</td>
<td>30</td>
</tr>
</tbody>
</table>

LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pedigree chart showing affected members of kindred</td>
<td>4</td>
</tr>
<tr>
<td>2. Diagram showing blood circulation, lymphatic circulation and innervation of the intestine</td>
<td>7</td>
</tr>
<tr>
<td>3. Schematic representation of an intestinal villous and immune system factors present</td>
<td>7</td>
</tr>
<tr>
<td>4. Natural Killer reactivity in Gardner's syndrome vs. controls</td>
<td>27</td>
</tr>
<tr>
<td>5. Natural Killer reactivity in males vs. females</td>
<td>29</td>
</tr>
</tbody>
</table>
Gardner's syndrome is an autosomal dominant disease presenting multiple colonic polyps with a predisposition for malignant change. In addition to colonic polyp formation by early adolescence, extracolonic lesions appear often prior to polyp formation. One theoretical mechanism for the origin of polyps and malignancies in Gardner's syndrome is a genetic defect in the natural killer cell activity of patients with this disease. Natural killer cells are a subpopulation of lymphocytes that spontaneously lyse tumor cells and virally transformed cells. A study was undertaken to determine the natural killer activity of patients with Gardner's syndrome.
A technique termed chromium release was used to determine natural killer cell reactivity. This assay involves incubating patient's lymphocytes (effector cells) with tumor cells from a myelogenous leukemia cell line (target cells) which has been previously labeled with the radioactive isotope chromium-51. The level of isotope in the supernatant is believed to correlate with the natural killer activity of the subject.

The results of this study indicate normal natural killer cell activity in patients with Gardner's syndrome. A trend toward lower natural killer cell activity was found at higher effector to target ratios in the patients. An incidental finding of higher natural killer activity in males (patients and healthy subjects) than in females was made. Higher natural killer cell activity of male lymphocytes against the cell line K562 has not been previously reported. In conclusion, the natural killer cell activity of lymphocytes from patients with Gardner's syndrome against the cell line K562, is no different from that found by lymphocytes of healthy subjects.
INTRODUCTION

Gardner's syndrome (GS) is an autosomal dominant disease presenting multiple colonic polyps with a predisposition for malignant change. GS was first reported by E.J. Gardner in Utah in the early 1950's (Gardner, 1951). Victims of this disease usually develop colorectal polyps by early adolescence which continue to grow in size and number and if untreated, transform into adenocarcinoma. The syndrome has been clinically characterized by the colorectal polyps and one to several of the following extracolonic lesions: epidermoid cysts, osteomas, subcutaneous fibromas, desmoid tumors and dental abnormalities.

One theoretical mechanism for the origin of the polyps and malignancies seen in GS, is a genetic defect in the natural killer (NK) cell activity of patients with this disease. Recently, a population of lymphocytes termed NK cells has been demonstrated to lyse, in vitro, tumor and virally transformed cells in man and experimental animals (Herberman and Holden, 1978). The NK cells require no prior priming to foreign antigens on the tumor or virally infected cells for lysis to occur. Since NK cells destroy tumor cells in vitro, it is believed that they have an important role in protecting against tumors. Hersey et al. (1979a) has found in some strains of mice that the inheritance of NK cell activity is autosomal
dominant as in segregation of susceptibility of GS in man. Warner and Dennert (1982) provide solid evidence for the role of NK cells as immune surveillants against tumors in vivo. They injected cyclophosphamide into two groups of mice making them NK cell deficient. One of the groups then received cloned NK cells three hours prior to injection with pulmonary tumor cells. The other group of mice did not receive NK cells. The mice were sacrificed three weeks later and those treated with cloned NK cells had 50-85% fewer pulmonary tumor colonies than the control group and had no metastases as compared to liver metastases found in the mice not receiving NK cells. The above findings led to an investigation of NK cell activity in GS.

NK cell reactivity is determined by incubating lymphocytes from a subject (effector cells) with tumor cells (target cells) that have previously been labeled with an isotope of chromium-51. The NK cells lyse the target cells by damaging the target cell membrane. The label is released from the target cells and aliquots of culture medium are assayed for the amount of chromium released. The amount of chromium released correlates with the level of NK activity.
REVIEW OF LITERATURE

Gardner's Syndrome

Colon cancer is the most frequent form of malignancy in the United States today. GS has been under investigation for the past thirty years. Much of the previous work on GS has involved defining the disease, pedigree analysis, documenting case studies and investigating the possibility of chromosomal abnormalities (Gardner et al., 1982).

In 1947, E.J. Gardner began studies on a family in which the gene for GS was segregating. This family has been designated as kindred 109, an abbreviated pedigree for this kindred is shown in Figure 1. The pedigree includes the individuals tested for NK cell activity. At the onset of the study, eight people had died from colon cancer with the ninth soon to follow. All victims were in their mid-thirties at the time of death. The pedigree clearly demonstrates the autosomal dominant transmission of the disease. Six people in the pedigree were found to have multiple polyps in their large intestine at the first screening. Of these six individuals, two have died, (one was the ninth victim and the other lived for 22 years after surgery and then died of an unrelated cause) and the rest remain alive and in good health (Gardner, 1983). Gardner has found the onset of colonic polyps in
ABBREVIATED KINDRED 109

Figure 1. Pedigree chart showing affected members of kindred 109.

