A Study of Lymphocyte Subpopulations in Allergic and Asthmatic Patients

Fall 1978

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A STUDY OF LYMPHOCYTE SUBPOPULATIONS IN ALLERGIC AND ASTHMATIC PATIENTS

BY

CLEORA W. BARNWELL
B.S., University of Florida, 1969

THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Sciences: Biological Sciences in the Graduate Studies of the Program of the College of Natural Sciences of Florida Technological University at Orlando, Florida

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1978
ABSTRACT

The role of T and B lymphocytes in the human immune response has been studied in allergic and asthmatic patients by many investigators. Theories of suppressed cellular immunity have been reported showing decreased levels of T cells in peripheral blood. Elevated levels of IgE, an antibody, have been reported in these conditions, but not shown to be significant in distinguishing the different types of asthma.

A double blind study of 19 allergic and 42 asthmatic individuals was conducted measuring T and B cell levels and IgE levels. The asthmatic group was subdivided into three types, intrinsic, extrinsic and mixed. The possible effects of corticosteroids were considered with each group since 47 out of the total 60 patients studied were steroid dependent. T and B cells were enumerated by rosette method. This method was developed and normal values established. IgE levels were measured by radioimmunoassay.

Results showed all steroid dependent patients, allergic and asthmatic, had normal or slightly elevated T and/or B cell levels and IgE levels. The corticosteroids appeared to have a stimulative effect rather than a suppressive effect on T and B cells and IgE as previous investigators have reported. Allergic or asthmatic conditions cannot be distinguished on the basis of T and B cell levels. IgE levels were found to be highly significant at a 95% confidence level in distinguishing intrinsic asthma from extrinsic asthma.
ACKNOWLEDGEMENTS

I would like to dedicate this thesis to a very special person, my mother, Pauline. She died before the completion of my degree, but was my most enthusiastic supporter. Equally, to my wonderful family, my father, brother, and sister-in-law, I thank a thousand times over for their patience and continuous support.

No graduate student was ever blessed with so many great and wonderful friends. To these, I want to extend my most heartfelt thanks with a special remembrance to:

My graduate committee for putting up with me and giving me expert guidance. A special thank you to a great teacher, Dr. Michael J. Sweeney.

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Subject
Identification number of patients

Age
Expressed in years

Sex
M = male; F = female

T%
T cell percent value

Tabs
T cell absolute number value

B%
B cell percent value

Babs
B cell absolute number value

Mean and s.d.
Arithmetic mean and standard deviation

IgE
Immunoglobulin E expressed in ln and U/ml
(International Units per ml of serum)

Confidence Level
Statistical probability level

s
Steroid dependent

n
Nonsteroid dependent

c
Control normals
Individuals who develop a cough, wheeze, shortness of breath, recurrent colds, sinus infections, headaches, ear involvement, skin manifestations, gastrointestinal complaints, drenching sweats, hives or urticaria, eczema, or any combination of these, may be allergic* or atopic*. Ten to twenty per cent of the population of the United States suffers to a greater or lesser degree with allergies to allergens such as grass pollen, animal danders, mites in house dust, etc. (1). For those unfortunates sensitized to foods such as strawberries, the price of indulgence may result in a generalized urticaria or hives. In extreme cases, death can even occur in highly sensitized persons who react to such things as insect bites and injection of penicillin antibiotic through anaphylaxis.

These allergic or atopic symptoms are the result of the body's immune system responding to the foreign substance. The function of the immune system is to recognize foreign agents that might be damaging to the body and to neutralize these agents, thereby maintaining the internal metabolic environment in a steady state. The core of this defense is the lymphoid system, comprised of immunocompetent cells -- the lymphocytes, a type of white blood cell. The present concepts of the immune system were derived from numerous animal experiments and

*Allergic refers to individuals who have allergic symptoms, but do not have elevated blood levels of the antibody IgE. Atopic refers to individuals who exhibit allergic symptoms and have elevated IgE levels.
from studies of human immune deficiency diseases.

Development of the lymphoid system occurs along two independent pathways leading to functionally and morphologically distinct populations of immunocompetent cells (2). Some lymphocytes migrate from the bone marrow via the blood stream to the thymus gland for processing and are destined to become "T" cells. Others which pass through lymphoid tissue, the bone marrow, are the "B" cells.

The T cells make up about 70 to 80% of the circulating pool of lymphocytes in peripheral blood (3). These cells function as mediators of cellular immunity, and are active in such functions as tuberculin sensitivity, graft rejection, rejection to some virus and fungal infections, tumor surveillance, delayed contact sensitivities, immunological memory, and autoimmune disease (4-6). T cell also modifies B cell function (7-8).

The B cell comprises approximately 20% of the circulating lymphocytes in peripheral blood (9). B cells become plasma cells upon appropriate stimulation by a foreign agent. Plasma cells secrete proteins called immunoglobulins or antibodies. The B cell in association with T cell produces a variety of classes of immunoglobulins -- IgG, IgA, IgM, IgD, and IgE (10).

Many other diseases besides allergy exhibit the same kinds of symptoms, but the hallmark of the phenomenon of allergy or atopy is the increased levels of the immunoglobulin E. This immunoglobulin has a propensity to attach onto a tissue mast cell, blood basophils, and blood neutrophils. These cells, particularly abundant in the skin and bronchial tissue, contain many cytoplasmic granules which are the
reservoirs for the mediators of the allergic reactions. These mediators are histamine, slow reacting substance of anaphylaxis (SRSA), eosinophil chemotactic factor, prothrombin activating factor, and serotonin. When the IgE on the mast cell comes in contact with a specific allergen and aggregates, the cell undergoes degranulation and subsequent rupture with the release of the mediators. Release of the pharmacologically active mediators from the ruptured cells in the bronchial tree, nasal mucosa, or conjunctival tissue produces the symptoms of asthma or hayfever. In the case of food allergies, allergenic substances from the allergen such as strawberries are absorbed from the gut into the blood stream and react with the IgE bound cell in the skin producing urticaria as symptoms. There is a strong genetic predisposition to the development of allergic manifestations, usually parents or siblings of allergic patients will also have a history of allergies. The reasons for this are not yet understood.

In addition to increased levels of IgE, allergic individuals generally exhibit an increase in eosinophils, a type of white blood cell. Eosinophils can be detected in the peripheral blood and in nasal smears. The significance of increased eosinophil levels is unknown at this time.

THE ASTHMATIC STATE

An allergic state, asthma, is of particular importance since 2 to 3% of the general population develop this condition. Its etiology is still unknown. Asthma, although considered benign by many, is a chronic disease which has a yearly mortality rate of 0.2%. It is characterized as an obstructive bronchitis which has shown increased responsiveness of the trachea and bronchi to various stimuli. Characteris-
tic asthmatic symptoms are bronchospasms, bronchial edema, and excessive mucus secretions. These symptoms result from the action of histamine and SR5A on smooth bronchial tissue causing it to contact and on exocrine glands causing bronchial hypersecretion (11).

Asthmatics in general enjoy reasonably active lives with most experiencing frequent recurrent attacks. Generally asthma is potentially reversible unless an acute phase, status asthmaticus, occurs. The acute phase is characterized by bronchospasms which become unresponsive to the usual medications (11). Status asthmaticus can result from overwhelming exposure to an allergen, neglect of symptoms, inappropriate use of medications, emotional upsets, irritants, or respiratory tract infections.

Asthma is generally categorized into the following three groups, based upon reaction to allergens and the onset and duration of symptoms (11):

1. Extrinsic asthma is caused by demonstrable allergens such as pollen, spores, dust, etc. Skin testing with these allergens shows areas of hardness surrounded by erythema. These reactions are commonly referred to as wheal and flare and are considered as positive skin tests. Frequently extrinsic asthmatics exhibit atopic dermatitis and allergic rhinitis. Extrinsic asthma can persist from childhood into adulthood with a quiescent period at puberty.

2. Intrinsic asthma differs from extrinsic asthma in that it seems unrelated to any known allergen and skin tests are weak or negative to most antigens (allergens). Onset generally does
not develop until adult life and is associated with infections of the lower respiratory tract or paranasal sinus. It can be mistaken for chronic bronchitis.

