CELLULAR IMMUNITY IN CHILDREN WITH
DOWN SYNDROME (TRISOMY-21)

by

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Roger Lee Noble
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<td>AET</td>
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<td>B</td>
<td>humoral immunity-mediating</td>
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<td>CDC</td>
<td>complement-dependent cytotoxicity</td>
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<td>Con A</td>
<td>concanavalin A</td>
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<td>DS.</td>
<td>Down syndrome, trisomy-21</td>
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<td>FBS</td>
<td>fetal bovine serum</td>
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<td>IFN</td>
<td>interferon</td>
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<td>Ig.</td>
<td>immunoglobulin</td>
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<td>IL-2</td>
<td>interleukin-2, T cell growth factor</td>
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<td>2-ME.</td>
<td>2-mercapto-ethanol</td>
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<td>NK.</td>
<td>natural killer</td>
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<td>PBMC.</td>
<td>peripheral blood mononuclear cells</td>
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<td>PBS</td>
<td>phosphate buffered saline</td>
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<td>PHA</td>
<td>phytohemagglutinin</td>
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<td>PMA</td>
<td>4-phorbol 12-myristate 13-acetate</td>
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<td>PWM</td>
<td>pokeweed mitogen</td>
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<td>RFC</td>
<td>SRBC rosette-forming cells</td>
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<tr>
<td>S.D.</td>
<td>standard deviation</td>
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<td>s.e.m.</td>
<td>standard error of the mean</td>
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<td>SRBC.</td>
<td>sheep red blood cells</td>
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<td>T4:T8</td>
<td>ratio of OKT4+ cells to OKT8+ cells</td>
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<td>WBC</td>
<td>white blood cells</td>
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ABSTRACT

Cellular Immunity in Children with
Down Syndrome (Trisomy-21)

by

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Utah State University, 1985

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Department: Biology

Individuals with Down syndrome (DS) suffer from increased incidence of respiratory infections and lymphoblastic leukemia, and a death rate that is particularly high in the first 5 years of life. Relatively few studies have probed immune parameters in young DS children. Primary immune defects in DS may be masked by a degree of immune maturity in adults, and hygienic factors may have an effect on immune capability throughout the years. A study of young children can give clearer evidence of the actual primary immune defects in DS.

Blood samples were drawn from 20 DS children under 6 years old and from age-matched controls. Packed blood cell volume was measured, various blood cell subpopulations were enumerated and differential counts were performed. Several tests of cellular immune function were performed and plasma zinc levels were determined using atomic adsorption spectrophotometry.

Elevated hematocrit levels were observed in the DS group. White blood cell counts and proportions of rosette-forming cells were normal
in blood from DS children. Altered neutrophil and lymphocyte proportions in DS samples resulted from a depressed number of circulating lymphocytes in the these subjects. This indicated that T cell numbers are low in DS. DS individuals had a low number of circulating OKT4+ T cells which resulted in significantly depressed T4:T8 ratios. Cells from DS subjects exhibited a reduced proliferative response to phytohemagglutinin; a low response to the optimal concentration of concanavalin A was seen with DS samples, but at suboptimal doses the response was normal; suboptimal concentrations of pokeweed mitogen elicited normal responses by cells from DS children. Preliminary results suggest that interleukin-2 (IL-2) production in young children may correlate positively with age and that DS subjects may produce normal or elevated amounts of IL-2. This suggests that IL-2 receptor function may be defective in T cells from DS children. DS children had normal natural killer cell activity and cells from those children were no more sensitive to the augmenting effects of interferon-alpha than cells from control children. Plasma zinc levels in DS appeared to be normal.

These findings not only provide evidence that the primary immune defect in DS involves low levels of T cells, but they show depressed number and function of helper T cells and suggest defective IL-2 receptor expression in DS.
INTRODUCTION

Down syndrome (DS)\(^1\) is a common cause of mental retardation in humans resulting from an extra replicate of a portion or all of chromosome 21 in the genome of the affected individual. The mechanism leading to retardation is not understood. Approximately 0.1-0.2\% of all babies born worldwide have DS (Wahrman and Fried 1970; Sever et al. 1970; Lott 1982). A unique set of characteristics and conditions, mental, morphological and physiological, are observed in these individuals. An unusually high incidence of respiratory infections in patients with DS, as well as an elevated frequency of anti-thyroid antibodies and increased risk of acute lymphoblastic leukemia (Scholl et al. 1982; Fialkow 1970), implicate the immune system and suggest defective immune function. Consequently, many reports of the number and proportions of immunocompetent cells and the functional status of cellular immunity and the phagocytic system in DS have been published. However, the majority of those were studies of adults with DS, and relatively few studies have dealt with young DS patients. Since the increased rate of mortality is highest during the earliest years of life (Wahrman and Fried 1970), those earlier studies of older individuals were making observations in a select group of survivors.

The purpose of the study reported in this dissertation was to investigate immune parameters in young children to give a clearer view of the primary immune defects found in DS. The project involved sampling blood from children 5 years old and younger who had DS, \(^\text{1}A \text{ list of abbreviations can be found on pg. vii.}\)
assaying the samples for immune parameters and then comparing the results with data derived by studying samples from healthy age-matched controls.

When possible, the hematocrit was measured, a total white blood cell (WBC) count was made, and a differential smear was prepared and counted. Depending on the number of peripheral blood mononuclear cells (PBMC) obtained from each individual, T cells were enumerated and the proportions of T cell subsets (OKT4+ and OKT8+) determined.

Tests of several immune functions were performed to determine what abnormalities might exist in children with DS. The proliferative response of lymphocytes to phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) was assayed. Natural killer (NK) cell activity was tested and the level of augmentation of the NK activity by interferon (IFN) was investigated in order to determine the sensitivity of these cells to IFN. This was of interest because the genetic determination of sensitivity to the effects of IFN has been assigned to the distal end of the long arm of chromosome 21 (Epstein et al. 1980), the chromosome which is trisomic in DS.

The trace element zinc is a necessary cofactor for many metalloenzymes, including several which are required for the synthesis of DNA and RNA. Such synthesis is crucial in the proliferative function of lymphoid cells. It has been shown that zinc deficiency has a profound influence on the thymus resulting in thymic involution, depressed DNA content and reduced levels of thymic hormone (Good 1981). Also, zinc deficiency leads to alterations in thymus-mediated immune mechanisms such as depressed helper and killer T cell function (Good 1981). In DS, thymuses often are small, depleted of lymphocytes and
exhibit diminution of the cortex and a loss of corticomedullary demarcation (Levin et al. 1979). Because of the immune deficiency in DS and these histological abnormalities, plasma zinc levels were determined in this study.
REVIEW OF LITERATURE

Down Syndrome

DS was initially described by Seguin in 1846, and was characterized more definitely in 1866 by Sir Langdon Down, as "mongolian idiocy". The term "mongolism" has been all but dropped from common usage in favor of "Down syndrome". About 95% of patients with DS are trisomic for chromosome 21 (Lejeune et al. 1959), while other DS patients have either "translocation" or "mosaic" DS (National Institutes of Health 1979). The subjects included in this study were of the trisomic type only.

Although known primarily as a condition of physical abnormalities and profound mental retardation, DS also has a striking association with other diseases which have resulted in increased rates of mortality (Sever et al. 1970; Wahrman and Fried 1970; Deaton 1973; Seger et al. 1977). With the advent of antibiotic therapy, life expectancy for DS individuals increased from about age 9 years in 1929 to about 35 years of age in 1969 (Thase 1982; Deaton 1973). Recently, it was reported that an estimated 50% of all people born with DS now live to middle-age (Jones 1979). In spite of this increased survival time, the risks of infection and mortality remain elevated in these patients (Oster et al. 1975; Scholl et al. 1982). Respiratory infections and pneumonia occur in increased incidence and are the leading causes of death in all DS patients (Siegel 1948a; Donner 1954; Oster et al. 1964; Sever et al. 1970; Kaldor and Pitt 1971; Deaton 1973; Oster et al. 1975; Scholl et al. 1982). Lymphocytic leukemia, of uncertain etiology, also occurs at
higher frequency in the DS population than in the general population and is another common cause of death (Krivit and Good 1956; Miller 1964; Oster et al. 1975). Mortality caused by leukemia seems to be especially severe in the group of DS subjects < 5 years old (Scholl et al. 1982). The other leading cause of death in DS, cardiovascular disease, is due primarily to congenital anomalies (Scholl et al. 1982).

Chronic antigenemia for the hepatitis B virus surface antigen (also known as the Australia antigen) is observed more frequently in institutionalized DS patients than in their non-DS counterparts (Blumberg et al. 1967; Sutnick et al. 1972; Smith et al. 1982). However, no significant correlations between this condition and other immune abnormalities have been noted.

The above observations have led many researchers to suspect that a primary immunodeficiency exists in DS. Consequently, many studies of DS individuals have probed immune parameters.

**Effect of Age in Down Syndrome**

Of the many studies of immune functions in DS which have been performed, the majority deal strictly with adults or disregard age altogether. Only a few have specifically dealt with the young DS child. It has been pointed out that "the age problem deserves emphasis" (Walford 1982).

The death rate in DS is particularly high in subjects < 5 years old and in those over 40 years of age (Deaton 1973), and is highest during the first year of life (Sever et al. 1970; Wahrman and Fried 1970). Prior to the antibiotic era, about 75% of the DS patients died before puberty (Seger et al. 1977). Specifically, 0-4 year old DS children
have a 3-fold greater incidence of leukemia than expected (Krivit and Good 1956) and mortality from leukemia is reported to be especially severe in the < 5 year old age group (Scholl et al. 1982).

Differences between the immune capacity of DS adults and that of DS children, when they are compared to age-matched normal controls, are evident. Some studies which report these differences will be mentioned in the section below. Also, there are several immune parameters for which no data from young DS children have been reported.