(E.J. Gardner, 1983)
kindred 109 to occur as early as 11 years of age and as late as 18 years with an average of 14 years. Presently there is no test available at birth to determine, conclusively, if an infant has GS, as shown in Table 1. However, extracolonic lesions may be present at birth with osteomas being the most prevalent lesion. All kindred 109 members have been found to have osteomas. If these osteomas are present at birth, they may be detected by a physical examination. There has been much success in Utah in extending the life of GS patients well beyond mid-thirties. This is done by regular proctoscopic examinations and surgical removal of polyps as the number found becomes too large to remove by cautery at proctoscopic examinations (Gardner, 1980). In Table 1., the treatment histories of the patients are presented.

The mechanism of polyp formation in GS has been investigated by Deschner and Lipkin (1975) using kinetic studies with tritiated thymidine. Their results illustrate a continuous proliferation of the polyps as they pass the mid-level of the Crypts of Lieberkuhn upward to the mucosal surface (Figure 2.). These polyps may be pedunculated (hang on stocks of mucosa) or villous adenomas which are sessile and broad based at the mucosal surface. GS is a specific disease with polyp formation but is not synonymous with familial polyposis coli (FPC). In 1980, Gardner addressed the differences and similarities of GS and FPC. He asserts that GS patients have fewer but larger adenomas than FPC patients who are known to have a few hundred to 5000
Table 1. Clinical Follow-up and Status of Patients and Internal Controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>G.S. lesion</th>
<th>Age first recorded</th>
<th>Age first colon adenoma</th>
<th>Status</th>
<th>Age of Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-8</td>
<td>*</td>
<td>7</td>
<td>14</td>
<td>colostomy</td>
<td>51</td>
</tr>
<tr>
<td>IV-10</td>
<td></td>
<td>6</td>
<td>13</td>
<td>colostomy</td>
<td>45</td>
</tr>
<tr>
<td>IV-20</td>
<td>*</td>
<td>10</td>
<td>14</td>
<td>subtotal colectomy</td>
<td>41</td>
</tr>
<tr>
<td>IV-21</td>
<td></td>
<td>2</td>
<td>18</td>
<td>subtotal colectomy</td>
<td>38</td>
</tr>
<tr>
<td>IV-30</td>
<td></td>
<td>2</td>
<td>14</td>
<td>died at 30 from mesenteric fibromatosis</td>
<td>30</td>
</tr>
<tr>
<td>IV-31</td>
<td>*</td>
<td>18</td>
<td>18</td>
<td>died at 23 from mesenteric fibromatosis</td>
<td>23</td>
</tr>
<tr>
<td>V-20</td>
<td></td>
<td>4</td>
<td>12</td>
<td>subtotal colectomy</td>
<td>20</td>
</tr>
<tr>
<td>V-21</td>
<td>*</td>
<td>5</td>
<td>11</td>
<td>subtotal colectomy</td>
<td>18</td>
</tr>
<tr>
<td>V-28</td>
<td>*</td>
<td>At birth</td>
<td>16</td>
<td>subtotal colectomy</td>
<td>22</td>
</tr>
<tr>
<td>V-29</td>
<td></td>
<td></td>
<td></td>
<td>control</td>
<td>20</td>
</tr>
<tr>
<td>V-30</td>
<td></td>
<td>6</td>
<td>15</td>
<td>multiple adenomas</td>
<td>18</td>
</tr>
<tr>
<td>V-31</td>
<td></td>
<td>2</td>
<td></td>
<td>Gardner syndrome</td>
<td>14</td>
</tr>
<tr>
<td>V-32</td>
<td></td>
<td></td>
<td></td>
<td>control</td>
<td>12</td>
</tr>
<tr>
<td>V-33</td>
<td></td>
<td>At birth</td>
<td></td>
<td>Gardner syndrome</td>
<td>9</td>
</tr>
<tr>
<td>V-34</td>
<td>*</td>
<td>At birth</td>
<td></td>
<td>Gardner syndrome</td>
<td>7</td>
</tr>
<tr>
<td>V-62</td>
<td></td>
<td>5</td>
<td>13</td>
<td>multiple adenomas</td>
<td>18</td>
</tr>
<tr>
<td>V-63</td>
<td>*</td>
<td>3</td>
<td>11</td>
<td>multiple adenomas</td>
<td>15</td>
</tr>
<tr>
<td>V-66</td>
<td></td>
<td>2</td>
<td></td>
<td>Gardner syndrome</td>
<td>6</td>
</tr>
<tr>
<td>V-84</td>
<td></td>
<td>7</td>
<td>14</td>
<td>subtotal colectomy</td>
<td>27</td>
</tr>
<tr>
<td>VI-13</td>
<td></td>
<td></td>
<td></td>
<td>At risk</td>
<td>2</td>
</tr>
<tr>
<td>VI-39</td>
<td></td>
<td></td>
<td></td>
<td>At risk</td>
<td>6</td>
</tr>
<tr>
<td>VI-39a</td>
<td></td>
<td></td>
<td></td>
<td>At risk</td>
<td>2</td>
</tr>
</tbody>
</table>

Average 4.7 14

*Gardner's syndrome patients tested for NK activity (E.J. Gardner, 1983)
Figure 2. Diagram showing blood circulation, lymphatic circulation and innervation of the intestine.