3. Mixed asthma is a combination of extrinsic and intrinsic asthma.

GENERAL DIAGNOSTIC PARAMETERS OF ALLERGY

The diagnosis of allergy or atopy depends largely on patient history. The various criteria that must be considered are family history (particularly parental), presence of several stigmata of allergy involving separate systems (such as chest and skin), active favorable response to antiallergenic medications (such as antihistamines and bronchodilators), demonstration of the patterns of allergic disease (such as known food or inhalant allergies, seasonal history, etc.), and finally presence of physical and laboratory corroboration (12).

Two laboratory tests, eosinophil and IgE levels, are used to aid in the diagnosis of the allergic state. Skin testing and RAST test provide more specific information of what is the cause (the offending allergen) of the allergenic state. These tests are described as follows:

1. Eosinophil levels. Eosinophils are found in peripheral blood and nasal smears. Eosinophilia is favored by the release of Eosinophil-Chemotactic Factor from degranulating IgE bound mast cells, blood basophils, and neutrophils.

2. IgE levels. Increased blood levels of IgE is the hallmark of most atopic responses, but the elevation may not be striking. Normal blood levels are extremely low and comprise very little
of the total immunoglobulins. A protective role for IgE has not been specifically shown, but there is some correlation with sino pulmonary infection.

3. Skin testing. Sensitivity to an allergen is assessed by the response to intradermal challenge. The skin provides a large area for testing for the presence of cytophilic IgE bound to tissue mast cells. The offending allergen will produce a wheal and erythema at the site of its injection due to the allergen binding to IgE bound mast cell. This binding causes degranulation and release of histamine or other mediators. Reactions are read as positive based upon the size and redness of the erythema plus the timing of the response.

4. RAST test. The RAST test measures the amount of IgE antibody to a specific allergen present in peripheral blood. The allergen suspected is covalently coupled to a paper disc which then is treated with patient's serum. The amount of specific IgE bound to the paper is then estimated by addition of labelled anti-IgE antibody.

ALLERGY -- AN IMMUNE DISORDER

Allergic diseases are now recognized as disorders of the immune system. IgE is similar to other immunoglobulins in that its production is controlled by B cells. T cells have been shown to have both helper and suppressor functions on B cell activity (7–8) through biologically active mediators (13). An activated helper T cell can interact with the precursor cell into a mature B cell (plasma cell) which secretes IgE (14). Suppressor T cells may interfere with the activation of
the helper T cells, 2.) the actual function of the helper T cell in terms of its facilitating interactions with the B cell, 3.) in the actual differentiation of the precursor B cells into a mature plasma cell, or 4.) the antibody production by exerting a direct inhibitory effect on the fully matured plasma cells (14).

The critical role of T cells in helping and suppressing B cell antibody production has been demonstrable in many animal models. Tada and his colleagues' studies on rats demonstrated for the first time the existence of suppressor T cells exerting a very powerful negative regulatory effect over production of IgE antibodies in this species (14-15). Katz's studies in mice of the effects of irradiation or cyclophosphamide on T cells provided direct evidence for the existence of a suppressor T cell mechanism involved in nonspecific control of IgE production (14). Watanabe, Kojima, and Ovary reported similar findings (15).

The suppressor T cell mechanism in mice has been shown by Katz (14) to exist as circulating suppressor molecules, as yet undefined biochemically and immunologically, which do not appear to be immunoglobulin in nature, do not display any antigen specificity, but are exquisitely specific in terms of their capacity to negatively regulate responses of one immunoglobulin class, IgE.

Studies of T cell immunodeficient diseases of humans such as Wiskott-Aldrich syndrome and DiGeorge's syndrome have shown a relationship between decreased T cell function and increased serum IgE (16-17). Numerous investigators of allergic states such as atopic dermatitis and atopic eczema have reported decreased numbers of circulating T cells as well as other defects in cellular responses (18-24). Occasional
reports, however, have described normal numbers of T cells and intact cellular function in conditions with increased IgE, including atopic dermatitis and allergic rhinitis (25-26). Increased understanding of the control mechanisms involved in cellular interactions has evolved from studies of lymphocyte membrane markers involved in recognition and subsequent activation.

SURFACE MARKERS OF T AND B LYMPHOCYTES

THE ROSETTE PHENOMENON

Human T and B lymphocytes can be differentiated on the basis of their membrane surface markers. Well recognized surface markers for B lymphocytes are membrane-bound immunoglobulin (Ig), complement (C3), and aggregated IgG (Fc fragment) (27-29). However, the identity of the surface markers on the T cells has been poorly defined. None of the B cell markers are found on activated T cells. T lymphocytes are identified by their ability to spontaneously bind with sheep erythrocytes (red blood cells, RBC) through their surface marker for sheep erythrocytes, and also by their ability to bind fluoresceinated T cell specific heteroantisera through their Theta surface marker (30-32).

Discovery that T cells bear a receptor for sheep erythrocytes (33), while B cells carry a receptor for the third component of complement (34), has made possible the detection of lymphocyte subpopulations by rosette formation. A rosette is 3 or more sheep erythrocytes bound to a lymphocyte (35). (See Figure 1). Because of the limited availability of adequate specific heteroantisera, the sheep erythrocyte rosette technique has become the major method for identifying and thereby studying human T cells. B cells can be identified with other methods than
FIGURE 1. A SHEEP ERYTHROCYTE ROSETTE.

L: LYMPHOCYTE
E: SHEEP ERYTHROCYTE

FIGURE 2. SURFACE RECEPTORS INVOLVED IN ROSETTE FORMATION.

≤ T CELL RECEPTOR FOR E
△ E SURFACE DETERMINANT
△ lgM AGAINST E
△ C3b
the rosette method such as with fluorescent antisera designed to
detect the specific immunoglobulin (Ig) on the B cell surface. There
are now differing opinions as to which method should be used to give
the best results.

T cell rosettes are commonly referred to as E rosettes, the E
representing erythrocyte (sheep). T cell receptors for sheep erythro-
cytes bind directly with the erythrocytes. B cell rosettes are re-
ferred to as EAC rosettes, the E for erythrocyte, the A for antibody,
and the C for complement. B cells cannot bind sheep erythrocytes di-
rectly, but can bind to erythrocytes precoated with antibody and com-
plement. Antibody specifically against sheep erythrocytes is bound to
receptors on the sheep erythrocyte, while the complement binds to a
portion of the antibody. B cells then can bind the membrane of the
complement-antibody coated sheep erythrocyte through their complement
receptor (See Figure 2).

Factors such as pH, temperature, incubation time, and sheep ery-
throcyte to lymphocyte ratio have been shown to be critical in rosette
formation. Variations of the technique have lead to widely disapparable
results (33, 36-39).

Identification of T and B cells with surface markers is a qualita-
tive and quantitative measurement. The presence of normal numbers of T
and B cells is just one of many parameters of an intact cellular and
humoral immunity, respectively. Enumeration of the cells does not in-
dicate their functional or regulatory roles.

THE TREATMENT OF ALLERGIES

Allergies are commonly treated by hyposensitization. Hyposensi-
Hyposensitization is injection of the offending allergen(s) in small diluted concentrations over a long period of time. These injections are thought to activate the normal IgE inhibitory mechanisms that are dormant in allergic individuals who are producing large amounts of IgE. These inhibitory mechanisms include IgG feedback and suppressor cells. IgG antibody made against the injected allergen may prevent the allergic response by either competing with IgE for the allergen or by feedback inhibiting IgE production. IgE suppressor cells may be activated by hyposensitization thereby reducing the production of IgE (40).

Antihistamines block the action of histamine released from IgE-bound mast cells, hence the allergic symptoms are reduced or eliminated. Bronchodilators work on the smooth muscle of the bronchi which have contracted due to histamine, SRSA, etc. released from IgE-bound mast cells. The smooth muscle responds by relaxing thereby dilating or opening the bronchi allowing normal breathing. Both antihistamines and bronchodilators are temporary treatments relieving symptoms but not curing them (41).