It is possible that DS subjects "grow out of" some immune deficiencies, a concept which could account for the decreased mortality seen in middle-aged patients. This maturation of the DS immune system may mask primary immune defects. It has been suggested that environmental factors, particularly poor personal hygiene due to mental retardation and crowding of institutionalized patients, may lead to a heavy load of infections in early life, and that this may result in a "stress-deficiency" of the cell-mediated immune system (Whittingham et al. 1977). Such age and environmental factors may cloud the issue in older DS subjects and make it difficult to distinguish primary immune defects caused by trisomy-21 alone. The current study of immune capacity in young DS children avoids selecting only those individuals who survived beyond the fifth year and more clearly identifies abnormalities caused primarily by the genetic defect of trisomy-21.

**Immunity and Down Syndrome**

Immune factors and immunopotent cells are circulated via the peripheral blood. WBC are the cells most directly involved in immunity. The various types of WBC perform extremely diverse functions in an
immune response. It is useful to think of the immune system as four interrelated subsystems: the phagocytic system, the antibody-mediated system, cell-mediated immunity, and the complement system. All but the complement system are under control of the thymus by means of thymic "hormones" and factors, and by thymic influence on certain stem cells as they mature and become T lymphocytes (T cells). The immune system includes populations of specialized WBC which maintain surveillance and detect, respond to, and neutralize in various ways foreign substances and abnormal conditions which may arise in the body. Measurable indicators of the competence of immunity include observations of cell morphology, absolute and proportional enumeration of the specialized cells and functional assays. The extra chromosome 21 in DS likely has a direct or indirect effect on most immune abnormalities which are observed in DS.

Blood Cell Populations

The microhematocrit method is used widely to yield precise estimates of the percentage of the blood which is occupied by the cellular component (Davidsohn and Nelson 1969). Abnormally high hematocrits have been reported in association with DS (Miller and Cosgriff 1983), suggesting some hematopoietic defect. Establishing hematocrit levels will also give information regarding the physiological condition of each individual studied.

A total WBC count provides a basis for interpreting enumeration of subpopulations of leukocytes. Fluctuations in WBC counts are common at all ages, but are greatest in infants and children (Davidsohn and Nelson 1969). Total numbers of WBC and lymphocytes have been reported to be
low in DS children in one study (Hann et al. 1979) while other studies have found them to be present in normal amounts in both adults and children (Spina et al. 1981; Dela Nuez et al. 1982).

Differentiation of granulocytes, lymphocytes, and monocytes is made by counting cells on a stained smear. Information regarding the relative proportions of the cell types is useful when looking for a deficiency in the immune response, but care must be taken to interpret these results in terms of the total WBC count. Differential leukocyte counts in DS have been reported to be similar to those in normal controls (Rosner et al. 1973).

The Phagocytic System

In DS, the phagocytic system appears deficient. Although the quantitative neutrophilic response to infection generally appears normal (Levin 1979), there has been a report that the percentage of polymorphonuclear leukocytes is low in DS adults (Spina et al. 1981). Both monocytic and neutrophilic chemotaxis appear decreased in DS children and young adults (Barroeta et al. 1983; Barkin et al. 1980a; Bjorksten et al. 1980). Phagocytic and bactericidal capabilities of neutrophils are diminished in DS (Rosner et al. 1973; Kretschmer et al. 1974; Costello and Webber 1976; Barkin et al. 1980b), however, monocyte killing seems normal (Barkin et al. 1980b).

The Antibody-mediated System

The number of circulating B lymphocytes (B cells) in DS is controversial; some workers report low levels in adults (Walford et al. 1981; Hann et al. 1979; Spina et al. 1981; Franceschi et al. 1978) while others report normal or elevated levels in children and adults
(Whittingham et al. 1977; De la Nuez et al. 1982; Levin et al. 1975; Gershwin et al. 1977). Reports concerning the levels of the different immunoglobulin (Ig) classes in DS are conflicting (Nishida et al. 1978; De la Nuez et al. 1982), but the most consistent findings are that IgM is decreased, IgG increased, with IgA increased occasionally (Steihm and Fudenberg 1966; Hann et al. 1979; Kaldor and Pitt 1971). In contrast, Miller and co-workers (1969) observed low IgG in DS newborns, and suggested that the increased IgG seen often in older subjects may be compensatory to a primary defect in IgM synthesis.

Siegel (1948b) noted that antibody response to specific vaccines is suppressed in DS patients, but more recently normal responses have been reported (Griffiths and Sylvester 1967; Hawkes et al. 1978; Hawkes et al. 1980). Also naturally occurring antibodies, specific for various antigens, are present at normal levels in DS individuals (Sever et al. 1970; McKay et al. 1978). Observations of increased incidence of anti-thyroid antibody have been reported (Fialkow 1970), but differences between DS patients and healthy subjects are small, and the mothers and siblings of DS individuals also exhibit anti-thyroid antibody (Fialkow et al. 1965). Therefore, the importance of these findings is uncertain.

The Cell-mediated System

Cell-mediated immunity for the most part is mediated by T cells. One characteristic of T cells is their possession of surface receptors for sheep erythrocytes (SRBC). Most studies of DS individuals, including newborns and young children, have shown a deficiency in the number of cells which will form rosettes with SRBC (Burgio et al. 1974; Levin et al. 1975; Franceschi et al. 1978; Griffiths et al. 1969;

Franceschi et al. (1978) reported that with the reduction in T and B cells in young DS adults, there is an increased proportion of third population cells ("non-T, non-B" cells; a.k.a. "null" cells) which lack both T (SRBC receptors) and B cell (surface Ig) markers. In contrast, one group recently reported normal numbers of rosetting cells in young DS adults (Spina et al. 1981) while another group reported normal T cell numbers as identified by a pan-T cell (9.6) monoclonal antibody in children (Gupta et al. 1983). When SRBC were treated with 2-amino-ethyl-isothiouronium bromide (AET) prior to rosetting with lymphocytes, levels of rosetting cells in DS adults appeared elevated (Whittingham et al. 1977). The putative effect of such pretreatment is to enhance the size and stability of the rosettes (Bloom et al. 1976).

An interesting finding is that many of the third population cells in DS possess low-avidity receptors for SRBC and membrane receptors for autologous erythrocytes (Burgio et al. 1978). Perhaps AET affects the low-avidity receptors in such a way that the avidity for SRBC is increased. It has been suggested that most third population cells in DS are immature T cells differentiating along the thymus-dependent axis. Because of this idea and the conflicting reports in DS, a comparison of rosetting methods with and without AET-pretreatment of SRBC may be worthwhile.

Assays of in vitro T cell function are especially useful when dealing with human subjects. In DS, mitogenic stimulation of lymphocytes by PHA has been studied extensively. A vast majority of studies show a decreased lymphocyte response to PHA mitogenesis in DS adults (Agarwal et al. 1970; Burgio et al. 1975; Gershwin et al. 1977;
Whittingham et al. 1977; Mellman et al. 1970; Franceschi et al. 1978; Spina et al. 1981; Walford et al. 1981). It has been suggested that this deficient response may reflect excessive suppressor cell activity (Thase 1982), but deficient helper cell function could also be the cause. However, decreased numbers of the suppressor/cytotoxic T cell subset defined by the OKT8 monoclonal antibody have been reported in 2-12 year old DS subjects, while normal levels of the OKT4+ helper/inducer subset were seen (Gupta et al. 1983). More recently, DS individuals whose cells showed a low response to PHA were shown to have a "reversed" T4:T8 ratio (i.e. < 1.0). The low ratio appeared to primarily be due to a higher than normal proportion of OKT8+ cells in the group of "low responders". DS individuals which responded normally to PHA had T4:T8 ratios > 1.0 (Karttunen et al. 1984). It is not known whether the abnormal proportions in the OKT8+ subset are due to altered numbers of suppressor cells or cytotoxic cells. Helper function has also been reported to be intact in DS adults (Whittingham et al. 1977). Studies which have looked specifically at DS children report that PHA-induced proliferation is normal, declining only at some time after the first decade of life (Dela Nuez et al. 1982; Burgio et al. 1974; Seger et al. 1977; Nishida et al. 1981). There are only a few reports of the mitogenic effect of other plant lectins in DS. Whereas PHA preferentially stimulates T helper cells, Con A and PWM exert a more general stimulation on T cells. Con A is known to stimulate both the helper and suppressor T cell subsets. The responses to Con A and PWM are reported to be reduced in DS adults (Whittingham et al. 1977; Gershwin et al. 1977). One study found that these reductions are age-dependent (Nishida et al. 1978). Another showed that PWM, at
optimal and supra-optimal doses, preferentially stimulates T cells, while sub-optimal doses will stimulate B cells (Franceschi et al. 1978). The T cell response to PWM is reported to be low in DS while the B cell response appears normal.

T cell proliferation in response to autologous cells in DS adults may be quite reduced (Franceschi et al. 1981), but it has been reported that DS children 2-12 years old respond normally (Gupta et al. 1983). Similar confusion exists regarding the proliferative response to alloantigens, with one group reporting an abnormally high response in DS (Sasaki and Obara 1969) and another observing low response in 2 of 3 age groups (Walford et al. 1981).

The production of certain lymphokines in DS has been investigated. In vivo production of leukocyte migration inhibition factor is reported to be low in DS infants (Levin et al. 1977). In vitro PHA-induced leukocyte migration inhibition factor is depressed in DS infants and children, while it appears normal in DS adults (Levin et al. 1979). The production of both PHA-induced immune IFN (gamma) and classical viral IFN (alpha), induced by poly I:C, is reported to be reduced in DS (Levin et al. 1979; Boyer and Fontes 1975). However, in children 4-10 years old various IFN inducers triggered higher levels of production of alpha and gamma IFN than in normal controls (Funa et al. 1984). Production of interleukin-2 (IL-2) by PHA-stimulated mononuclear cells was reported to be normal in a group of 18 DS individuals ranging in age from 5 to 37 years (Karttunen et al. 1984). Also, levels of cyclic adenosine monophosphate, an immune mediator, have been reported to be decreased in DS lymphocyte membranes (Levin et al. 1979).