Figure 3. Schematic representation of an intestinal villous and immune system factors present.
adenomas carpeting their large intestine. Polyps found in both groups are similar if not identical in terms of histology, pathology, and genetics but, the major difference between the two diseases is the lack of extracolonic lesions in FPC patients and the presence of one or more of these lesions in GS patients. In addition, St. Mark's Hospital in London has placed the average age of onset in FPC patients to be ten years later than that of GS patients.

To date there has been no immunological research on GS except for one study done by Berlinger et al. in 1977. Mixed lymphocyte culture assays focusing on lymphocyte responsiveness and the modulatory role of adherent mononuclear cells were completed on a mother and daughter with GS. Results of these studies suggest decreased responses in these women as compared to those subjects without GS. The investigators theorize a deficit of recognitive immunity may be an unsuspected component of GS.

Review of the Immune System

The primary components of the immune system in man are the B cells and the T cells. B cells originate in the bone marrow and produce antibodies whereas T cells are derived from the thymus and can be delineated into at least three subpopulations:

1. Cytotoxic T cells which lyse foreign cells.
2. Helper T cells which are necessary for antibody production and the development of cytotoxic T lymphocytes (CTL).

3. Suppressor T cells which suppress expression of the immune system.

T cells are also known to produce humoral factors called lymphokines which assist in the functioning of the immune system. Lymphokines should not be mistaken as antibodies. Two important lymphokines that cause proliferation of NK activity levels in the peripheral blood of man are interferon and T cell growth factor also known as Interleukin-2.

Macrophages are not considered to be part of the immune system but are thought to work in conjunction with B and T cells in the production of antibody and development of cell-mediated immunity. Macrophages destroy 90-98% of the foreign substance which enters the body and then deliver the remaining antigenic substance to the B and T cells.

Cytotoxic T lymphocytes (CTL), K cells and NK cells are similar in function but their mechanisms of killing differ. CTL need to be exposed to an antigen for seven to fourteen days before they become cytotoxic. K cells and NK cells are not classified as either B or T cells but are considered more primitive cell forms. They both have receptors for IgG and need no prior sensitization for mediating cytotoxicity, however; K cells require antibody coated cells to mediate lysis and NK cells do not. Table 2. summarizes the cells of
the immune system. (Reviewed by Herberman and Ortaldo (1981)).

Origin of NK Cells

Major controversies over the origin of NK cells have been in existence since the discovery of these cells in the early 1970's. Are NK cells immature T cells or are they a separate lymphoid population? Also, are NK cells and K cells the same or divergent lymphoid lines? These questions have not been answered but many have speculated and conducted experiments in attempt to answer these questions.

Timonen et al. (1981) have isolated large granular lymphocytes (a subpopulation of lymphocytes) that are 50% larger than most other lymphocytes and have kidney-bean shaped nuclei and nuclear azurophilic granules. These granular lymphocytes express NK activity and antibody-dependent cellular cytotoxicity. These lymphocytes are found in highest concentration in the peripheral blood and lungs each (7% of the total), the spleen (4%), peritoneal exudates (3%), and lymph nodes (1%) but few if any are found in the thymus or bone marrow. Based on experiments with the T cell growth factor, Herberman (1982) proposes that large granular lymphocytes have diverged from "T stem cell lineage". NK cells retain NK morphology and activity for seven days, at which time, the cell surface markers shift to those found on mature T-cells. Timonen et al. suggest that T cell growth factor may "push" the large granular lymphocytes to further differentiation.
Table 2. Review of the Immune System

<table>
<thead>
<tr>
<th>Morphology</th>
<th>NK cells</th>
<th>T cells</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>12-15 microns</td>
<td>9-12 microns</td>
<td>12-16 microns</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Slightly indented</td>
<td>Round</td>
<td>Markedly indented</td>
</tr>
<tr>
<td>Adherence-plastic</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cell surface markers for sheep RBC</td>
<td>Yes on about 50% (low affinity)</td>
<td>Yes (high affinity)</td>
<td>No</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Antigens (Human)</td>
<td>Most or all cells: HNK-1, OKM1, asialo-GM1, OKT10 subsets: 9.6, 1a</td>
<td>Most or all cells: 9.6, OKT3 subsets: OKT4, OKT8</td>
<td>Most or all cells: OKM1, asialo-GM1 subset: 1a</td>
</tr>
<tr>
<td>Functional Characteristics</td>
<td>Spontaneous reactivity</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Response time</td>
<td>In vivo: within 4 hrs. in vitro: within 1 hr.</td>
<td>Primary response: 5-7 days memory response: 2-5 days</td>
<td>In vivo: 5-10 days in vitro: about 18 hrs.</td>
</tr>
<tr>
<td>Nature of target</td>
<td>At least several widely distributed antigenic specificities</td>
<td>Wide array of specific antigens; important role of MHC restricted antigens</td>
<td>Specificity not clearly defined; selectivity for tumor targets</td>
</tr>
<tr>
<td>Growth factors</td>
<td>TCGF</td>
<td>TCGF</td>
<td>Colony stimulating factor</td>
</tr>
<tr>
<td>Possible mechanism of cytotoxicity</td>
<td>Protease, phospholipase, cytotoxin</td>
<td>Protease, osmotic</td>
<td>Reactive oxygen species, protease, lysozyme, phagocytosis, PGE, IFN</td>
</tr>
<tr>
<td>Production of soluble mediators</td>
<td>IFN, TCGF</td>
<td>Wide array of lymphokines</td>
<td>LAF, PGE, IFN, Colony stimulating factor</td>
</tr>
</tbody>
</table>