Some allergy patients do not respond to hyposensitization or drugs such as the antihistamines and bronchodilators. In these patients, allergy symptoms are relieved temporarily with corticosteroids (42). Corticosteroids are thought to reduce symptoms through their action of suppressing the allergic response (43). Several immunosuppressive effects of these agents have been observed in man including mild lymphocytopenia (44), decreased immunoglobulin production (45), and impaired expression of skin tests (46). Despite these observations, little is known about the precise mechanism of the action of cortico-
steroids on the immune response in humans (42).

THE SIGNIFICANCE OF THE STUDY

A detailed search of the literature has revealed numerous studies of T and B cell levels in atopic diseases, but very few in bronchial asthma. Only three known studies of asthma have examined levels of T and B cells in peripheral blood using surface markers. Two of these studies were with children and adolescents and the other was with adults. Each study explored the hypothesis that decreased levels of T cells were associated with the asthmatic state, hoping to relate depression in numerical numbers with suppressed cellular immunity. In each study attention was given to the possible effects of corticosteroids on the levels of T and B cells in peripheral blood.

Saraclar and associates (47) studied 59 children and adolescents who were diagnosed as having rhinitis and/or asthma and atopic eczema. All results were compared to a normal control group of 30 children and adolescents. They found no significant difference in T cell numbers between the patients ages 2 to 10 years and the normal control group. In adolescents ages 10 years and up, significantly fewer T cells were seen when compared in relative percentages to normals, but when comparing absolute numbers no such difference was seen. Children ages 2 to 10 years had significantly higher B cells in both relative percentages and absolute numbers than did the normal control group. When those subjects treated with corticosteroids were separated from the total atopic group, there were no significant differences seen between the atopic and normal group. The effect of corticosteroids, bronchodilators, antihistamines and immunotherapy were considered
and could be shown to produce no consistent effect on lymphocyte numbers. No attention was given to IgE levels of the patients.

A study conducted by Kue-Hsiung Hsieh (48) consisted of 60 children ages 5 to 16 years with asthma, 52 extrinsic and 8 intrinsic. Two normal control groups were used, one healthy children (20 individuals) and one healthy adults (20 individuals). T cell relative percentages showed no differences between asthmatics and normals. Absolute numbers of T cells showed an increase in asthmatics as compared to normals. No B cell levels were done. Some of the patients were taking bronchodilators or corticosteroids, but none had taken any steroids during the two weeks preceding the T and B cell studies. IgE levels were studied in this survey. Correlation of IgE levels and E rosettes was sought, but none was found.

Gupta, et. al. (49) made the only survey of adult asthmatics, 23 individuals in all. Sixteen of the patients were considered both extrinsic and intrinsic (mixed) asthmatics, while 7 were classified as extrinsic asthmatics. This study showed T cell levels of asthmatics were depressed significantly in relative percentages and in absolute numbers when compared to their normal control group. B cell numbers, absolute and percentages, were not significantly different from the control group values. Of the 23 subjects studied, only one was receiving corticosteroids as treatment at the time of the study. IgE levels were considered in this study with attention given to any correlation of IgE and T and B cell levels in peripheral blood. No correlation was found.

A study of IgE levels of asthmatics by Grove and associates (50)
stated intrinsic and extrinsic asthma could not be differentiated on the basis of IgE levels. No T and B cells levels were done, but other immunological testing such as tetanus immunization was done. They reported on the basis of their data that asthma may be associated with immunodeficiency states.

The effects of corticosteroids on normal human subjects have been studied by Yu (51) and Fauci (42). Yu showed a decrease in T cell percentages, an increase in B cell percentages, and a decrease in both T and B cell absolute numbers. The blood samples were drawn 6 hours after administration of the corticosteroids. Fauci showed a profound decrease in the absolute numbers of T and B cells 4 to 6 hours after administration of corticosteroids with the levels returning to normal base line values within 24 hours. (See Figure 3, personal communique).

This study was undertaken to investigate the clinical implication of the relationship of T and B cell levels in asthmatics. Specific areas needing extensive research were:

1. To develop a reliable rosette technique for enumerating T and B cell levels in peripheral blood.
2. To establish normal values of T and B cell levels for normal individuals with the developed rosette technique.
3. To determine T and B cell levels in allergic and in asthmatic individuals.
4. To determine if T and B cell levels differed in asthmatics and in allergic individuals compared to normals.
5. To determine if there was any interrelationship between sex, age, family history, hyposensitization, duration of symptoms,
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014
January 30, 1978

S.D. Klotz, M.D.
303 East Par
Orlando, Florida 32804

Dear Dr. Klotz:

Thank you for your letter of January 5, 1978. I can answer your question with regard to the effects of steroids on T and B cells by the following statement. Even with chronic steroid therapy, T and B cells usually return to normal within 12 to 18 hours after the last dose unless patients are on extraordinarily high doses. If a patient is on a single dose of prednisone, for example 20 milligrams once in the morning, then his T and B cells should be normal just before his next dose in the morning. There are a lot of variations on this scheme depending upon whether the patient is on divided dose, how high the divided dose is, which particular steroid preparation is used, etc.

I would be happy to discuss this with you further, if I see you in Phoenix. Best regards.

Sincerely,

Anthony S. Fauci, M.D.
Head, Clinical Physiology Section
Laboratory of Clinical Investigation
Deputy Clinical Director
National Institute of Allergy and Infectious Diseases

Figure 3. Personal Communique
eosinophil levels, IgE levels and T and B cell levels.

6. To determine if any relationship existed between corticosteroid treatment and T and B cell levels in asthmatic individuals.

7. To determine if IgE levels were significantly different in the three types of asthma, intrinsic, extrinsic, and mixed.
MATERIALS AND METHODS

SELECTION OF PATIENTS AND CONTROLS

Sixty adults, 43 females and 17 males, were selected from the allergy clinic of Drs. Klotz and Moeller, Orlando, Florida. These patients were classified as either asthmatic (subdivided into intrinsic, extrinsic, and mixed) or allergic. Their ages ranged from 20 to 87 years with 12 patients between 20 and 40 years, 27 patients between 41 to 60 years, and 21 patients were 61 years and up. Of the sixty patients, 18 were allergic while 42 were asthmatic. Of the 42 asthmatics, 12 were extrinsic, 15 intrinsic and 15 mixed.

The normal control group consisted of 27 persons, 11 females and 16 males, selected from the general population including college students, teachers, secretaries, and laboratory workers. Each normal was carefully screened to exclude any allergies or past history of allergies, malignancies, recent infections (within 2 weeks), history of any chronic infection, and any recent drugs or drug therapy. The age range of the normals was from 19 to 61 years with one person being 19 years, 23 persons between 20 to 40 years, 2 persons between 41 to 60 years, and one person 61 years old.

COLLECTION OF PERIPHERAL BLOOD SAMPLES

Venous blood was drawn from the donor with either 10 ml vacuum blood tubes (no anticoagulant) or with disposable 20 ml syringes using 20 gauge needles. Two blood smears were made for leukocyte differential immediately upon collection of the blood, prior to addition of the
anticoagulant. Sodium heparin (free of preservative) was added in the amount of 0.4 ml of sodium heparin concentration 2000 Units/ml to each 10 ml of whole blood collected, then mixed gently. The heparinized blood was kept at room temperature and separation of the lymphocytes was done within 4 hours of collection. The blood smears for differentials were stained with Wright's stain within 4 hours of collection.

TECHNICAL CONSIDERATIONS

Interpretation of much of the published data concerning T and B cells in health and disease has been questionable due to the uncertainty regarding technical procedure used (51). All techniques used in this study followed the recommendations laid down by the World Health Organization/International Agency for Research on Cancer (WHO/IARC) in their special technical report of 1974 (35). An effort was made to standardize, as much as possible, the technical procedure to help eliminate many of the variables. In this study special consideration was addressed to the value of reporting lymphocyte levels in percentages and in absolute numbers. The methods for separation and preparation of lymphocytes were adapted from the works of Dr. E.J. Shannon and Associates, Carville, Louisiana (52).