The ability to spontaneously kill certain types of tumor cells
been attributed to a subset of the third population cells, usually referred to as NK cells. Several studies report normal NK function in DS (Spina et al. 1981; Matheson et al. 1981; Nurmi et al. 1982; Walford 1982), however all of these studies were done in adults. Speculation that NK cells are an immature form of T cells is common.

All the observations of abnormal T cell numbers and function in DS have led researchers to suspect defective thymic capacity. In fact, morphologic derangement in the thymus and low levels of thymic hormones have been reported in DS (reviewed by Ugazio 1981).

**Zinc and Immunity in Down Syndrome**

It is known that nutrition has a complex influence on immune function; specifically, the single element zinc has a profound effect on thymus-derived immunity (Good 1981). The thymus, T cells and cellular immunity in both humans and animals appear to be uniquely sensitive to this single trace element. Cattle with genetically caused malabsorption of zinc have a markedly hypoplastic thymus, deficient T cell immunity and increased susceptibility to zinc (Brummerstedt et al. 1977). The human disease acrodermatitis enteropathica is also a genetically caused zinc deficiency with associated problems including extreme susceptibility to infections. Supplementation of patients diets with zinc will completely cure the patient of the disease (Moynahan and Barnes 1973).

Because of the extensive thymic abnormalities observed in DS (Levin et al. 1979; Ugazio 1981) it is a possibility that a zinc deficiency may exist. Conflicting reports have appeared regarding zinc levels in the plasma of DS individuals. Two studies showed significantly decreased
levels of zinc (Milunsky et al. 1970; Bjorksten et al. 1980). In one of those studies (Bjorksten et al. 1980) the diets of DS patients were supplemented with zinc. Supplementation resulted in increased serum zinc levels, normalized phagocytic cell function and partial restoration of certain T lymphocyte-mediated immune functions. Another study of plasma zinc found no evidence of altered levels in DS (Neve et al. 1983a).

Gene-loci on Chromosome 21

It is interesting to note that the location of the gene which codes for the IFN surface receptor in human cells has been determined to be on the distal end of the long arm of chromosome 21, the same segment which seems to be responsible for the DS phenotype (Epstein and Epstein 1976; Revel et al. 1976). In cultures of fibroblasts from trisomic DS individuals the antiviral activity of IFN is increased (DeClerq 1978). It has been observed that there is increased IFN-induced inhibition of thymidine uptake by lymphocytes from DS adults, when stimulated by PHA, Con A, or PWM (Epstein and Epstein 1980; Cupples and Tan 1977; Matheson et al. 1981). However, uptake is enhanced when cells are stimulated by tetanus toxoid (Epstein and Epstein 1980) or Staphylococcus Cowan I (Matheson et al. 1981). Monocytes from DS patients are more sensitive to IFN inhibition of their maturation to macrophages (Epstein et al. 1980). IFN is also known to augment NK activity. Reports are somewhat conflicting as to whether cells from DS patients are more sensitive to the NK-augmenting effect of IFN. One group found no difference between DS and control individuals (Matheson et al. 1981) while another group found that the augmentation of NK activity in cells from adults with DS
is dependent on the presence of monocytes (Nirmi et al. 1982). A more recent report states that NK cells from DS children are more sensitive to augmentation by IFN (Funa et al. 1984). Where IFN is more effective in DS, the effects are not always proportional to the gene-dosage (a 50% increase), a phenomenon yet to be explained.

The gene-locus coding for the enzyme superoxide dismutase (SOD) is also assigned to the long arm of chromosome 21 (Tan et al. 1973). Superoxide is an oxygen metabolite produced by phagocytic cells for its bactericidal activity. The normal role of SOD is the conversion of the superoxide radical to a less toxic chemical species so as not to render harm to the host tissues. Increased activity has been demonstrated in several DS cell types (Neve et al. 1983b; Feaster et al. 1977). The SOD activity in DS is usually a predictable 50% higher than in normal controls. This increased SOD co-exists with many features of DS that may be secondary to oxidative damage. Such damage is thought to contribute to rapid aging, which in turn may lead to immunodeficiency (Sinet 1982).

**Immunity in Down Syndrome Children**

In summary, only a few studies have reported findings specifically about immunity in DS children. Total numbers of WBC and lymphocyte numbers have both been reported to be low, but WBC counts normally fluctuate in this age group. Decreased phagocytic cell chemotaxis has been observed in a group of DS children. The number of circulating B cells is reported to be normal or elevated, but there is one report of low serum IgM in DS newborns. DS children and their siblings and mothers have increased thyroid antibody levels. Newborns and young
children with DS are reported to have low numbers of rosette-forming cells (RFC or T cells), however there has been a report of normal numbers of T cells, in DS children, as detected by a pan-T monoclonal antibody. One group reports that DS children have a low proportion of OKT8+ cells and normal proportions of OKT4+ cells. Normal proliferative responses to PHA and autologous cells have been reported in DS children. Decreased production of leukocyte migration inhibition factor has been reported in children with DS, but production of IFN alpha and gamma has been observed to be elevated. Finally, one report found NK cells from DS children to exhibit increased sensitivity to IFN-augmentation of NK activity. Most of these reports are solitary reports which await confirmation or otherwise, a major purpose of this study.
MATERIALS AND METHODS

Down Syndrome Subjects and Controls

Blood samples were drawn from children, 5 years old and younger, who have trisomy-21 DS. Most of these children attend classes at the Developmental Center for Handicapped Persons affiliated with Utah State University. Parental consent was obtained prior to sampling according to the stipulations of the University Institutional Review Board, which gave approval to this project.

For each DS child sampled, another healthy child about the same age (within a year), but not having DS, was sampled as a control. Sex was not matched in every case.

Collection and Separation of Blood Samples

Approximately 10-30 ml of blood was drawn with syringes containing 10 units of preservative-free heparin per milliliter of blood to be collected. Plasma was removed by centrifugation at 500 x g for 15 minutes and a portion of the plasma was frozen at -70°C for zinc determinations described below. The cell pellet was reconstituted to the original volume with RPMI-1640 (GIBCO, Grand Island, New York) containing 100 U/ml penicillin and 100 ug/ml streptomycin (medium). About 5 to 7 ml of the cell suspension was then carefully layered over 3 ml of ficoll-diatrizoate (Histopaque-1077, Sigma, Saint Louis) in 15 ml conical centrifuge tubes. Enough tubes were used to overlay the entire sample. The mononuclear cells were then separated by density gradient
centrifugation at 800 x g for 20 minutes.

The PBMC interface was carefully removed using a pasteur pipet and washed 2 times in medium, the first wash at 500 x g to get rid of the ficoll and the second wash at 200 x g to remove the platelets. The PBMC were then resuspended in medium for use in as many of the assays described below as possible.

Hematocrits, Total White Cell Counts, and Differential Counts

Hematocrits, total WBC counts and differential smears were performed on some of the whole blood samples before the centrifugation for removing the plasma.

The microhematocrit method was used by filling two capillary tubes per sample and centrifuging them for 5 minutes on a special centrifuge to pack the cells. Linear measurements were made to calculate the percentage of the total blood volume occupied by the packed cells.

For the total WBC count, the sample of whole blood was mixed well and a small volume was diluted with acidic crystal violet solution. The WBC were counted on a hemacytometer and calculations were made to obtain total WBC concentrations.

Smears were made on microscope slides with a drop of the whole blood sample. Wright's stain was used to stain the cells and a differential count was performed to enumerate lymphocytes, monocytes, neutrophils, eosinophils and basophils.

SRBC-rosetting With and Without AET-treatment

Cells forming rosettes with SRBC, RFC or T cells, were enumerated
using a rosetting technique. Sheep blood was collected aseptically in a syringe or tube with 10 units of heparin per milliliter of blood collected. The sheep blood was then centrifuged at 1000 x g for 20 minutes and the buffy coat and plasma were removed. The SRBC remaining were washed once more in phosphate buffered saline (PBS) or medium at 1000 x g for 20 minutes and then reconstituted with medium and stored at 4°C for up to 1 month. The PBS used here and in other procedures described below contained CaCl₂ (0.10 g/1), KCl (0.20 g/1), KH₂PO₄ (0.20 g/1), MgCl₂-hexahydrate (0.10 g/1), NaCl (8.00 g/1) and Na₂HPO₄-heptahydrate (2.16 g/1).

Some of the SRBC were pretreated with AET prior to enumerating RFC. A 4% (w/v) solution of AET in distilled water was first adjusted to a pH of 9.0 with 4N NaOH. One volume of packed SRBC was incubated with 4 volumes of the basic AET solution for 15 minutes in a 37°C water bath. The cells were then washed 4 times with cold PBS and a 10% suspension of AET-treated SRBC was prepared in medium and stored up to 1 week at 4°C.

Human PBMC were suspended in medium at 5 x 10⁶/ml. Equal volumes of the PBMC suspension and SRBC (AET-treated or untreated), suspended at a concentration of 1% in medium containing 20% fetal bovine serum (FBS), were mixed gently in a small tube, centrifuged 5 minutes at 200 x g and refrigerated at least 15 minutes. For each 0.5 ml of the mixture, 1 or 2 drops of 0.1% toluidine blue in PBS was added. The pellet was then carefully resuspended by rocking gently so as not to disturb the rosettes which had formed. Using a pipettor with the end of the tip cut off so the orifice is not so small, a part of the suspension was put on a slide and covered with a cover slip. Rosetting cells (cells attached to 3 or more SRBC) and non-rosetting cells were counted microscopically,
until a total of at least 200 lymphocytes had been counted so that the percentage of the lymphocytes which are T cells could be calculated.

Rosetting of lymphocytes using both AET-treated SRBC and untreated SRBC was done concurrently. This allowed for a comparison of the two methods.

Enumeration of T Lymphocyte Subsets

Proportions of T cell subsets were determined using the complement-dependent cytotoxicity (CDC) assay. PBMC were depleted of monocytes by incubating them in a plastic culture flask for 1 hour at 37°C. Non-adherent cells (lymphocytes) were then poured off into a tube and adjusted to approximately $1 \times 10^7$ cells/ml in medium with 10% FBS.