(R.B. Herberman, 1982)
Perhaps not all populations of NK cells in mammals have diverged from the T stem cell lineage. Hersey et al. (1979a) discovered in mice having a congenital lack of thymus (nude mice) and in mice neonatally thymectomized, that higher NK activity exists than is found in other strains of mice. Also when T cells are depleted from mice with anti-sera directed against T cells, NK cells demonstrate an increased activity. These experiments suggest that NK cells do not originate from the T cell lineage in some cases but instead arise from bone marrow.

In recent years, there has been a proliferation of papers concerning NK cells and K cells and their similarities and differences. Gupta (1980) believes that three different subpopulations of lymphoid cells exist including, cells capable of mediating both NK and antibody-dependent cellular cytotoxicity, cells capable of mediating NK cell cytotoxicity alone, and cells capable of mediating antibody-dependent cellular cytotoxicity alone. Helms and Bull (1980) prefer a model of a single effector cell acting through two separate mechanisms. They found that the elimination of monocytes from an effector cell population resulted in an increase in the NK cell cytotoxicity and antibody-dependent cellular cytotoxicity. Enzyme treatments showed marked differences between NK and K cell activity which may suggest that two different receptor sites are involved. Pretreatment of effector cell populations with neuraminidase
diminished K cell activity and increased NK activity, whereas, pretreatment with trypsin reduced NK killing but had no effect on K cell killing. Hahn et al. (1978) agree with Helms and Bull on their proposed model and cite some additional evidence that one effector cell mediates both K and NK cell activity. They found when passing peripheral blood lymphocytes over nylon wool columns, that K cell killing increased and NK cell killing did not. If lymphocytes are stored at room temperature overnight in medium with fetal calf serum, antibody-dependent cellular cytotoxicity increases while NK activity remains unaltered. Lastly, a fluctuation in NK activity is found from month to month but no fluctuation occurs in K cell activity. Hahn et al. theorize the fluctuation is present because of the heterogeneity of the NK cell population.

Until recently, fragment crystalline receptors were found only on K cells, but now they can be demonstrated on both NK and K cells. In 1980, Nelville successfully separated NK cells from K cells by buoyant density centrifugation. Together NK and K cells represent 2-3% of the lymphocyte population or 20-30% of the receptor bearing cells. The NK and K cells are activated to lyse target cells by different mechanisms, Nelville found that NK cells bind to P-815-Ab cells but do not lyse them, inversely, K cells bind to K562 cells but do not lyse them either. Therefore, binding is not sufficient to cause lysis. This researcher proposes that two different fragment crystalline receptor bearing lymphocyte populations exist, where in K cells, these fragment
crystalline receptor populations activate the K cells to become cytotoxic and lyse antibody coated cells, and the other receptor population signals cytotoxicity to NK cells for NK sensitive targets.

Mechanisms of NK

There is no general consensus among researchers on the mechanism of natural killing. Wright and Bonavida (1983b) have published a series of papers proposing that natural killer cytotoxic factors are involved in NK. They found that nude mice with high NK activity also possessed high levels of cytotoxic factors, and, conversely, other mice with low NK levels had low cytotoxic factor levels. They define natural killer cytotoxic factors as non-adherent, Thy-1.2 negative factors, with asialo-GM markers on surface membranes. These factors are found to be active on target cells sensitive to NK lysis. Their model of cytolysis is based on sequential steps as follows: first the effector cell recognizes and binds to the target cell. The target cell then emits a signal to the effector cell which activates the lytic cycle. The effector cell releases cytotoxic factors which bind to the target cell and lysis occurs.

Roder (1980) found that dinitrophenol and sodium azide inhibited NK cell lysis but did not block effector-target cell binding. He hypothesizes that energy is necessary to expose the enzyme's active site to the cell's exterior and suggests an esterase as the enzyme
responsible for this activity. Hiserodt et al. (1982) agree that effector-target cell binding may play a passive role with no temperature requirements and little energy or membrane movement necessary. They feel that interferon steps up the programming for lysis by changing the membrane of effector cells and allowing increased expression of the lytic elements.