STATISTICAL ANALYSIS

The data of the patient group and normal group was subdivided into numerous categories as follows:

Allergic, all (steroid and nonsteroid dependent)
Allergic, steroid dependent
Allergic, nonsteroid dependent
Asthmatic, all (steroid and nonsteroid dependent; all 3 types)
Asthmatic, all types, steroid dependent
Asthmatic, all types, nonsteroid dependent
Intrinsic asthmatic, all (steroid and nonsteroid dependent)
Intrinsic asthmatic, steroid dependent
Intrinsic asthmatic, nonsteroid dependent
Extrinsic asthmatic, all (steroid and nonsteroid dependent)
Extrinsic asthmatic, steroid dependent
Extrinsic asthmatic, nonsteroid dependent
Mixed asthmatic, steroid dependent
Mixed asthmatic, nonsteroid dependent
Mixed asthmatic, all (steroid and nonsteroid dependent)
Normal, all (male and female)
Normal, female
Normal, male

The Student's t test was performed on all data. Analyses of the patient group for age, sex, IgE levels, eosinophil levels, steroid or nonsteroid dependency, type of disease state, skin testing reactions, hyposensitization, duration of symptoms, family history and levels of T and B cells were performed. The normal control group was analyzed for age, sex, and levels of T and B cells. All T and B cell data of the patient group was compared to the T and B cell levels of the normal control group. Male and female T and B cell levels were combined in the analysis. T and B cells were reported in percentages and in absolute numbers.

IgE levels were determined by the Pharmacia IgE PRIST method and their prescribed calculations. Since the range of IgE was great, values
were calculated in ln then converted to the international units per milliliter of blood serum, U/ml.

Tables 1, 2 and 3 contain the raw data on the allergic, asthmatic, and normal groups. Table 4 contains the arithmetic means and standard deviations for all groups for T and B cell levels.

SEPARATION AND PREPARATION OF LYMPHOCYTES

**Separation procedure.** 15 ml heparinized blood was diluted with 15 ml cold Hank's Balanced Salt Solution (HBSS) and placed into a 30 ml plastic syringe for dispensing. The mixture was then layered quickly from the syringe onto 7 ml Lymphoprep(R) in a 50 ml round-bottomed clear plastic Sorvall centrifuge tube. The tube was then centrifuged at room temperature in a horizontal swinging head for 40 minutes at 400 x g at the blood-Lymphoprep(R) interface level. The lymphocyte-rich layer (the very top of the buffy layer) was removed with a Pasteur pipette (approximately 1 ml) and transferred to a 10 ml tube. 7 to 10 ml cold HBSS was added to the tube and then centrifuged at 800 x g for 10 minutes. This was repeated for a total of 3 times with the supernatant being removed each time by vacuum flask. On the last wash, 0.5 ml of the supernatant was retained for resuspending the cells.

**Preparation procedure.** Cell volume was adjusted to $2 \times 10^6$ cells/ml. A cell count of the lymphocyte concentration was done on the Coulter Counter Model ZBI; results were in cells/mm$^3$. These results were converted to cells/ml by multiplying by $10^3$ mm$^3$/ml. The number of lymphs in the concentrate was divided by the number of cells desired, $2 \times 10^6$ cells/ml to yield a ratio of cells to one milliliter of volume. The ratio was then multiplied by the number of mls of lymphs concentrate to
Table 1. NORMAL CONTROL GROUP

<table>
<thead>
<tr>
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<td>Sex</td>
<td>Type</td>
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<td>Tabs</td>
<td>%B</td>
<td>Babs</td>
<td>U/ml</td>
<td>ln</td>
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<td>1719</td>
<td>20</td>
<td>446</td>
<td>180</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Mean and s.d.: 68.0+10.9 1388+557 15.2+9.7 321+297

IgE levels geometric mean and s.d.:  
- Intrinsic, all: 12.5+2.8 2.5  
- Extrinsic, all: 456.4+1.8 6.1  
- Mixed, all: 125.7+2.7 4.8  
- Nonsteroid, mixed: 70.8+1.7 4.3  
- Steroid, mixed: 157.8+2.7 5.6
<table>
<thead>
<tr>
<th>Subject</th>
<th>T%</th>
<th>Tabs</th>
<th>B%</th>
<th>Babs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals, all</td>
<td>68.5 ± 8.6</td>
<td>1348 ± 403</td>
<td>14.9 ± 5.3</td>
<td>295 ± 129</td>
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<tr>
<td>Normal, female</td>
<td>66.4 ± 10.1</td>
<td>1269 ± 338</td>
<td>12.4 ± 4.1</td>
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<td>Normal, male</td>
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<td>335 ± 137</td>
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<td>Allergic and asthmatic, all</td>
<td>67.6 ± 10.9</td>
<td>1402 ± 558</td>
<td>15.8 ± 10.0</td>
<td>341 ± 304</td>
</tr>
<tr>
<td>Allergic, all</td>
<td>66.9 ± 11.4</td>
<td>1434 ± 574</td>
<td>17.4 ± 10.5</td>
<td>390 ± 325</td>
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<tr>
<td>Asthmatic, all</td>
<td>68.0 ± 10.9</td>
<td>1388 ± 557</td>
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<td>321 ± 297</td>
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<tr>
<td>Allergic and asthmatic, steroid</td>
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<td>Allergic and asthmatic, nonsteroid</td>
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<td>14.3 ± 8.7</td>
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<tr>
<td>Allergic, steroid</td>
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<td>17.9 ± 12.2</td>
<td>429 ± 395</td>
</tr>
<tr>
<td>Allergic, nonsteroid</td>
<td>73.6 ± 10.0</td>
<td>1447 ± 653</td>
<td>16.6 ± 8.1</td>
<td>328 ± 179</td>
</tr>
<tr>
<td>Asthmatic, all, steroid</td>
<td>67.2 ± 10.5</td>
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<td>16.2 ± 10.0</td>
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</tr>
<tr>
<td>Asthmatic, all, nonsteroid</td>
<td>72.7 ± 13</td>
<td>1427 ± 356</td>
<td>11.7 ± 9.2</td>
<td>240 ± 208</td>
</tr>
<tr>
<td>Intrinsic, all</td>
<td>66.1 ± 10.1</td>
<td>1262 ± 440</td>
<td>13.9 ± 8.4</td>
<td>253 ± 161</td>
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<tr>
<td>Extrinsic, all</td>
<td>70.5 ± 9.6</td>
<td>1513 ± 766</td>
<td>15.8 ± 11.3</td>
<td>358 ± 417</td>
</tr>
<tr>
<td>Subject</td>
<td>T%</td>
<td>Tabs</td>
<td>B%</td>
<td>Babs</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>Mixed, all</td>
<td>67.8 ± 12.8</td>
<td>1415 ± 473</td>
<td>16.0 ± 10.3</td>
<td>359 ± 296</td>
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<td>Intrinsic, steroid</td>
<td>66.8 ± 10.1</td>
<td>1262 ± 456</td>
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<td>Intrinsic, nonsteroid*</td>
<td>56.0</td>
<td>1267</td>
<td>14.0</td>
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<tr>
<td>Extrinsic, steroid</td>
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<td>1518 ± 803</td>
<td>16.5 ± 11.5</td>
<td>380 ± 430</td>
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<tr>
<td>Extrinsic, nonsteroid*</td>
<td>75.0</td>
<td>1458</td>
<td>6.0</td>
<td>117</td>
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<tr>
<td>Mixed, steroid</td>
<td>64.7 ± 11.8</td>
<td>1399 ± 502</td>
<td>17.3 ± 10.2</td>
<td>399 ± 311</td>
</tr>
<tr>
<td>Mixed, nonsteroid</td>
<td>76.3 ± 13.0</td>
<td>1459 ± 448</td>
<td>12.5 ± 11.3</td>
<td>251 ± 255</td>
</tr>
</tbody>
</table>

*One person
yield total volume. Total volume minus the number of mls of lymphs
concentrate equals the number of mls of diluent needed to adjust cell
volume to a final concentration of $2 \times 10^6$ cells/ml. The lymphocytes
were checked with acridine orange stain for purity. If greater than
1% were cells other than lymphocytes, a correction was made in the fi-
nal results. All lymphocytes were tested for viability using trypan
blue. If lymphocytes were less than 90% viable, they were rejected
for testing.