Commercially prepared monoclonal antibodies (OKT4 and OKT8, Ortho Diagnostics, Raritan, New Jersey) had previously been diluted 1:150 and stored in aliquots at -70°C. An aliquot of each was thawed and 12.5 ul were placed in duplicate wells of a flat-bottomed 96-well microplate (Corning). As controls, a similar quantity of medium alone and a pan-leukocyte monoclonal antibody (7.29.5, New England Nuclear, Boston, Massachusetts) were also placed in separate duplicate wells. Fifty ul of lymphocytes were then added to each of the wells. The plate was incubated 1 hour at 4°C and then 50 ul of rabbit complement (Pel-Freez, Rogers, Arkansas), which had been tested previously for it's cytotoxic capability, was added to each well. The plate was then incubated another 2 hours at room temperature.

After incubation, 50 ul of the cell suspension from one of the wells was mixed with 25 ul of a 0.4% (w/v) trypan blue solution in PBS.
A portion of this mixture was loaded onto a hemacytometer and dead and viable cells were enumerated microscopically. Dead cells take up the stain and appear blue, while viable cells exclude the stain. Each of the several wells were stained and counted in this manner and the proportions of OKT4+ and OKT8+ cells were calculated.

To test the reliability of the CDC system described above, a commercial kit for quantifying OKT4+ and OKT8+ cells (Quantigen, Bio-Rad, Richmond, CA) was obtained, and CDC and Quantigen were compared on a few samples. Directions which came with the Quantigen kit were followed for the most part. Briefly, washed PBMC were suspended in medium at approximately $1 \times 10^7$/ml. One hundred ul of the cell suspension was added to a small test tube. A suspension which had microscopic yellow latex beads coated with OKT8 monoclonal antibody and red beads coated with OKT4 antibodies was provided with the kit. Two hundred ul of the latex bead suspension was added to the tube and it was centrifuged at 150 x g for 3 minutes, then incubated 30 minutes at 37°C. At this point, 200 ul of a vital stain (erythrosin B) solution, provided with the kit, was added. Using a pasteur pipet, the pellet was resuspended, being careful not to use force sufficient to break up any latex bead rosettes that may have formed around OKT4+ or OKT8+ cells. The suspension was then examined microscopically and the number of lymphocytes forming rosettes (3 or more attached latex beads) with yellow beads (OKT8+) or red beads (OKT4+) or not forming rosettes, were counted. A total of 200 cells were counted and the percentages and ratios were calculated.
Mitogen-stimulated Lymphocyte Proliferation

The blastogenic response of PBMC to various concentrations of 3 different mitogens was tested. The mitogens and concentrations used were as follows: PHA (GIBCO) at 1.0, 0.5 and 0.25% (by volume of commercial stock concentrate); Con A (Flow Laboratories, McLean, Virginia) at 5.0, 2.5 and 1.25 ug/ml; PWM (Sigma) at 0.06, 0.03 and 0.015% (w/v). The mitogens were diluted in medium to twice the concentrations listed above. They were then added to triplicate wells of a 96-well flat-bottomed microplate (Corning), 0.1 ml/well. Plain medium was added to control wells.

PBMC were suspended in medium with 20% FBS at a concentration of $1 \times 10^6$/ml and 0.1 ml of the cell suspension was added to each well. The plates were incubated for 72 hours at 37°C. During the last 4 hours of incubation, the cells were pulsed with 0.4 uCi of $[^3]$H-thymidine which had a specific activity of 2 Ci/mmol (New England Nuclear). The cells were then harvested on glass fiber filter paper disks using a Skatron cell-harvester (Flow, McLean, Virginia). The disks were placed in scintillation vials, about 2 ml of scintillation cocktail (Scintiverse E, Fisher Scientific, Fairlawn, New Jersey) was added to each vial and the radioactivity was determined using a Packard Model 3003 Tri-Carb Scintillation Spectrometer (Packard Instruments, Downers Grove, Illinois).

Interleukin-2 (IL-2) Production and Assay of IL-2

When PBMC are stimulated by a variety of agents, they will produce
T cell growth factor, now known as IL-2. A combination of PHA (Type VS; Sigma) and 4-phorbol 12-myristate 13-acetate (PMA; Sigma) was used in this case. PMA was dissolved in pure ethanol and aliquots containing 1 ug/ml were prepared in medium and stored at -20°C until used. PBMC were suspended at 1 x 10^7/ml in medium containing 2% FBS and 0.05 mM 2-mercaptop-ethanol (2-ME) (diluent). A solution containing 4 ug/ml PHA and 100 ng/ml PMA was prepared in diluent and 0.5 ml was incubated with 0.5 ml of the cells in a well on a 24-well culture plate for 24 hours at 37°C. The supernatant was then harvested, centrifuged at 500 x g for 5 minutes, to remove any cells, and stored at 4°C until assay.

The IL-2-dependent cell line, HT-2 (a murine [BALB/c] cloned cell line originally prepared by Dr. James Watson) was kindly provided by the laboratory of Dr. C. Garrison Fathman of Stanford University. HT-2 cells were maintained in culture using a mixture of 6 parts medium with 5% FBS, and 4 parts of medium conditioned by Con A-stimulated rat spleen cells. This conditioned medium was prepared by suspending 1.5 x 10^6 spleen cells/ml in 100-200 ml of medium with 1% FBS, 0.05 mM 2-ME, 15mM HEPES and 1.0 ug/ml Con A. This suspension was incubated 48 hours at 37°C and harvested by centrifugation at 16,000 x g for 10 minutes at 4°C. It was then sterilized by filtration through a 0.22 u membrane and stored at -70°C.

The assay of IL-2 activity in supernatants was determined in a microassay based on the IL-2-dependent proliferation of HT-2 cells. The sample suspected of containing IL-2 was thawed and two-fold serial dilutions in diluent were prepared in triplicate wells of a 96-well flat-bottomed microplate (Corning), 0.1 ml per well. Then 0.1 ml of HT-2 cells at 4 x 10^4/ml in diluent was added to each well. The plate
was incubated for 24 hours and during the last 4 hours, the cells were pulsed with 0.4 uCi of $[^3]$H-thymidine which had a specific activity of 2 Ci/mmol (New England Nuclear). The cells were harvested onto filter paper disks as described in the proliferation assay above and radioactivity was counted using liquid scintillation.

Each time unknown samples were tested, a serial dilution of a standard IL-2 solution was also tested. Results were analyzed by probit analysis, as described in the statistical methods below, and expressed as half-maximal units/ml. One unit is defined as that amount of IL-2 activity which will stimulate a response by indicator cells which is 50% of the maximal response.

Initially, the HT-2 cells in the IL-2 assay would not incorporate the labelled thymidine and appeared dead. Finally, as described above, 2-ME was included in the medium for stimulating IL-2 production and for the IL-2 assay. For comparison, the assay of IL-2 production was done with one sample of PBMC, both with and without 2-ME included.

**Assay of Natural Killer Cell Activity and Interferon Augmentation**

A $^{51}$Cr-release assay was used to quantify NK activity. This assay was performed routinely the day after blood samples were drawn and PBMC were incubated overnight (18 hours) at room temperature in medium with 10% autologous plasma. With some of the samples an equal number of PBMC were incubated with 1000 U/ml of human IFN-alpha (Sigma).

The next day, target cells, K562 cells (myeloid tumor line), were labelled with $^{51}$Cr. Five to 10 ml of a suspension of K562 cells, which had been maintained in medium supplemented with 10% FBS, was
centrifuged for 5 minutes at 500 x g in a 50 ml centrifuge tube. The supernatant was decanted and 0.1 ml of \[^{51}\text{Cr}\]-sodium chromate solution containing 1 mCi/ml and having a specific activity of 300 to 500 mCi/mg (New England Nuclear) was added to the cell pellet. The pellet was then gently dispersed and this mixture was incubated for 1 hour at 37°C.

During the incubation to label the target cells, the PBMC (effector cells) were washed 1 time in medium and suspended, in medium supplemented with 20% FBS, at a concentration of 5 x 10^6/ml. A volume of 0.1 ml of effectors was placed in triplicate wells on a 96-well round-bottomed microplate (Corning) for a 50:1 effector to target cell ratio. Two-fold dilutions were then carried out, using medium with 20% FBS as diluent, and triplicate wells were prepared for effector to target cell ratios of 25:1, 12:1 and 6:1. Medium with 20% FBS was used for background release wells and 0.5% saponin was used for maximum release wells.

After 1 hour of incubation, the target cells were washed 3 times in a 50/50 mixture of medium and PBS and were resuspended at a concentration of 1 x 10^5/ml. One-tenth ml of the labelled target cells was then added to each well and the plate was incubated at 37°C for 4 hours.

After the incubation, 0.1 ml was carefully harvested from each well so as not to get any of the cells, which had settled to the bottom of the well. These supernatants were placed in separate vials to be counted in a gamma-counter (Beckman, Irvine, California). The percent of \[^{51}\text{Cr}\] released due to NK activity was calculated according to the following equation:
\[ \% \text{release} = \frac{\text{experimental release} - \text{background release}}{\text{maximum release} - \text{background release}} \times 100, \]

where experimental release was the mean counts per minute (cpm) from the triplicate sample wells, background release was mean cpm from the wells with medium and target cells only and maximum release was the mean cpm from the wells with saponin and targets cells only.

Results from IFN-treated cells were compared with the results from corresponding untreated cells and the extent to which the NK activity was augmented by the IFN was calculated.