Pucetti et al. (1979) have followed work started by Djeu et al. (1979) regarding a functional relationship between NK and macrophage activity. They agree with Djeu et al. that macrophages are necessary for the production of interferon and an increase of NK activity when poly I:C is used. Pucetti et al. theorize that a common link between NK and macrophages may only occur upon release of interferon. When using a large dose of pyran copolymer, a reticulo-endothelial stimulating agent and interferon inducer, to activate macrophages, they found a decrease in NK activity. This observation agrees with that of others who find that macrophages are able to both activate and suppress NK activity. They are not ruling out a suppressor cell acting to lower NK activity, but prefer their theory that a cellular association exists between NK cells and macrophages.

In addition to interferon, interleukin-2 is being investigated as a lymphokine of primary importance to the NK mechanism. Domzig et al. (1983) have examined in vitro, the effect of interleukin-2 in humans by utilizing a monoclonal antibody against interleukin-2. Depleting
anti-interleukin-2, did not effect the capacity of NK cells to rejuvenate, if the cells were treated for less than twenty hours. However, depleting for more than twenty hours, resulted in NK cells completely devoid of interleukin-2. Only with the addition of extraneous interleukin-2 did NK activity reappear. No interferon was detected in these samples of interleukin-2 or anti-interleukin-2 so that all subsequent findings could be attributed solely to interleukin-2. Kawase et al. (1983) found that interleukin-2 preparation from human or mouse tissues is able to stimulate interferon production in mice spleen cells. As much interferon was produced in nude mice as in CBA mice suggesting mature T cells play no role in interferon production. When macrophages and NK like cells are exposed to interleukin-2, a cooperative effort exists between these cell types to produce interferon. Kawase et al. suggest a two stage approach to interferon production and subsequent NK boosting. In the first stage, one to several soluble mediators are generated from the macrophages that may act alone or in conjunction with interleukin-2 to produce interferon from these NK like cells which bear asialo-GM-1 and NK-1.2 markers. The second step involves boosting NK activity from the newly produced interferon. The mechanism of interferon and interleukin-2 on NK activity are unknown, but many agree that these lymphokines are important in maintaining a healthy, active NK cell population.
The researchers whose work was discussed above have tried to delineate the NK mechanism under normal circumstances, but what, if any, changes occur under adverse conditions such as cancer or other disease conditions? A profound defect of NK cell activity has been found in humans (Roder et al., 1980) and is termed Chediak-Higashi syndrome. This syndrome is caused by an autosomal recessive gene which is analogous to the recessive "beige" gene in mice. These individuals have normal humoral immunity and delayed type hypersensitivity, but suffer pyrogenic infections in childhood and have high risks of contracting lymphoproliferative disorders if adulthood is reached. Given high doses of purified interferon over a twenty-four hour period, these patients are able to respond like controls. Targan and Oseas (1983) suggest a defect in their post-binding mechanism of lysis to cause the low levels and delay in NK activity.

Tumors are theorized to secrete prostaglandins that may lower NK activity. Roder and Klein (1979) using an inhibitor of prostaglandin synthesis termed indomethacin, demonstrated a small but significant increase of the NK cytolytic response in mice.

Steinhauer et al. (1982) have found a difference in NK activity in patients with advanced cancer. The number of large granular lymphocytes and effector-target binding cells was no different in advanced cancer patients than it was in other cancer patients and normal subjects. In a normal functioning system, the individual NK
cell is able to make a lethal hit, recycle and induce another lethal hit. In these advanced cancer victims, the defect results from the inability of the NK cell population to recycle despite normal numbers and binding capacities.

NK Activity in the Gut Lymphoid Tissue

Studies of NK activity or antibody-dependent cellular cytotoxicity in gut lymphoid tissue are pertinent when investigating GS and its localization of polyp formation in the colon. Clancy and Pucci (1978) have looked at antibody-dependent cellular cytotoxicity in healthy and inflamed human gut mucosa and conclude that no activity is present. Arnaud-Battandier et al. (1978) have extended the former study and have investigated NK and antibody-dependent cellular cytotoxicity in guinea pigs. The guinea pig gut lymphoid tissue was separated into the lamina propria and the intraepithelial tissue (Figure 2. and 3.). Lamina propria contains plasma cells for antibody production and the intraepithelial tissue contains 90% of the lymphocytes. The researchers assessed NK and antibody-dependent cellular cytotoxicity in the spleen cells, lamina propria, intraepithelial lymphocytes, Peyer's patches and mesenteric lymph nodes. The NK activity was at the highest level in the spleen cells following with a moderate level of activity in the intraepithelial
lymphocytes, a minimal level in the mesenteric lymph nodes and no activity in the Peyer's patches. Similar results were obtained with the antibody-dependent cellular cytotoxicity assay. Arnaud-Battandier et al. conclude that the lack of NK and antibody-dependent cellular cytotoxicity in mesenteric lymph nodes and Peyer's patches is due to a lack of fragment crystalline receptor positive cells in these tissues.