ACRIDINE ORANGE STAIN

Acridine orange stain, a fluorescent stain, was prepared according
to Animal Tissue Techniques by Humason (53). Lymphocytes appeared as
green mononuclear cells with one reddish-orange granule. Monocytes,
mononuclear like lymphocytes, appeared as pale green cells with no
granules. Granulocytes appeared as green cells with many reddish-orange
granules and multilobed nuclei.

A stock solution was made by adding .01 g acridine orange to 5.0
ml 95% ethanol. Excess dye was allowed to settle to the bottom and
then was stored in the dark at $4^\circ$ C until ready to use. A working so-
lution was prepared fresh for each time used. The working solution was
made by adding 5 drops of the stock solution to 5.0 ml 95% ethanol.
This solution was then flooded onto clean microscope slides, allowed to
sit a few seconds, then the slides were placed vertical to dry. Slides
were then stored in a dust free box at room temperature until used.

A drop of lymphocyte solution ($2 \times 10^6$ cells/ml) was added to an
acridine orange stained slide, then a coverslip was placed on top. The
fresh wet mount was read immediately under an ultraviolet microscope.
200 cells were counted and the percent of cells other than lymphocytes was recorded. If greater than 1%, a correction was made in the final results.

VIABILITY - TRYPAN BLUE (52)

A stock solution of 1% was made by adding 1 g trypan blue to 100 ml of HBSS. This was stored in a Nalgene plastic bottle to prevent leaching; the bottle was rinsed with 70% alcohol prior to the stain to eliminate bacterial contamination. The stock solution was stored at 4° C. A working solution of 0.4% was made by adding 0.4 ml filtered stock solution to 9.6 ml HBSS; this was made fresh for each testing day.

0.5 ml of a 0.4% trypan blue was placed in a small disposable tube (12 x 75 mm) and allowed to equilibrate in a 37° C water bath. 100 microliters of the 2 x 10^6 cells/ml lymphocyte solution was added to the trypan blue and incubated for 5 minutes at 37° C. The solution was then placed in a Neubauer hemacytometer and 100 cells were counted at random. Nonviable cells took up the stain and appeared blue. Viable lymphs remained clear. If viability was less than 90%, lymphocytes were rejected for rosette assay.

CELL COUNTS, HEMOGLOBINS, WBC DIFFERENTIALS

All patients and normal controls had total WBC counts, RBC counts, hemoglobins, and WBC differentials done. Dilutions for cell counts were made with a Coulter automatic dilutor using Coulter reagents. The cell counts were performed using a Coulter Counter Model ZBI. Hemoglobin samples were obtained from the WBC dilution with Coulter Zaptoglobin(R). The hemoglobin concentrations were determined using a Coulter hemoglobinometer. All equipment was quality controlled with commercial
Coulter 4C control cell suspensions prior to each day's use. All cell counts were corrected using Coulter's coincidence charts.

WBC differentials were determined on the blood smears stained with Wright's stain. 100 to 200 leukocytes were counted with each type recorded as a percent of the total counted.

PREPARATION OF SHEEP ERYTHROCYTES

Sheep erythrocytes in Alsever's solution were obtained from Colorado Serum Company Laboratories, Denver, Colorado. 15 ml of sheep erythrocytes (SRBC) were removed aseptically from the stock bottle and transferred to two 10 ml tubes. The SRBC were washed and centrifuged 3 times using a volume of 9 ml of 0.85% saline (or until the supernatant was clear) at 3500 rpm for 5 minutes each. The supernatant was removed after each wash with a vacuum flask.

HUMAN AB SERUM, 10% (52)

Human AB serum was obtained from Grand Island Biological Company, Grand Island, New York. This serum was heat inactivated for complement at 56° C for 30 minutes. The serum was then absorbed with packed SRBC (10 ml serum to 1 ml packed SRBC) at 4° C for 30 minutes, then centrifuged at 800 x g for 10 minutes. The serum once absorbed was transferred to another 1 ml of packed SRBC, incubated at 37° C for 30 minutes, then centrifuged at 800 x g for 10 minutes. The supernatant serum was removed and stored at -20° C in aliquots of 250 microliters.

19s ANTI-SHEEP ERYTHROCYTE ANTIBODY (52)

The source of the 19s (IgM) antisheep erythrocyte antibody was Cordis Laboratories, Miami, Florida. This antibody was used to prepare
the sheep erythrocyte-antibody-complement (EAC) complex for the B cell rosette method. Microtiter technique was used to titer the 19s antibody to determine the maximum subagglutinating dose in order to dilute the antibody accordingly for testing. The Cordis 19s antibody was serially diluted with HBSS in a microtiter tray. SRBC suspensions of 1% and 2% were added to the serially diluted antibody. Each microtiter well was observed for hemagglutination. The end point of the hemagglutination reaction was read to the highest dilution. The 2% SRBC suspension yielded the best end point of 1:32 dilution. The 19s antibody was then diluted with HBSS 1:32 and stored at -20°C in aliquots of 1 ml.

MOUSE COMPLEMENT

Mice from the common animal stock were anesthetized with 0.1 ml Nembutol injected intraperitoneally using a tuberculin syringe and 24 gauge needle. Each mouse was dissected to reveal the heart while still beating. Blood was drawn directly from the heart with a 20 gauge needle and tuberculin syringe. Each mouse yielded approximately 1 ml whole blood. Blood was allowed to clot on ice, then the serum was removed. 5 mice yielded approximately 2 ml serum which was ample supply for one month's testing. Serum was absorbed twice, first with 0.5 ml packed SRBC per 1 ml mouse serum at 4°C for 30 minutes, then with another 0.5 ml packed SRBC per 1 ml mouse serum at 37°C for 30 minutes. The absorbed mouse serum was aliquoted into volumes of 250 microliters and stored at -20°C. At time of use, the frozen serum was rapidly thawed in a 37°C water bath just prior to use. No mouse serum was used if more than one month old.
SOLUTIONS FOR SRBC - PREPARATION FOR EAC AND EC

For the T cell rosette, a 0.5% solution of SRBC was prepared by adding .05 ml packed SRBC to 9.95 ml HBSS.

For the B cell rosette, EAC cells and EC cells were made from a 2.5% solution of SRBC (0.25 ml packed SRBC to 9.75 ml HBSS).

To prepare EAC cells (sheep erythrocyte-antibody-complement) and EC cells (sheep erythrocyte-complement), a control for immune adherence, the following was done: Two 10 ml tubes were labelled, one EAC and one EC. To the EC tube, 1.0 ml of a 2.5% SRBC solution and 1.0 ml of cold HBSS was added. To the EAC tube, 1.0 ml of a 2.5% SRBC solution and 1.0 ml of 19s antisheep erythrocyte antibody was added. Both tubes were incubated in a 37°C shaking water bath at 60 cpm for 30 minutes. Tubes were allowed to cool to room temperature (5 to 10 minutes). Cells were then washed 3 times with 3 ml cold HBSS each time at 300 x g for 5 minutes. Supernatant was removed each time with a vacuum flask.

After the last wash, the packed pellet of cells was resuspended in 2 ml cold HBSS. 100 microliters of mouse complement was added to each tube and the tubes were incubated in a 37°C shaking water bath at 60 cpm for 30 minutes. Cells were washed 3 times with 3 ml cold HBSS, removing the supernatant with vacuum flask each time, at 300 x g for 5 minutes. After the last wash, the packed pellet of cells was resuspended in 5 ml cold HBSS to yield a final concentration of 0.5% suspension of EAC and EC cells. These solutions were stored at 4°C and used for two days as long as no hemolysis occurred. Cell suspensions were kept on ice during testing.

THE B CELL (EAC) METHOD
Two 12 x 75 mm plastic tubes were labelled, one EAC and EC.

The following steps were taken:

1. **Tube EC control**
   - Added: 100 μl 0.5% EC cells
   - + 100 μl 2 x 10⁶ lymphs/ml

2. **Tube EAC test**
   - Added: 100 μl 0.5% EAC cells
   - + 100 μl 2 x 10⁶ lymphs/ml

2. Tubes were incubated for 5 minutes in a 37°C shaking water bath at 60 cpm.

3. Tubes were centrifuged at 200 x g for 5 minutes.

4. The pellet of cells was resuspended in its supernant by gentle rocking until all of the pellet was off the bottom of the tube. One drop of 0.33% methylene blue was added for ease in reading.