**Assay of Plasma Zinc**

Zinc content of the plasma was assayed by atomic absorption spectrophotometry using an Atomic Absorption/Atomic Emission Spectrophotometer 457 with a single slot burner head and a hollow cathode lamp (Instrumentation Laboratory, Wilmington, Massachusetts). The assay used an oxidizing lean flame with an air-acetylene gas mixture and the spectrophotometer was set at a wavelength of 213.9 nm. A standard curve and the concentrations of the samples were calculated automatically by the instrument. The spectrophotometer was standardized with solutions containing 0.1, 0.2, 0.3 and 0.4 mg of zinc per liter. They were prepared from a stock solution of 1000 mg/l by diluting with a glycerol/distilled water mixture (5/95 by volume) to correct for viscosity of plasma samples according to the method of Smith and Butrimovitz (1979).

Samples of plasma which had been collected and frozen at \(-70^\circ\text{C}\) as described above were then thawed and diluted 1:10 in distilled water. After thorough mixing, the zinc content was determined with the atomic
absorption spectrophotometer.

Statistical Methods

Data from the assays enumerating the various subsets of cells were analyzed using a paired sample t-test (Zar 1984). An exception was the chi-square analysis (Bishop 1980) used to test for significant differences in proportions of cells obtained by the differential counts.

A 2-way factorial randomized block split plot design was used to analyze variances in the several tests which involved multiple treatments within groups of age-matched subjects. These included mitogen proliferation, enumeration of SRBC-rosetting cells with and without treatment by AET and NK cell function and it's augmentation with IFN.

The model for this analysis expressed as:

\[ Y_{ijk} = u + P_i + C_i + \varepsilon_{ij} + T_k + CT_{jk} + \delta_{ijk}, \]

where \( u \) is the mean, \( P \) designates age-matched pairs, \( C \) represents the two conditions of DS and the control group, \( T \) is derived from the multiple treatments in each of the experiments, \( CT \) is the interaction between conditions and treatments and \( \varepsilon \) and \( \delta \) are error terms. This analysis was computed using a program called FCTCVR.

In addition, the slopes of the regression lines of NK cell activity with and without IFN treatment were compared using analysis of covariance (Snedecor and Cochran 1967).

Results from the assay for IL-2 activity were derived using probit analysis (Gillis et al. 1978). Briefly, this was done by calculating what percentage of the maximal response each individual response gave. This produced a sigmoidal response curve which was then linearized by
transforming the percentage values to probit units. Tables for this transformation have been published (Finney 1971). The resulting values were plotted with probit units on the vertical axis and \( \log_{10} \) of reciprocal dilutions on the horizontal axis. The original percentages can also be plotted directly on probability paper. A probit regression line was then calculated and the dilution which contained 1 half-maximal unit was the anti-log of the value on the x-axis which corresponded with 50% (5 probit units) on the y-axis. From this, the concentration of IL-2 activity in the undiluted sample was obtained. The results thus derived were then analyzed using the paired sample t-test mentioned above.
RESULTS

Enumeration of Various Cell Populations
in the Blood

For cells in the blood to be effective in defending the body against invasive organisms or oncogenic transformation, they not only must be functional, but the must also be present in adequate and appropriate numbers. For this reason various cell types were enumerated in this study. Table 1 lists the results of studying erythrocyte and leukocyte levels in the blood of young DS and control subjects. Hematocrits of children with DS were significantly higher than those of healthy children (p < 0.05). Total WBC concentrations for the two groups were similar and well within the normal range. A differential count of the WBC revealed a very significant difference between the proportion of cells from DS and control subjects, as analyzed by chi-square analysis (Table 2). Partitioning of this chi-square (Table 3) revealed that all of the difference was due to differences in the proportions of the lymphocyte and neutrophil subsets. However, when the absolute numbers of circulating lymphocytes and neutrophils were calculated by multiplying the percentage obtained in the differential count by the total WBC count for each individual, it became apparent that there was no significant difference in the numbers of neutrophils (Table 1). In addition, when analyzed by the paired t-test, the difference in the number of lymphocytes in samples from DS and control children is only marginal (p < 0.1).
Table 1. Enumeration of blood cell subsets in young children with Down syndrome (DS)

<table>
<thead>
<tr>
<th>Cell population</th>
<th>Number of matched pairs studied</th>
<th>Subject Group&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Down syndrome</td>
</tr>
<tr>
<td>Hematocrit (% of vol.)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>13</td>
<td>42.6 (6.0)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total white blood cell count (X 10&lt;sup&gt;6&lt;/sup&gt;/ml)</td>
<td>14</td>
<td>7.6 (2.6)</td>
</tr>
<tr>
<td>Differential count&lt;sup&gt;c&lt;/sup&gt; (% of total WBC)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes ***</td>
<td>35.3 (12.8)</td>
<td>49.6 (10.1)</td>
</tr>
<tr>
<td>Neutrophils ***</td>
<td>53.9 (9.7)</td>
<td>39.5 (13.7)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1.5 (1.6)</td>
<td>1.4 (1.7)</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.6 (0.7)</td>
<td>0.8 (1.2)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>8.8 (6.0)</td>
<td>8.8 (9.0)</td>
</tr>
<tr>
<td>(X 10&lt;sup&gt;6&lt;/sup&gt;/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes +</td>
<td>2.9 (1.8)</td>
<td>4.7 (1.9)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4.2 (1.6)</td>
<td>4.2 (3.9)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.6 (0.4)</td>
<td>0.7 (0.7)</td>
</tr>
<tr>
<td>SRBC-rosetting&lt;sup&gt;c&lt;/sup&gt; cells (% of lymphocytes)</td>
<td>9</td>
<td>66.3 (15.8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> DS patients and age-matched controls, < 6 yrs old.

<sup>b</sup> Mean (standard deviation).

<sup>c</sup> SRBC were treated with AET before rosetting procedure.

<sup>+</sup> p < 0.1, by paired t-test analysis.

<sup>*</sup> p < 0.05, by paired t-test analysis.

<sup>***</sup> p < 0.001, by chi-square analysis.
Table 2. Chi-square analysis\textsuperscript{a} of data from differential blood cell counts in young children with Down syndrome

H\textsubscript{0}: There is no difference in the proportion of cell subsets in the blood from Down syndrome (DS) and age-matched control (CN) children.

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>Monocytes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>Obs. 564</td>
<td>862</td>
<td>24</td>
<td>10</td>
<td>140</td>
<td>1600</td>
</tr>
<tr>
<td></td>
<td>Exp. 679</td>
<td>747</td>
<td>23</td>
<td>11</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dev. -115</td>
<td>115</td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>Obs. 794</td>
<td>632</td>
<td>22</td>
<td>12</td>
<td>140</td>
<td>1600</td>
</tr>
<tr>
<td></td>
<td>Exp. 679</td>
<td>747</td>
<td>23</td>
<td>11</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dev. 115</td>
<td>-115</td>
<td>-1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Obs. 1358</td>
<td>1494</td>
<td>46</td>
<td>22</td>
<td>280</td>
<td>3200</td>
</tr>
</tbody>
</table>

\[\text{chi-square} = \sum \frac{(\text{Obs} - \text{Exp})^2}{\text{Exp}} = 74.7\]

\[\text{df} = (\# \text{ of columns} - 1)(\# \text{ of rows} - 1) = 4\]

\[p < 0.001\]

\textsuperscript{a}See Bishop 1980, pp. 72-78.
Table 3. Partitioning of chi-square analysis\textsuperscript{a} of data from differential blood cell counts in young children with Down syndrome

$H_0$: There is no difference the proportion of cells subsets in the blood from Down syndrome (DS) and age-matched control (CN) children.

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Total Obs.</th>
<th></th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>Monoocytes</th>
<th>Total Obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS Exp.</td>
<td>564</td>
<td>862</td>
<td>1426</td>
<td>DS Exp.</td>
<td>24</td>
<td>10</td>
<td>140</td>
<td>174</td>
</tr>
<tr>
<td>Dev.</td>
<td>-115</td>
<td>115</td>
<td></td>
<td>Dev.</td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CN Exp.</td>
<td>794</td>
<td>632</td>
<td>1426</td>
<td>CN Exp.</td>
<td>22</td>
<td>12</td>
<td>140</td>
<td>174</td>
</tr>
<tr>
<td>Dev.</td>
<td>115</td>
<td>-115</td>
<td></td>
<td>Dev.</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total Obs.</td>
<td>1358</td>
<td>1494</td>
<td>2852</td>
<td>Total Obs.</td>
<td>46</td>
<td>22</td>
<td>280</td>
<td>348</td>
</tr>
</tbody>
</table>

chi-square = 74.4, df = 1

\[ p < 0.001 \]

chi-square = 0.3, df = 2

not significant

\textsuperscript{a}See Bishop 1980, pp. 72-78.
Among the lymphocytes from the DS children, a lower mean percentage of cells forming rosettes with SRBC was observed (Table 1), but the difference was not significant. The SRBC used in this study were pretreated with AET, reputedly resulting in more stable rosette formation. In earlier studies of RFC in DS, SRBC may or may not have been treated with AET. Since some of the SRBC receptors on T cells from DS individuals might have a lower avidity for SRBC, rosettes formed with non-AET-treated SRBC may be more prone to disrupted during the procedure prior to counting. This would result in erroneous counts and reports of lower levels of RFC. A comparison of T cell enumeration with and without AET treatment did not reveal any difference when lymphocytes from healthy control children were tested (Table 4). When lymphocytes from DS children were tested, a lower mean percentage of RFC was observed by using untreated SRBC as opposed to treated SRBC, but again, there was not a significant difference (Table 4).

Figure 1 shows that young DS children have significantly lower T4:T8 ratios than control children (p = 0.01). Of 16 children with DS, 4 had "reversed" ratios; i.e. T4:T8 < 1.00. The imbalance could be due to a low proportion of OKT4+ cells, a high proportion of OKT8+ cells, or both. In fact, both an increased proportion of OKT8+ cells (p < 0.02) and a decreased proportion OKT4+ cells (p < 0.05) were observed in the DS group. However, when the absolute numbers of these two subsets were calculated, by multiplying the percentage of each subset by the number of circulating lymphocytes (this was determined from the differential count and the total WBC count) for each individual, it was seen that the numbers of OKT8+ cells in samples from the DS children were similar to the numbers seen in control samples (Figure 2). The paired t-test
Table 4. Comparison\(^a\) of two techniques\(^b\) for enumerating rosette-forming cells in young Down syndrome children

<table>
<thead>
<tr>
<th>Subject Group(^c)</th>
<th>Percent Rosette-forming Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated SRBC</td>
</tr>
<tr>
<td>Down Syndrome</td>
<td>58.3 (3.1)(^d)</td>
</tr>
<tr>
<td>Control</td>
<td>69.1 (2.7)</td>
</tr>
</tbody>
</table>

\(^a\)No statistically significant differences were observed between the two techniques being compared.