Age, Sex and Environment Affecting NK Levels in Man

Uksila et al. (1983) studied NK activity in premature human newborns and aborted fetuses. They found a progressive development of NK cell function during the last third of intrauterine life. In a nineteen week old fetus, NK activity was evident in the liver and spleen but not in the thymus or bone marrow suggesting that NK cells do not evolve from B or T progenitor cells. Lymphocytes from cord blood at birth show levels of cytotoxicity that are higher than that of the peripheral blood lymphocytes from premature infants but lower than that of cells found in adults. Toder et al. (1983) initiated a study looking at NK activity in healthy pregnant women and pregnant women with edema-proteinuria-hypertension. This condition is a partial or total malfunction of either the fetal or the maternal immune system. A normal pregnancy does not specifically alter the NK level whereas in women with this condition, the NK level is doubled. Upon addition of interferon, the normal pregnant woman's NK level is more
than doubled but little or no enhancement is found in edema-proteinuria-hypertension gestosis women. Toder et al. propose that the pre-NK or undifferentiated NK cells in these women may undergo some activation by an unknown factor, other than interferon. Whether this activation is a manifestation of the disease or a secondary response is unknown.

A study done by Hersey et al. (1979b) examined the NK level of patients with melanoma and their families. The findings suggest a lower NK activity in melanoma patients and closely related relatives. In families free of melanoma, the influence of genetic and environmental factors on NK levels was not as apparent as in the melanoma families.

In 1981, Nagel et al. published their findings on a longitudinal study done in Baltimore on 150 men and 50 women aged 20-95 years. Nagel concluded that NK activity against a myelogenous leukemia cell line K562, was independent of age, sex and whether or not the NK cell donor smoked. In patients forty to seventy-five years of age, a decrease in the level of cytotoxicity was evident but, this decrease was not significant. However, a higher NK activity was found for those 75 years of age and older. Nagel theorizes that the higher NK activity gives the subject a survival advantage. One could consider that individuals with lower NK activity, if it does indeed render immune surveillance, have previously died. No data were compiled in
this study for individuals under twenty years and no other studies have been done at this time. Penschow and Mackay (1980) have demonstrated an increase in NK activity in males and younger healthy controls when measuring NK activity with the Chang cell line. The K cell activity did not differ in their study according to sex or age. The different findings of Nagel and Penschow and Mackay are most likely due to the different cell lines used.

Herberman (1982) has published his own ideas on possible future clinical applications of NK cells in disease conditions such as cancer. Peripheral blood of a cancer patient could be drawn and NK cells isolated from the other lymphocyte populations. These isolated NK cells could then be cultured in T cell growth factor until the cells increase by $10^6$. These cloned cells may then be injected back into the cancer patient and theoretically, the increased NK cell population should eradicate the tumor.
MATERIALS AND METHODS

The Natural killer activity is determined by utilizing the chromium release assay with $^{51}$Cr. The procedure is given below and modified from Jondal (1976).

Preparation of Lymphocytes

1. Thirty to sixty ml of heparinized blood is drawn from each subject (.2ml of 1000 μ/ml of preservative free heparin (Sigma St. Louis, Mo.)).

2. The blood is either used undiluted or mixed with an equal volume of phosphate buffered saline (.01M phosphate and .15M NaCl). If the plasma is to be retained, the blood is centrifuged at 2000 rpm for 15 minutes in an IEC centrifuge. Next the diluted blood is layered over 3.5 ml of Ficoll-Hypaque gradient (specific gravity 1.077-1.080 g/ml) in a 15 ml centrifuge tube and centrifuged at 2000 rpm for 20 minutes.

3. The lymphocyte population, located at the interface on the gradient, is removed and subsequently washed at 1500 rpm for 10 minutes in phosphate buffered saline. The cells are
decanted and then are resuspended in the saline and centrifuged at 1000 rpm for 5 minutes. The cells are then counted and suspended in RPMI-1640 (Gibco, Grand Island, N.Y.) that has been supplemented with 10,000 units of aqueous penicillin and 10 mg. streptomycin/100 ml solution. If stored overnight, the medium is supplemented with 20% autologous plasma and the lymphocytes are held at a concentration of no more than 2x10^6/ml in solution. The lymphocytes are stored at room temperature.

Target Cell Preparation

1. The target cells are subcultured 24 hours before use to assure a healthy population. The target cells are from a human myelogenous leukemia line termed K562 and are maintained in RPMI-1640 with 10% fetal calf serum (Sterile Systems, Logan, Ut.) in 5% CO₂ and 37°C. The cells are passed twice weekly.

2. On the morning of the assay, the subcultured K562 cells are centrifuged and 0.1 ml of autologous plasma and 0.1 ml of sodium chromate (Na₂⁵¹CrO₄) are added at a concentration of 3 miCi/ml and a specific activity of 300-400miCi/mg (New England Nuclear, Boston, Ma.). The target cells are then
incubated for one hour without shaking in a 5% CO₂ incubator at 37°C.

3. After one hour, the target cells are resuspended in 15 ml RPMI with 10% autologous plasma and centrifuged at 1000 rpm for 3 minutes. The cells are then washed 3 more times using the same procedure and suspended at a final concentration of 1x10⁵/ml.