5. The lymphocytes were counted under a light microscope using a Neubauer hemacytometer for each cell suspension of EAC and EC. The cell suspensions were added by Pasteur pipette to both chambers of the hemacytometer. A total of 200 cells, rosetted and nonrosetted, were counted, 100 cells per chamber. The total rosette number was divided by 2 to give the % EAC and % EC rosettes. The % of B cells was determined by subtracting % EC from % EAC. A sample calculation:

   EAC tube
   - 30 rosettes / 170 nonrosettes = Total 200 cells
   - EC tube
   - 10 rosettes / 190 nonrosettes = Total 200 cells

   20 rosettes

   10 = % B cell

   For the absolute number of B cells, the total WBC count was multiplied by the percentage of lymphocytes determined from the WBC differential
to give the total absolute lymphocyte number. The total absolute lymphocyte number was then multiplied by the % B cell to yield the total B cell absolute number. A sample calculation:

\[
10,000 \, \text{WBC/mm}^3 \times 30\% \, \text{lymphs (differential)} = 3000 \, \text{total absolute lymphocytes}
\]

\[
3,000 \, \text{total absolute lymphs} \times 10\% \, \text{B cells} = 300 \, \text{total absolute B cell number}
\]

**THE T CELL (E) ROSETTE METHOD**

One 12 x 75 mm plastic test tube was labelled E. The following steps were taken:

1. To the E tube was added 50 μl absorbed 10% human AB serum
   
   \[
   50 \, \mu l \, 2 \times 10^6 \, \text{lymphs/ml}
   \]
   
   \[
   100 \, \mu l \, 0.5\% \, \text{SRBC}
   \]

2. The tube was incubated for 30 minutes in a 37° C shaking water bath at 60 cpm.

3. The tube was centrifuged at 200 x g for 5 minutes.

4. The tube was then incubated for 60 minutes at 4° C.

5. The pellet of cells was resuspended by gentle rocking until all the pellet was off the bottom of the tube. One drop of 0.33% methylene blue was added for ease in reading.

6. The lymphocytes were counted under the light microscope using a Neubauer hemacytometer for the cell suspension. The cell suspension was added to the hemacytometer with a Pasteur pipette to both chambers of the hemacytometer. A total of 200 cells, rosetted and nonrosetted, were counted, 100 cells per chamber. The total
rosette number was divided by 2 to give the % E rosettes. The % T cells equalled the % E rosettes. A sample calculation:

E tube. 150 rosettes / 50 nonrosettes = Total 200 cells 

2 ) 150

75 = % T cell

The absolute number of T cells was obtained by multiplying the total WBC count by the percentage of lymphocytes determined from the WBC differential to give the total lymphocyte absolute number. This total lymphocyte absolute number was then multiplied by the % T cells to yield the total T cell absolute number. A sample calculation:

10,000 WBC/mm³ x 30% lymphs (differential) = 3000 total absolute lymphocytes

3,000 total absolute lymphs x 75% T cells = 2250 total absolute T cell number

IMMUNOGLOBULIN E

Serum IgE levels were measured using the radioimmunoassay method, PRIST of Pharmacia Laboratories, Piscataway, New Jersey.
RESULTS AND DISCUSSION

The patient population, allergic and asthmatic, was considered first on the basis of sex, age, family history, hyposensitization, duration of symptoms, and eosinophil levels. No interrelationships were seen in these areas. T and B cell levels (percent and absolute number) and IgE levels were considered in greater detail with attention being given to the possible effects of corticosteroid treatment on these levels.

The normal values for the rosette assay were established as follows:

- T cell percent \((T\%)\) = 68.5 \(\pm\) 10.6%
- T cell absolute number \((\text{Tabs})\) = 1345 \(\pm\) 403 cells/mm\(^3\)
- B cell percent \((B\%)\) = 14.9 \(\pm\) 5.3%
- B cell absolute number \((\text{Babs})\) = 295 \(\pm\) 129 cells/mm\(^3\)

The normal value for adults for IgE levels set by Pharmacia Laboratories was 20 U/ml.

The numerous categories of patient groups as listed on pages 18 and 19, were compared to T cell percent, T cell absolute number, B cell percent, and B cell absolute number to normal values, respectively. Chart 1 is a summary of these comparisons.

THE ALLERGIC GROUP – T AND B CELLS

The allergic group, 18 individuals, was considered first as a whole including steroid and nonsteroid dependent. No significant differences were seen in T and B cell percent and absolute numbers of
this group when compared to normal values at a 95% confidence level. B cell percent and B cell absolute numbers were elevated above normal values at a 70% and 83% confidence level, respectively. (See Table 5).

The allergic group was then considered on the basis of steroid or nonsteroid dependency. The nonsteroid dependent allergics consisted of 7 individuals. In this group no significant differences were seen in T and B cell percent and absolute numbers when compared to normal values at a 95% confidence level. T cell percent values of nonsteroid dependent allergics were elevated above normal values at an 83% confidence level. (see Table 6). The steroid dependent allergics consisted of 11 individuals. No significant differences were noted in T and B cell percent and absolute number when compared to normal values at a 95% confidence level. B cell percent and B cell absolute numbers were elevated above normal values at a 71% and 89% confidence level, respectively. (See Table 7).

THE ASTHMATIC GROUP - T AND B CELLS

The asthmatic group was first considered as a whole, 42 individuals, including all types (intrinsic, extrinsic, and mixed) and steroid and nonsteroid dependent. No significant differences in T and B cell percent and absolute numbers of this group were seen when compared to normal values at a 95% confidence level. (See Table 8).

The asthmatic group was then considered including all types (intrinsic, extrinsic, and mixed) on the basis of steroid and nonsteroid dependency. The nonsteroid dependent group consisted of 6 individuals, while the steroid dependent group consisted of 36 individuals. No significant differences were seen in either group in T and B cell percent
### Table 5  NORMAL VS. ALLERGIC, STEROID AND NONSTEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Allergic*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>66.9 ± 11.4</td>
<td>39%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1434 ± 574</td>
<td>46%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>17.4 ± 10.5</td>
<td>70%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>390 ± 325</td>
<td>83%</td>
</tr>
</tbody>
</table>

*Values expressed are means ± s.d.

### Table 6  NORMAL VS. ALLERGIC, NONSTEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal</th>
<th>Allergic, nonsteroid</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>73.6 ± 10.0</td>
<td>83%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1447 ± 653</td>
<td>40%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>16.6 ± 8.1</td>
<td>49%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>328 ± 179</td>
<td>42%</td>
</tr>
</tbody>
</table>

*Values expressed are means ± s.d.
Table 7  NORMAL VS. ALLERGIC, STEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Allergic, steroid*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>62.6 ± 11.4</td>
<td>16%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1426 ± 552</td>
<td>39%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>17.9 ± 12.2</td>
<td>71%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>429 ± 395</td>
<td>89%</td>
</tr>
</tbody>
</table>

*Values expressed are means ± s.d.

Table 8  NORMAL VS. ASTHMATIC, ALL TYPES, STEROID AND NONSTEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Asthmatic*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>68.0 ± 10.9</td>
<td>14%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1388 ± 557</td>
<td>27%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>15.2 ± 9.7</td>
<td>10%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>321 ± 297</td>
<td>33%</td>
</tr>
</tbody>
</table>

*Values expressed are means ± s.d.
and absolute number when compared to each other at a 95% confidence level. (See Table 9).

Each type intrinsic, extrinsic, and mixed was considered separately with each type including both steroid and nonsteroid dependent individuals. Intrinsic asthmatics (15 individuals), extrinsic asthmatics (12 individuals), and mixed asthmatics (15 individuals) with each type including steroid and nonsteroid dependent, showed no significant differences in T and B cell percent and absolute numbers when each type was compared to normal values at a 95% confidence level. (See Tables 10, 11, 12). The intrinsic group, steroid and nonsteroid dependent, showed at a 66% confidence level decreased B cell absolute number when compared to normal values. (See Table 10). Extrinsic asthmatics steroid and nonsteroid dependent, showed an increase in T cell absolute number when compared at a 63% confidence level to normal values. (See Table 11). The mixed asthmatic group, steroid and nonsteroid dependent, showed an elevated B cell absolute number when compared to normal values at an 83% confidence level. (See Table 12).