\(^b\)Sheep red blood cells (SRBC) which had not been treated with AET (see methods) were used in the standard rosetting technique in parallel with AET-treated SRBC.

\(^c\)Eight age-matched individuals < 6 yrs old from each group were tested.

\(^d\)Mean (standard error of the mean).
Figure 1. Depressed ratio of T cell subsets (OKT4+:OKT8+) in young children with Down syndrome (p < 0.01). The number of PBMC bearing each of the T cell markers were enumerated with a complement-mediated cytolytic assay. Sixteen age-matched pair of children were studied. Each point represents the ratio derived from an individual child. Mean ± s.e.m. is shown.
Figure 2. The number of circulating OKT4+ cells in young children with Down syndrome (DS) is significantly depressed (p < 0.05). OKT8+ cell numbers are the same as in healthy controls (C). Mean concentration ± s.e.m. is indicated for 7 individuals from each group.
analysis revealed that DS children do, in fact, have a significantly lower number of OKT4+ cells in their peripheral blood (p < 0.05).

After the T4:T8 ratios had been determined for several of the age-matched samples, a question arose regarding the validity of results from the CDC assay. Therefore, a commercial kit (Quantigen), which is not dependent on complement cytotoxicity for enumeration of OKT4+ and OKT8+ cells, was obtained. A few samples were tested using both methods in parallel for comparison. As can be seen from data in Table 5, the two methods gave similar results.

**Proliferative Response to Mitogens**

The response of cells from the DS children to PHA was low when compared to cells from age-matched controls (p < 0.05) as seen in Figure 3. The overall response of cells from the DS children to Con A (Figure 4) was not significantly different from the control response when analysis of variance was performed. However, a paired t-test revealed that the response by cells from DS individuals to the highest (optimal) dose of Con A was significantly lower than the control cells (p < 0.05). Figure 5 shows that the mitogenic response to suboptimal concentrations of PWM by cells from DS subjects is not significantly different from the response by cells from normal children.

The variation in the mitogen-stimulation data is considerable, especially for PWM mitogenesis. Often results are converted to log_{10} for statistical analysis on the assumption that variances are proportional to the means. It was not apparent that that assumption was true for these results, so no such transformation was done, and statistical analysis was carried out on the raw data.
Table 5. Comparison of complement-dependent cytoxicity (CDC) and the Quantigen kit as methods for determining OKT4+ and OKT8+ cells and T4:T8 ratios

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>CDC</th>
<th>Quantigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>33.8 (2.6)</td>
<td>35.7 (6.3)</td>
</tr>
<tr>
<td>OKT4+ cells (% of lymphocytes)</td>
<td></td>
<td>23.4 (3.5)</td>
<td>25.1 (4.7)</td>
</tr>
<tr>
<td>Mean T4:T8 ratio</td>
<td></td>
<td>1.47 (0.29)</td>
<td>1.42 (0.15)</td>
</tr>
</tbody>
</table>

*No statistically significant differences were observed between the two methods being compared.*

*Means of 3 paired samples ± S.D.*

*Percent of cells lysed; detected by trypan blue exclusion.*

*Percent of cells rosetting with appropriate latex beads.*
Figure 3. Depressed proliferative response to phytohemagglutinin (PHA), as counts per minute of $^3$H-thymidine uptake, by lymphocytes from young children with Down syndrome ($p < 0.05$). (■■) Down syndrome group; (■■) control group. Lines extend 1 s.e.m. above the mean of 10 individuals from each group.
Figure 4. Concanavalin A (Con A)-stimulated $^3$H-thymidine uptake by lymphocytes from young Down syndrome and control children. The difference between the DS group and the control group that occurred with the 5.0 $\mu$g/ml Con A concentration was significant ($p < 0.05$). (■) Down syndrome; (□) controls. Lines extend 1 s.e.m. above the means of 9 individuals in each group.
Figure 5. Stimulation of lymphocyte proliferation by pokeweed mitogen, measured as counts per minute of $^3$H-thymidine uptake, in young children with Down syndrome. No significant differences were observed. (■) Down syndrome; (□) controls. Lines extend 1 s.e.m. above the means of 9 subjects from each group.
Interleukin-2 (IL-2) Production

Each time an IL-2 assay was done, a laboratory standard of rat IL-2 was also assayed as a positive control. Figure 6 illustrates the response curve and the method for probit analysis to derive the activity in half-maximal units/ml which were present in the original sample. This method was used to derive each of the values in Table 6.

The assay for IL-2 did not work until it was discovered that 2-ME was an essential component of the assay medium. When IL-2 production and assay were performed using medium without 2-ME, incorporation of $^3$H-labelled thymidine by the indicator cells was never substantially greater than by the cells in negative control wells which contained only medium with the cells. The simple inclusion of 2-ME in the medium provided culture conditions in which the murine cell line, HT-2, could respond to IL-2 making assay of IL-2 possible.

Because of the delay caused by leaving 2-ME out of the diluent, only three DS children were tested for IL-2 production. Table 6 shows the results from the three age-matched pairs. Though the data are not statistically different, there is a trend which suggests that T cells from DS children produce IL-2 at higher levels than the matched controls. In addition, there is a hint that IL-2 production may be age-related since the 1 year old infants produced far less IL-2 than did the 4 and 5 year old children.

Natural Killer Cell Activity and the Response of Natural Killer Cells to Interferon

Figure 7 shows results of the NK assay comparing DS and age-matched
Figure 6. Probit analysis of the sigmoidal response curve from assay of a laboratory standard solution (supernatant from concanavalin A-stimulated rat splenocytes) of interleukin-2 (IL-2). Counts per minute are converted to percentage of maximum response and are plotted against log₁₀ of reciprocal of dilutions on probability paper. The 50% response gives the dilution containing 1 unit/ml of IL-2.
Table 6. Interleukin-2 production\textsuperscript{a} by stimulated\textsuperscript{b} lymphocytes from young children with Down syndrome (DS)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Age (mos.)</th>
<th>Subject Group\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Down syndrome</td>
</tr>
<tr>
<td>1</td>
<td>8/12</td>
<td>17.6</td>
</tr>
<tr>
<td>2</td>
<td>55/55</td>
<td>48.3</td>
</tr>
<tr>
<td>3</td>
<td>60/60</td>
<td>98.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Units/ml IL-2 produced in 24 h as derived by probit analysis.

\textsuperscript{b}1 \times 10^6 PBMC/ml were incubated 24 h with 2 \mu g/ml PHA and 50 ng/ml PMA in RPMI-1640 with 2% FBS and 5 \times 10^{-3} \text{M} 2-ME.

\textsuperscript{c}No statistically significant differences between DS and controls.
Figure 7. Baseline natural killer cell activity, induction of $^{51}$Cr-release from K562 cells by PBMC from young Down syndrome children and age-matched controls. No significant differences were observed. (▲) Down syndrome group; (▲▲) controls. Lines extend 1 s.e.m. above the mean of 17 individuals from each group.
controls. Although there seems to be a trend of lower NK activity by cells from the DS children, the small difference in mean $^{51}$Cr-release at four effector to target cell ratios was insignificant. A significant increase in $^{51}$Cr-release resulted from pre-treatment of cells with IFN (Table 7). However, the mean percent increase with IFN pretreatment in the DS group was not significantly different from the control group. In order to probe further, regression lines of these data were drawn. In Figure 8, it can be seen that IFN-treatment of DS cells may have increased the regression slope. An analysis of covariance was used to compare slopes of the four regression lines to determine if this difference was significant. When a comparison of all four regression slopes was made (Table 8), significant differences were apparent. However, when the slope of regression for DS IFN-augmented NK activity was compared directly with that of DS baseline NK (Table 9) or control IFN-augmented NK (Table 10) activity, no significant differences were observed. Because of this it cannot be concluded that the regression slope was changed by IFN-treatment of the DS cells, neither can it be concluded that DS NK cells respond any differently to IFN than control cells.

**Plasma Zinc Levels**

The concentration of elemental zinc in plasma samples from young children with DS and age-matched controls was determined. Figure 9 shows a higher mean for the DS group, but a paired sample t-test failed to demonstrate any significant difference ($p < 0.1$).
Table 7. Interferon\textsuperscript{a} augmentation of natural killer activity in young children with Down syndrome

<table>
<thead>
<tr>
<th>E:T\textsuperscript{c}</th>
<th>Subject Group\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Down syndrome</td>
</tr>
<tr>
<td>NK activity without</td>
<td>50:1</td>
</tr>
<tr>
<td>IFN pre-treatment</td>
<td>25:1</td>
</tr>
<tr>
<td>(% \textsuperscript{31}Cr-release from K562 cells)</td>
<td>12:1</td>
</tr>
<tr>
<td></td>
<td>6:1</td>
</tr>
<tr>
<td>NK activity with\textsuperscript{e}</td>
<td>50:1</td>
</tr>
<tr>
<td>IFN pre-treatment</td>
<td>25:1</td>
</tr>
<tr>
<td>(% \textsuperscript{31}Cr-release from K562 cells)</td>
<td>12:1</td>
</tr>
<tr>
<td></td>
<td>6:1</td>
</tr>
</tbody>
</table>

* * * * * * * * * * * * * * * * * * * * * * * * * *

Mean % increase

<table>
<thead>
<tr>
<th>E:T\textsuperscript{c}</th>
<th>Subject Group\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Down syndrome</td>
</tr>
<tr>
<td>with IFN pre-treatment</td>
<td>50:1</td>
</tr>
<tr>
<td></td>
<td>25:1</td>
</tr>
<tr>
<td></td>
<td>12:1</td>
</tr>
<tr>
<td></td>
<td>6:1</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Human leukocyte (alpha) interferon obtained from Sigma Chemical Co., Saint Louis, Missouri.