Effector Cell Preparation

1. While labeling the target cells, a Linbro 96 well round-bottomed microtiter plate is prepared by adding 0.1ml of .5% saponin detergent (maximum release) and 0.1ml of 20% autologous plasma in RPMI (control release) in triplicates to the wells.

2. The lymphocytes are then pipetted into the microtiter plate at concentrations of 50 effectors to 1 target cell, 25:1, 12:1, and 6:1.

3. A multiple pipetter is used to add the targets to the wells. The plate is then incubated for 4 hours at 37°C and 5% CO₂.
4. After the incubation, 0.1ml of supernatant is pipetted from each well and counted for five minutes in a Beckman gamma counter. The spontaneous release is calculated by dividing the control release by the maximum release. The spontaneous release averaged 26% for this assay.

5. The calculation for % release is as follows:

\[
\text{% release} = \frac{\text{experimental release} - \text{control release}}{\text{maximum release} - \text{control release}} \times 100
\]

Experimental release is obtained from incubating the lymphocytes with the target cells, the control release is from target cells incubated in medium alone and the and the maximum release from target cells incubated in a detergent.
RESULTS AND DISCUSSION

Results

Figure 4. shows the results of studying seven patients with GS and seven healthy subjects who were age and sex matched with the patients. An analysis of variance (Table 3.) indicates that no statistical difference exists between the NK activity of controls and GS patients at any of four effector to target ratios. However, at the higher effector to target ratios, a suggestion of difference is evident with the controls demonstrating a higher NK activity level. Another analysis of variance was computed for a difference in NK reactivity between males and females. The men had a higher NK activity at all four ratios (p< .1 F test, Table 4.).

The mean age of the ten women sampled was 28.8 ± 16.2 years which included two girls aged 8 and 10 years. These younger women had higher NK activities than the older women. The men assayed (including patients and healthy subjects) had a mean age of 34.73 ± 14.35 years with a concentration of men over 40 years of age. A lower level of NK was found in the men over 40 years than in the younger men; there were too few women over 40 years in this study to make the same assessment.
Figure 4. Natural Killer reactivity in Gardner's syndrome vs. controls
Table 3. Analysis of Variance for Gardner's Syndrome vs. Controls

<table>
<thead>
<tr>
<th>Source</th>
<th>dF</th>
<th>Sum of Squares</th>
<th>MSE</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair</td>
<td>6</td>
<td>4849.356</td>
<td>808.226</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>1</td>
<td>980.294</td>
<td>980.294</td>
<td>1.102</td>
</tr>
<tr>
<td>Error A</td>
<td>6</td>
<td>5335.237</td>
<td>889.206</td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>3</td>
<td>8049.268</td>
<td>2683.089</td>
<td></td>
</tr>
<tr>
<td>G x R</td>
<td>3</td>
<td>334.313</td>
<td>111.438</td>
<td>2.236</td>
</tr>
<tr>
<td>Error B</td>
<td>36</td>
<td>1794.321</td>
<td>49.842</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>21342.791</td>
<td>388.051</td>
<td></td>
</tr>
</tbody>
</table>
NATURAL KILLER REACTIVITY AND SEX

Legend

\[ \Delta \text{ MALES} \]
\[ \times \text{ FEMALES} \]

Figure 5. Natural Killer reactivity in males vs. females
### Table 4. Analysis of Natural Killer Activity in Males vs. Females

#### A. Analysis at 50:1 between male and female

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>MSE</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>737.489</td>
<td>737.489</td>
<td>3.15 *</td>
</tr>
<tr>
<td>Error</td>
<td>19</td>
<td>4454.749</td>
<td>234.461</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at .90 level

#### B. Analysis at 25:1 between male and female

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>MSE</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>988.099</td>
<td>988.099</td>
<td>3.85 *</td>
</tr>
<tr>
<td>Error</td>
<td>19</td>
<td>4884.819</td>
<td>257.096</td>
<td></td>
</tr>
</tbody>
</table>

#### C. Analysis at 12:1 between male and female

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>MSE</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>479.467</td>
<td>479.467</td>
<td>3.07 *</td>
</tr>
<tr>
<td>Error</td>
<td>19</td>
<td>2968.959</td>
<td>156.261</td>
<td></td>
</tr>
</tbody>
</table>

#### D. Analysis at 6:1 between male and female

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>MSE</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>285.016</td>
<td>285.016</td>
<td>3.29 *</td>
</tr>
<tr>
<td>Error</td>
<td>19</td>
<td>1645.456</td>
<td>86.603</td>
<td></td>
</tr>
</tbody>
</table>
Attempts were made to use CCL-218 (ATCC, Rockville, Md.), a colorectal adenocarcinoma cell line as target cells for NK cell reactivity because there are no GS adenocarcinoma lines available at present. Labeling of these cells was attempted using the procedure described in "Methods" but the spontaneous release was always in an excess of 50%. In an effort to lower this release, the target cells were labeled, washed and then allowed to incubate in a Linbro 96 flat bottomed plate for several hours before effector cells were added as suggested by Brunner et al. (1976). This procedure did not remedy the problem of high spontaneous release, so study of this cell line was discontinued.