The three asthmatic types were next considered on the basis of type and whether or not the individuals were steroid or nonsteroid dependent. The intrinsic and extrinsic nonsteroid dependent groups consisted of only one individual each. The intrinsic individual had lower T cell percent and absolute number than normal values with no differences noted in B cell percent and absolute number. (See Table 13). The extrinsic individual had T cell percent and absolute number within normal range, but the B cell percent and absolute number were lower.
### Table 9  ASTHMATIC, STEROID DEPENDENT VS. ASTHMATIC, NONSTEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Asthmatic, steroid*</th>
<th>Asthmatic, nonsteroid*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>67.2 ± 10.5</td>
<td>72.7 ± 13.0</td>
<td>13%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1382 ± 588</td>
<td>1427 ± 356</td>
<td>1%</td>
</tr>
<tr>
<td>B%</td>
<td>16.2 ± 9.6</td>
<td>11.7 ± 9.2</td>
<td>13%</td>
</tr>
<tr>
<td>Babs</td>
<td>344 ± 308</td>
<td>240 ± 208</td>
<td>6%</td>
</tr>
</tbody>
</table>

### Table 10  NORMAL VS. INTRINSIC ASTHMATIC, STEROID AND NONSTEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Intrinsic*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>66.1 ± 10.1</td>
<td>57%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1262 ± 440</td>
<td>47%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>13.9 ± 8.4</td>
<td>39%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>253 ± 161</td>
<td>66%</td>
</tr>
</tbody>
</table>

*Values expressed are means ± s.d.*
Table 11 NORMAL VS. EXTRINSIC ASTHMATIC, STEROID AND NONSTEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Extrinsic*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>70.5 ± 9.6</td>
<td>51%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1513 ± 766</td>
<td>63%</td>
</tr>
<tr>
<td>BZ</td>
<td>14.9 ± 5.3</td>
<td>15.8 ± 11.3</td>
<td>25%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>358 ± 417</td>
<td>53%</td>
</tr>
</tbody>
</table>

*Values expressed are means ± s.d.

Table 12 NORMAL VS. MIXED ASTHMATIC, STEROID AND NONSTEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Mixed*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>67.8 ± 12.8</td>
<td>14%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1415 ± 473</td>
<td>39%</td>
</tr>
<tr>
<td>BZ</td>
<td>14.9 ± 5.3</td>
<td>16.0 ± 10.3</td>
<td>35%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>359 ± 296</td>
<td>83%</td>
</tr>
</tbody>
</table>

*Values expressed are means ± s.d.
Table 13 NORMAL VS. ASTHMATIC, NONSTEROID DEPENDENT BY TYPE

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>*<em>Intrinsic</em></th>
<th>*<em>Extrinsic</em></th>
<th>Mixed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>56</td>
<td>75</td>
<td>76.3 ± 13.0</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1267</td>
<td>1458</td>
<td>1459 ± 448</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>14</td>
<td>6</td>
<td>12.5 ± 11.3</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>317</td>
<td>117</td>
<td>251 ± 255</td>
</tr>
</tbody>
</table>

**One person.**

Normal vs. Mixed, Nonsteroid

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>89%</td>
</tr>
<tr>
<td>Tabs</td>
<td>40%</td>
</tr>
<tr>
<td>B%</td>
<td>54%</td>
</tr>
<tr>
<td>Babs</td>
<td>43%</td>
</tr>
</tbody>
</table>

*Values expressed are mean ± s.d.
than normal values. (See Table 13). The mixed nonsteroid dependent group consisted of 4 individuals. No significant differences in T and B cell percent and absolute number were seen in this group when compared to normal values at a 95% confidence level, but T cell percent was elevated above normal values at an 89% confidence level. (See Table 13).

The intrinsic steroid dependent asthmatic group consisted of 14 individuals. No significant differences were seen at a 95% confidence level in T and B cell percent and absolute number of this group when compared to normal values. B cell absolute number was lower than normal when compared at a 69% confidence level (See Table 14).

Eleven individuals made up the extrinsic steroid dependent asthmatic group. No significant differences were seen in T and B cell percent and absolute number of this group when compared to normal values at a 95% confidence level. T cell absolute number and B cell absolute number were elevated above normal values at a 63% and 65% confidence level, respectively. (See Table 15).

The mixed steroid dependent asthmatic group consisted of 11 individuals. No significant differences in T and B cell percent and absolute number were noted when compared to normal values at a 95% confidence level. T cell percent for this group was lower than normal at a 71% confidence level. B cell percent and absolute number were elevated above normal values at a 65% and 86% confidence level, respectively. (See Table 16).

T AND B CELLS IN SUMMARY

The values of T and B cell levels in this study did not show
Table 14 NORMAL VS. INTRINSIC ASTHMATIC, STEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Intrinsics*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>66.8 ± 10.1</td>
<td>41%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1262 ± 456</td>
<td>47%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>13.9 ± 8.7</td>
<td>38%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>248 ± 166</td>
<td>69%</td>
</tr>
</tbody>
</table>

Confidence Interval

Table 15 NORMAL VS. EXTRINSIC ASTHMATIC, STEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Extrinsic*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>70.1 ± 9.9</td>
<td>41%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1518 ± 803</td>
<td>63%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>16.5 ± 11.5</td>
<td>48%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>380 ± 430</td>
<td>65%</td>
</tr>
</tbody>
</table>

*Values expressed are mean ± s.d.
Table 16 NORMAL VS. MIXED ASTHMATIC, STEROID DEPENDENT

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Normal*</th>
<th>Mixed, Steroid*</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>T%</td>
<td>68.5 ± 8.6</td>
<td>64.7 ± 11.8</td>
<td>71%</td>
</tr>
<tr>
<td>Tabs</td>
<td>1348 ± 403</td>
<td>1399 ± 502</td>
<td>27%</td>
</tr>
<tr>
<td>B%</td>
<td>14.9 ± 5.3</td>
<td>17.3 ± 10.2</td>
<td>65%</td>
</tr>
<tr>
<td>Babs</td>
<td>295 ± 129</td>
<td>399 ± 311</td>
<td>86%</td>
</tr>
</tbody>
</table>

*Values expressed are mean ± s.d.
statistically at a high level of confidence, 95%, significant differences between the patient groups and normals. What was seen at lower confidence levels was best depicted visually with scattergram diagrams. (See Charts 2-21). Each category of disease state was diagrammed with steroid (s) dependent, nonsteroid (n) dependent, and normal control (c) values plotted against age in order to give a well distributed diagram for easy visual examination. Although definite differences were not seen, there were trends as follows: (See Chart 1).

1. Allergic individuals when considered as a whole, steroid and nonsteroid dependent, appeared normal in their T cell percent and absolute number while their B cell percent and absolute number appeared slightly elevated.

2. Asthmatic individuals including all three types (intrinsic, extrinsic, and mixed) and steroid and nonsteroid dependent when considered as a whole appeared normal in their T cell and B cell percent and absolute number.

3. No significant differences were seen in T and B cell percent and absolute number between intrinsic, extrinsic, and mixed asthmatics.

4. Intrinsic asthmatics, both steroid and nonsteroid dependent combined, appeared to have slightly lower B cell absolute number. They appeared normal in B cell percent, T cell percent and T cell absolute number.

5. Extrinsic asthmatics, steroid and nonsteroid dependent, appeared to have slightly elevated T cell absolute numbers. They appeared normal in T cell percent, B cell percent, and B cell absolute number.