\textsuperscript{b}PBMC from Down syndrome patients and age-matched controls, < 6 yrs. old; 11 individuals per group; no significant differences were observed between the two groups.

\textsuperscript{c}Effector to target cell ratio; effectors were PBMC from subjects and targets were \textsuperscript{31}Cr-labelled K562 cells.

\textsuperscript{d}Mean (standard deviation).

\textsuperscript{e}Cells were pre-incubated 18 h at 37°C with interferon (1000 units/ml).
Figure 8. Regression lines to assay the effects of increasing effector to target cell ratios (E:T) and of interferon treatment on natural killer cell activity in Down syndrome subjects. Each point represents the mean chromium release at each E:T for 11 individuals. Slopes are compared in Tables 8-10.
Table 8. Comparison of regression slopes for baseline and interferon-augmented natural killer cell activity of cells from Down syndrome and control subjects by analysis of covariance.

<table>
<thead>
<tr>
<th>Line</th>
<th>df</th>
<th>$\Sigma d^2_{xx}$</th>
<th>$\Sigma d^2_{xy}$</th>
<th>$\Sigma d^2_{yy}$</th>
<th>Slope</th>
<th>Deviation from Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df  SS  MS</td>
</tr>
<tr>
<td>1</td>
<td>43</td>
<td>4.98</td>
<td>192.9</td>
<td>16158</td>
<td>38.7</td>
<td>42  8687  207</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>4.98</td>
<td>272.1</td>
<td>24313</td>
<td>54.6</td>
<td>42  9445  225</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>4.98</td>
<td>195.7</td>
<td>19467</td>
<td>39.3</td>
<td>42  7691  183</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>4.98</td>
<td>223.0</td>
<td>23279</td>
<td>44.8</td>
<td>42  13294  317</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>168  39116  233</td>
</tr>
<tr>
<td>6</td>
<td>173</td>
<td>19.9</td>
<td>883.7</td>
<td>83217</td>
<td>44.4</td>
<td>171  44015  257</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4899  1633</td>
</tr>
</tbody>
</table>

\[ F = \frac{MS L7}{MS L5} = \frac{1633}{233} = 7.01; \text{df} = 3, 168 \]

\[ p < 0.001 \]

\textsuperscript{a}See Snedecor and Cochran 1967, pp. 432-436.
Table 9. Comparison of regression slopes for baseline and interferon-augmented natural killer cell activity of cells from Down syndrome subjects by analysis of covariance

<table>
<thead>
<tr>
<th>Line</th>
<th>df</th>
<th>Σd²_{xx}</th>
<th>Σd²_{xy}</th>
<th>Σd²_{yy}</th>
<th>(Regr. coeff.) Slope</th>
<th>Deviation from Regression</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Within DS/No</td>
<td>43</td>
<td>4.98</td>
<td>192.9</td>
<td>16158</td>
<td>38.7</td>
<td>42</td>
<td>8687</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>2 Within DS/IFN</td>
<td>43</td>
<td>4.98</td>
<td>272.1</td>
<td>24313</td>
<td>54.6</td>
<td>9445</td>
<td>225</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Pooled</td>
<td>86</td>
<td>9.96</td>
<td>465</td>
<td>40471</td>
<td>46.7</td>
<td>85</td>
<td>18762</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>5 Difference between slopes</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ F = \frac{MS L5}{MS L3} = \frac{630}{216} = 2.92; \text{ df } = 1, 84 \]

not significant

Table 10. Comparison of regression slopes for interferon-augmented natural killer cell activity of cells from Down syndrome and control subjects by analysis of covariance

<table>
<thead>
<tr>
<th>Line</th>
<th>df</th>
<th>$\sum d^2_{xx}$</th>
<th>$\sum d^2_{xy}$</th>
<th>$\sum d^2_{yy}$</th>
<th>(Regr. coeff.)</th>
<th>Deviation from Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 DS/IFN</td>
<td>43</td>
<td>4.98</td>
<td>272.1</td>
<td>24313</td>
<td>54.6</td>
<td>42</td>
</tr>
<tr>
<td>2 CN/IFN</td>
<td>43</td>
<td>4.98</td>
<td>223.0</td>
<td>23279</td>
<td>44.8</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Pooled</td>
<td>86</td>
<td>9.96</td>
<td>495</td>
<td>47592</td>
<td>49.7</td>
<td>85</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ F = \frac{\text{MS L5}}{\text{MS L3}} = \frac{252}{271} = 0.93; \text{df} = 1, 84 \]

not significant

\[ ^a \text{See Snedecor and Cochran 1967, pp. 432-436.} \]
Figure 9. Zinc levels in plasma from young children with Down syndrome (DS). There was no significant difference between the DS group and controls. Twelve individuals per group are represented with the mean plasma zinc concentration ± s.e.m.
There have been many studies of immunocompetence in DS, but confusion and contradictory results persist. The immune system in DS probably changes with age much the same as it does in other human beings. The unusually high rates of morbidity and mortality in very young DS children and older DS adults is evidence of that. Environmental factors may influence the immune system of such individuals as the years go by, and the immune system itself may appear to compensate as the individual matures. It would make sense to study the DS immune system at a time of life when alteration by both of these factors is at a minimum. Therefore, this study of DS children 5 years old and younger with age-matched controls has been undertaken and is reported in this dissertation.

A number of difficulties were encountered during this project. Only a limited number of DS patients under 6 years old live near enough for it to be practical to obtain a blood sample from them. There were problems which made it impossible to get samples from some of these children. Although most parents were willing to give consent, some did decline to subject their child to the drawing of blood. Sometimes it was impossible to schedule a convenient time to draw a sample from a certain child. A few times when a DS child was available, blood could not be obtained since the poor muscle tone which is characteristic in DS can make venipuncture difficult. Finally, samples of blood from children under 6 years old were usually only 5 to 15 ml. With these small volumes the number of WBC was limited so that only a few of the
several assays and tests could be performed with each sample.

This study probed questions primarily about T lymphocyte immunocompetence in DS. However, the observation that the hematocrit is slightly higher in DS children is consistent with past findings (Miller and Cosgriff 1983). Neither blood pressure nor total blood volume was studied in these DS patients. Therefore, it is not certain whether these factors contribute to a relative polycythemia resulting in the observed hematocrit levels. The author knows of no report stating that hypertension or decreased fluid volume occur generally with DS. The hyperviscosity of this apparent polycythemia may contribute to the development of congenital disease, but it is not clear how it may affect immune function. It has been suggested that increased hematocrits in DS may arise from dysfunction in the release of hematopoietic elements in the bone marrow (Miller and Cosgriff 1983). If this is the case, then it is likely that the defect also exerts influence on the development of marrow-derived immune cells as well.

There is considerable confusion about whether the concentration of WBC in DS blood is abnormal. No significant abnormality in WBC count was observed in this study. Contrary to a previous report (Rosner et al. 1973), a very significant alteration of the proportions of lymphocytes and neutrophil levels was observed. However, careful interpretation of the data showed that numbers of circulating neutrophils was normal, while lymphocyte numbers were marginally depressed. This caused the altered proportions of these two cell types seen in the differential counts. The marginal depression of lymphocytes observed in DS suggests the possibility that lymphopenia is a characteristic of DS. Such a condition would be in agreement with other
reports (Hann et al. 1979) and would have a detrimental effect on immune mechanisms.

The possibility of a low level of lymphocytes led to the inspection of lymphocyte subsets. Forming rosettes with SRBC is a characteristic of T lymphocytes; this characteristic was used to differentiate between T and B cells. There have been several reports of depressed levels of RFC (T cells) in DS, but there are also some conflicting reports (see references in literature review). In this study no significant difference in the proportion of RFC between DS children and the controls was observed. However, if the number of circulating lymphocytes was low and the proportions of rosette-forming and non-RFC normal, it may be deduced that there are low T and possibly low B cell concentrations in DS.

The effect that treating SRBC with AET has on results obtained when using the SRBC-rosetting technique was investigated. Although no significant differences in results obtained using AET-treated SRBC and untreated SRBC were demonstrated while testing lymphocytes from the control children, a marginal difference was seen while testing cells from the DS group. The possibility remains that in the enumeration of RFC in DS subjects, earlier investigators may have inadvertently excluded some T cells with low-avidity SRBC receptors.

If a defect of T cell function exists in DS, further dissecting of the T cell population by functional characteristics may help determine this defect. Unique surface markers on T cells characterize different functional subsets and monoclonal antibodies to these surface markers aid in quantifying different T cell subsets. Helper T cells are known to express a marker defined by the monoclonal antibody OKT4 and
suppressor T cells express the marker defined by the OKT8 monoclonal antibody. The abnormal balance of these subsets in DS which were observed in this study is evidence for functional imbalance. Lower T4:T8 ratios imply that the immune system in DS is generally suppressed. A lower number of circulating helper T cells (OKT4+) appears to be the major cause of this oversuppression and could lead to the increased susceptibility to disease which is observed in DS.

These results agree with those reported by Karttunen et al. (1984) for DS individuals (5-37 years). However, the results of this study (0-5 yr) contrast those of Gupta et al. (1983) who reported a decreased level of OKT8+ cells and a normal level of OKT4+ cells in DS children (2-12 years). The effect of age on the abnormal expression of these T cell markers in DS is not known. It is difficult to determine a pattern based on the three studies discussed here since the report (Gupta et al. 1983) which contrasts most with this report is the one which involved the study of the most similar age group.