Discussion

Santoli et al. (1976) and Penschow and Mackay (1980) have reported that lymphocytes of men have a higher NK activity against the Chang cell line than do those of women. In the present study, a higher NK activity also was found in men against the K562 cell line which had never been reported before. Hersey et al. (1979b) reported familial inheritance of NK activity in melanoma patients and close relatives but no such inheritance was seen in the three GS families sampled.

There was no success using CCL-218 to measure NK cell activity, however; there are reports that HCT-8, a colorectal line, labels well with $^{51}$Cr. In retrospect it may have been a better choice of cell line
to use. The best colorectal line to use, if it could be labeled with $^{51}$Cr, would be tissue cultured from a GS adenocarcinoma. If such a line could be isolated and cultured, the GS adenocarcinoma would be disease specific and have the greatest promise for delineating a difference in NK activity between patients and controls. The K562 cell line used in the present study is a myelogenous leukemia cell line which is not disease specific but rather the most common line used to assay NK activity in humans.

No significant decrease in NK activity was found in GS individuals when peripheral blood lymphocytes were used in the assay. However, a difference might exist if lymphoid tissue from the gut were sampled. Polyps are not adenocarcinoma but, simply adenomas with the potential to transform. It is possible that these polyps secrete some inhibitor, such as prostaglandins, as has been suggested by Droller et al. (1978) which inactivates the immune system. Also, these polyps may not have any tumor associated antigens (TAA) on their surfaces or any markers that the immune system could recognize as foreign. GS patients may be specifically tolerant to the TAA or other markers possibly present on the polyp cell surface.

Testing peripheral blood lymphocytes for NK may not be a sensitive enough evaluation for the specialized polyp formation ongoing in the colon of GS patients especially when considering the possibility that NK cells may not be dispersed evenly through all gut
tissue. The polyps normally found in the gut of GS patients have a lamina propria base and are covered on the surface by intraepithelial lymphocytes. Could there be an inherent genetic defect in the intraepithelial lymphocytes which are unable to stop the progressive formation of these gut adenomas? For this to be ascertained, gut NK cells separated by techniques of Arnaud-Battandier et al. (1978) could be tested for activity.

Other immune functions should be investigated in the future. They include mitogen stimulation with phytohemagglutinin, concanavalin A, lipopolysaccharide and pokeweed mitogen to discern if T cell subpopulations and B cells are functioning normally in these patients. Possibly the helper T or cytotoxic T cells are not functioning in the lamina propria and allowing formation of these polyps. Another important area of future investigation is to perform human leucocyte antigen (HLA) typing on all GS patients and see if there is a correlation of HLA with the disease.

Conclusion

The NK cell activity against myelogenous leukemia cell line K562 was tested in patients with GS and no significant difference was found in these patients as compared to findings in healthy subjects. A
difference was noted in NK activity between male and female subjects tested. Males exhibited a higher NK cell activity against K562 cells \((p < .10)\) than the females. NK cell activity should be considered for future study in patients with GS but, different and more specific cell lines should be tried in these investigations.
REFERENCES


Gardner, E.J. A genetic and clinical study of intestinal polyposis a


Hersey, P., Edwards, A., Trilivas, C., Shaw, H., Milton, G.W. Relationship of natural killer activity to rhesus antigens in


Activation of mouse macrophages by pyran co-polymer and role in 
augmentation of natural killer activity. Int. J. Cancer. 24: 
819-825; 1979.

Roder, J.C. Target-effector interaction in the natural killer cell 
system. VI. The influence of age and genotype on natural killer 

Roder, J.C., Haliotis, T., Klein, M., Korec, S., Jett, J.R., Ortaldo, 
disorder in humans involving NK cells. Nature. 284: 553-555; 
1980.

Roder, J.C. and Klein, M. Target-effector interaction in the natural 
killer cell system. IV. Modulation by cyclic nucleotides. J. 

Santoli, D., Trinchieri, G., Zmijewski, C.M., Koprowski, H. 
HLA-related control of spontaneous and antibody-dependent 
cell-mediated cytotoxic activity in humans. J. Immunol. 117: 
765-770; 1976.

Steinhauer, E.H., Doyle, A.T., Reed, J., Kadish, A.S. Defective 
natural cytotoxicity in patients with cancer: normal number of 
effector cells but decreased recycling capacity in patients with 

Targan, S.R. and Oseas, R. The lazy natural killer cells of 

Timonen, T., Ortaldo, J.R., Herberman, R.B. Characteristics of human 
large granular lymphocytes and relationship to natural killer and 

Toder, V., Blank, M., Gleicher, N., Volgovich, I., Mashbiah, S., 
Nebel, L. Activity of natural killer cells in normal pregnancy 
145: 7-10; 1983.


Wright, S.C. and Bonavida, B. Studies on the mechanism of natural killer cell-mediated cytotoxicity. Interferon induced inhibition of natural killer target cell susceptibility to lysis is due to a defect in their ability to stimulate release NKCF. J. Immunol. 130: 2965-2968; 1983.