6. Mixed asthmatics, steroid and nonsteroid dependent, appeared
<table>
<thead>
<tr>
<th></th>
<th>T%</th>
<th>Tabs</th>
<th>B%</th>
<th>Babs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic, All</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Allergic, Steroid</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Allergic, Nonsteroid</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthmatic, All</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthmatic, Steroid</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Asthmatic, Nonsteroid</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrinsic, All</td>
<td></td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Intrinsic, Steroid</td>
<td></td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Intrinsic, Nonsteroid*</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrinsic, All</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Extrinsic, Steroid</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Extrinsic, Nonsteroid*</td>
<td></td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Mixed, All</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed, Steroid</td>
<td></td>
<td>↑</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Mixed, Nonsteroid</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

*One person

Normal  Increase  Decrease
Chart 2 ALLERGICS, T CELL PERCENT
Chart 3  ALLERGICS, T CELL ABSOLUTE NUMBER
Chart 4 ALLERGICS, B CELL PERCENT

Age
90
80
70
60
50
40
30
20
10

0 10 20 30 40 50

B%
Chart 6  ASTHMATICS, ALL TYPES, T CELL PERCENT

Age
90
80
70
60
50
40
30
20
10

40 50 60 70 80 90 100
Chart 7  ASTHMATICS, ALL TYPES, T CELL ABSOLUTE NUMBER
Chart 8 ASTHMATICS, ALL TYPES, B CELL PERCENT
Chart 9  ASTHMATICS, ALL TYPES, B CELL ABSOLUTE NUMBER
Chart 11  INTRINSIC ASTHMATICS, T CELL ABSOLUTE NUMBER

Age
90
80
70
60
50
40
30
20
10

0  500  1000  1500  2000  2500
Tabs

58
Chart 12 INTRINSIC ASTHMATICS, B CELL PERCENT
Chart 13 INTRINSIC ASTHMATICS, B CELL ABSOLUTE NUMBER
Chart 14 EXTRINSIC ASTHMATICS, T CELL PERCENT
Chart 15  EXTRINSIC ASTHMATICS, T CELL ABSOLUTE NUMBER
Chart 16 EXTRINSIC ASTHMATICS, B CELL PERCENT
Chart 17  EXTRINSIC ASTHMATICS, B CELL ABSOLUTE NUMBER

Age
90
80
70
60
50
40
30
20
10

0 200 400 600 800 1000 1200 1400

Babs
Chart 18: MIXED ASTHMATICS, T CELL PERCENT
Chart 19  MIXED ASTHMATICS, T CELL ABSOLUTE NUMBER

Age
90
80
70
60
50
40
30
20
10

Tabs
0 500 1000 1500 2000 2500
Chart 21 MIXED ASTHMATICS, B CELL ABSOLUTE NUMBER

Age
90
80
70
60
50
40
30
20
10

0 200 400 600 800 1000

Babs
to have slightly elevated B cell absolute number. They appeared normal in B cell percent, T cell percent and T cell absolute number.

7. In all steroid dependent individuals, regardless of the general disease state, allergic or asthmatic, T cell percent and absolute number appeared normal while B cell percent and absolute number appeared elevated.

8. In all steroid dependent individuals, regardless of the general disease state, allergic or asthmatic, T cell percent appeared elevated while T cell absolute number appeared normal. The allergics of this group appeared normal in their B cell percent and absolute number. The asthmatics of this group, however, appeared to have lower B cell percent while their B cell absolute number appeared normal.

9. Intrinsic asthmatics, nonsteroid dependent, appeared lower in T cell percent and absolute number, while their B cell percent and absolute number appeared normal.

10. Extrinsic asthmatics, nonsteroid dependent, appeared normal in T cell percent and absolute number, while their B cell percent and absolute number appeared lower.

11. Mixed asthmatics, nonsteroid dependent, appeared to have elevated T cell percent, while their T cell absolute number appeared normal. B cell percent and absolute number appeared normal.

12. Intrinsic asthmatics, steroid dependent, appeared normal in T cell percent and absolute number, and B cell percent levels, but showed a decrease in B cell absolute number.

13. Extrinsic asthmatics, steroid dependent, appeared normal in T cell percent and B cell percent levels, but were elevated in T cell
absolute number and B cell absolute number.

14. Mixed asthmatics, steroid dependent, showed decreased T cell percent levels, normal T cell absolute levels, and increased B cell percent and absolute number.

The effects of corticosteroids on T and B cell levels in asthmatics were considered in this study. Other investigators (48-50) made the assumption that asthma was a state of suppressed cellular immunity, the T cell side, and used levels of T and B cells in peripheral blood as indicators of this suppression. These investigators reported decreased levels of T cells in asthmatics, but their patient populations studied were primarily nonsteroid dependent individuals. Combining the theory of suppressed cellular immunity in asthmatics, the assumption that T cell levels can be an indicator of such suppression, and the known effects of corticosteroids in normal individuals (See Page 14), a conclusion that T and B cell levels should be decreased in steroid dependent asthmatics could be derived. This study, however, showed the opposite: T and B cell levels appeared normal or elevated in steroid dependent asthmatics. If asthma is truly a state of suppressed cellular immunity, then the corticosteroids appear to have the ability to stimulate the T and B cell production rather than suppress them. The action of the corticosteroids could be directly on the T helper cell to stimulate it or on the T suppressor cell to turn it off and allow the T helper cell to function. Clearly further studies of asthmatics in regard to the effect of corticosteroids on T helper and suppressor cell functions is an area worthy of attention, since such studies could have implications for future treatments.
THE ALLERGIC GROUP - IgE LEVELS

The allergic group consisting of 18 individuals ranged from IgE values of 5 to 1350 U/ml. The geometric mean for this group was 41.9 ± 5.1 U/ml. The normal IgE serum value is 20 U/ml.

THE ASTHMATIC GROUP - IgE LEVELS

The asthmatic group was considered by type for IgE levels. Intrinsic asthmatics, 11 steroid and 1 nonsteroid dependent, had a geometric mean of 12.5 ± 2.8 U/ml. Extrinsic asthmatics, 10 steroid and 1 nonsteroid dependent, had a geometric mean of 45.4 ± 1.8 U/ml. Mixed asthmatics, 11 steroid and 4 nonsteroid dependent, had a geometric mean of 125.7 ± 2.66. Since both the intrinsic and extrinsic groups had only one nonsteroid dependent individual each, no further analysis could be done. The mixed group, however, was divided further on the basis of steroid dependency. Mixed asthmatics, nonsteroid dependent had a geometric mean of 70.8 ± 1.7 U/ml., while the steroid dependent mixed asthmatics had a geometric mean of 157.8 ± 2.7 U/ml. Charts 22–24 show the IgE levels in histogram form.

IgE LEVELS IN SUMMARY

Eleven out of the 18 allergic individuals were steroid dependent. The geometric mean of 41.9 ± 5.1 U/ml indicates that steroids did not decrease the IgE levels in this group.

IgE levels in the asthmatics showed more dramatically the value of such a test in this disease state. IgE was shown to be significant at a 95% confidence level in distinguishing the three types of asthma, intrinsic, extrinsic, and mixed.

The effects of corticosteroids on asthmatics' IgE levels were
Intrinsic Asthmatics--IgE levels

NUMBER OF PEOPLE

GEOMETRIC MEAN: 12.5 ± 2.8 U/ml
Mixed Asthmatics--IgE levels

Geometric Mean: 125.7 ± 2.66 U/ml
NS 70.8 ± 1.7
S 157.8 ± 2.7
Extrinsic Asthmatics--IgE levels

GEOMETRIC MEAN: 157.8±2.7 U/ml
considered by type. All three types of asthma did not show a decrease in IgE levels with steroids, except intrinsic IgE values were lower than the normal 20 U/ml but not significantly lowered. Extrinsic and intrinsic asthmatics consisted of all steroid dependent individuals except two. Extrinsic asthmatics' IgE levels were much higher than the normal value. Mixed asthmatics showed a definite difference between steroid and nonsteroid dependent individuals, but still both IgE levels were much higher than the normal values. Steroids did not decrease IgE levels in the mixed asthmatic individuals. IgE levels in the steroid dependent mixed asthmatics were higher than in the nonsteroid dependent mixed asthmatics suggesting perhaps that a decrease in the T suppressor cell function has occurred which would correlate with the decrease in T cell percent observed in the steroid dependent patients, while the nonsteroid dependent patients showed an increase in T cell percent.
LITERATURE CITED