Different methods for detecting these cells have been employed by different investigators. Both Gupta's and Karttunen's groups used monoclonal antibodies in conjunction with fluorescently labelled anti-mouse IgG, while in this study, antibody-mediated CDC was employed. When CDC was compared to a commercial kit which uses monoclonal antibody-coated latex beads, it gave similar results. The commercial kit is claimed to give results comparable to methods which use fluorescently-labelled antibody. Additionally, when rabbit complement was obtained for use in this assay, different lots were screened for adequate cytolytic activity. These measures provided confidence in the results obtained by the CDC assay.
The ability of both B and T lymphocytes to proliferate *in vitro* in response to antigen and plant lectins is thought to reflect *in vivo* functional capability. Information regarding the functional status of lymphocytes from DS patients was obtained by studying the proliferative responses to three plant lectins. At suboptimal levels, PWM stimulates primarily B lymphocytes and the normal response observed in DS patients indicates that the patients had normal B cell function. The reduced proliferative response to PHA, which stimulates primarily helper T cells, indicates that young DS children may have defective T helper cell function. These results are clearly contradictory to reports that cells from DS children respond normally to PHA. A possible reason for this discrepancy is that this study has been restricted to studying DS patients under 6 years old while other studies make generalizations about the first 10 to 30 years of life. It is possible that helper T cell function in DS is defective only for the first few years of life. This function may be normal from ages 5 to 30 years and then decline as premature aging occurs. Unlike the findings of Karttunen et al. (1984), the results of this study showed no correlation between T4:T8 ratios and PHA responsiveness. This would indicate that low PHA responsiveness seen in DS patients is not just due to a smaller proportion of helper cells, but also to some defect in their functional capability.

Another mitogenic plant lectin, Con A, is known to stimulate both helper and suppressor T cells. At optimal doses, helper T cells are preferentially stimulated, and suppressor T cells are stimulated at suboptimal doses. The proliferative response by PBMC from DS children to Con A reflected this differentiation. The significantly lower proliferative response of cells from DS patients to the optimal level of
Con A confirms the functional defect which was seen in the response to PHA. The normal response to the suboptimal levels of Con A indicate that suppressor T cell function is intact.

Mitogen-stimulated T cell proliferation depends on the production of IL-2 and the expression of IL-2 receptors (Lotze and Rosenberg 1981). Since the proliferative response to PHA is defective in DS it would be worthwhile to test DS cells for their ability to produce IL-2.

The results obtained in the study of IL-2 production were preliminary at best (Table 6). The large variation due to age was unanticipated. Given the age discrepancy of the 3 matched pairs which were tested, variation in IL-2 production within the two groups was too great to allow any differences between the groups to become apparent. Therefore, no statistically significant difference between cells from DS children and age-matched controls was demonstrated. However, the trend suggests that DS individuals produce elevated, or at least normal, levels of IL-2. If this were the case, we could conclude that the proliferative defect seen in T lymphocytes from DS individuals is due to defective expression of IL-2 receptors or decreased receptor affinity for IL-2. Wakasugi and coworkers (1985) have recently demonstrated the existence of two forms of the IL-2 receptor, one with low affinity for IL-2 and the other with high affinity. A study testing many more DS children under 6 years old could reveal the actual situation. Time constraints and the difficulties (discussed at the beginning of this section) of obtaining enough samples for testing were the main reasons why more results are not reported here.

The initial difficulty in the assay for IL-2 activity was remedied by the inclusion of 2-ME in the medium. The use of 2-ME as an effective
replacement for monocytes in the culture media of various in vitro immune systems has been reported widely. The mechanism by which 2-ME replaces monocytic function is not well understood, however theories exist involving its reducing power and interactions with lymphocyte surface moieties that mimic antigen presentation. In the system used in this study, the mouse-derived IL-2-dependent cells required the presence of 2-ME in order to respond to IL-2. This confirms the fact that monocytes play a role in T cell clonal expansion. In their recent report Wakasugi et al. (1985) indicate that monocytes are necessary to induce the switch from low-affinity to high-affinity IL-2 receptors on the surface of peripheral blood lymphocytes. In light of this and the findings of the current study, 2-ME may be an effective replacement for this function.

The evidence of a defect of helper T cell function and increased suppressor T cell proportions suggest that thymic function may be altered in DS. As mentioned previously, thymic aberrations have been observed in DS and the thymus is acutely sensitive to zinc. DS children included in this study however, appeared to have normal levels of plasma zinc. The values of plasma zinc reported here are roughly 30-50% higher than those reported elsewhere for DS (Bjorksten et al. 1980; Neve et al. 1983a). Again, technique may account for the discrepancy since a method to correct for the viscosity of the plasma in atomic absorption spectrophotometry was used here.

Spontaneous cytotoxicity, or NK activity seems to represent a major surveillance mechanism which guards against tumor and virally-infected cells. The study of NK activity in DS is relevant in light of the increased incidence of leukemia and upper respiratory infections seen in
the DS population. Although the results presented here reveal no
difference in the NK activity of DS and control children, it was thought
that trisomic DS cells might be more sensitive to the NK-augmenting
effect of IFN, since they are more sensitive to other effects of IFN.
However, no difference in the response of NK cells from DS and control
children to IFN was observed in this project. Funa et al. (1984)
however, reported increased sensitivity to IFN in a slightly older group
of DS children (4-10 years). Their approach was somewhat different that
that reported here. Funa's group used four two-fold dilutions of IFN
and only one effector to target cell ratio, while the work reported here
had only one IFN concentration and four effector to target cell ratios.
Funa et al. compared the regression slope of their data from DS
individuals with that from the control subjects and reported a
significant difference. If there is in fact a significant difference
between the DS group and controls, the testing of only one concentration
of IFN should show that difference. This would be especially apparent
if the IFN concentration was within the range of concentrations which
Funa et al. used and therefore fell on their regression lines. The data
in the present study is from an NK assay using such a concentration of
IFN, yet no significant difference was observed. When the data for the
four different effector to target cell ratios were plotted and
regression lines calculated, a slightly increased slope for the
IFN-treated cell from the DS subjects resulted. If the increase of the
slope were significant, it would mean that IFN had increased the
synergistic efficiency of the NK cells from the DS subjects, while no
such response was observed in the control NK cells. However, analysis
of covariance did not verify that this slope was significantly different
from the others. Therefore, no evidence of hypersensitivity of NK cells to IFN was observed.

Further work in DS based on the findings of this study could prove fruitful. The elevated hematocrit raises questions about hematopoietic deficiency and its effect on cells destined to mediate immunity. Preliminary work should be done, however, to be sure more physiological factors such as blood pressure, total body fluid volume and blood viscosity in DS are well understood. These considerations would give guidance to any further investigation of the elevated hematocrit. Absolute numbers of B and T cells should be established with monoclonal antibody probes, and the relationship with B and T cell proportions should be elaborated.

Certainly, more should be done to follow up on the suggestion that T cells from DS children are hyporesponsive to IL-2. Such work ought to involve a quantitative study of the expression of IL-2 receptors by DS cells and the question of low and high affinity classes of IL-2 receptors. A probe into the effect of age on the ability of stimulated T cells to produce IL-2 would be important in understanding immunocompetence during early childhood. The relationship between the response to IL-2 by murine-derived IL-2-dependent cell lines and the requirement for 2-ME could also be a very interesting line of study.

An animal model for DS (trisomy-21) would be greatly helpful in elucidating the immune problems in DS. There are two possible systems currently being pursued. The Epsteins in California have developed a mouse strain with a trisomy which bears some resemblance to trisomy-21 in humans (Epstein et al. 1985). Also, Lenny Maroun in Illinois is currently working on a rabbit model for trisomy which shows promise
(personal communication). These represent possible animal models which may greatly accelerate research on trisomic syndromes.

The work presented in this dissertation has investigated several aspects of cell-mediated immunity in young children with DS. Evidence for a functional T helper cell defect has been found in addition to aberrant proportions of certain blood cell groups. These findings should provide direction for further investigation which will give us a clearer understanding of the primary abnormalities of the immune system in DS.
CONCLUSIONS

1. Young children (age < 6 years) with DS have significantly elevated peripheral hematocrits which may arise from hematopoietic dysfunction. This dysfunction may also affect differentiation of immune cells.

2. Marginally low lymphocyte levels and normal proportions of RFC in DS children imply that there may be low numbers of T cells in these children. This may result in depressed immune responsiveness and may lead to abnormal control mechanisms.

3. AET-treatment of SRBC for the rosetting technique does stabilize rosettes. There is a possibility that early studies of DS using the rosetting technique without AET may have been detecting only a subset of T cells with high avidity SRBC receptors.

4. With careful screening of complement and uniform execution, use of the CDC assay to mark and enumerate cell populations can provide data comparable to methods not dependent on complement cytotoxicity.

5. Young DS children have lower T4:T8 ratios which result from lower numbers of circulating OKT4+ cells. These may lead to suppressed immunocompetence in DS.

6. Cells from young DS subjects give a deficient response to PHA mitogenesis. This is evidence that T helper cell clones in DS individuals may be unable to expand at a rate sufficient for immune protection. The low response of DS cells to an optimal dose of Con A tends to confirm a functional helper T cell impairment.
7. Low T4:T8 ratios and low PHA responsiveness apparently do not correlate in young DS children. This supports the theory that low response to the lectin is a functional defect rather than resulting from low numbers of T helper cells.

8. Preliminary evidence indicates that IL-2 production by T lymphocytes may be age dependent. Also suggested is the possibility that DS cells produce more IL-2 than controls. Such an abnormality would indicate that the defective proliferative response is caused by inadequate expression and/or function of IL-2 receptors on the cell surface, possibly a class of low affinity receptors.

9. Zinc was present in plasma samples from DS children at normal levels; therefore, no evidence was found that a restricted supply of zinc is the cause of thymic abnormalities in DS.

10. NK function in young children with DS is normal; therefore, no evidence was seen that faulty NK activity results in the increased rates of infection and leukemia which are observed in DS.

11. NK cells from DS children are not more sensitive to the augmenting effect of IFN-alpha than cells from other children.

12. Mouse-derived IL-2-dependent cytotoxic T cell lines require 2-mercapto-ethanol for optimal response to IL-2.
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