THE FUNCTIONS AND MEASUREMENT OF HUMAN B- AND T-LYMPHOCYTES

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Much of the current information regarding the function and characteristics of human lymphoid cells has been derived from studies of lymphocytes from other mammalian and avian species and from patients with immunodeficiency and other diseases [1-4]. These data collectively provide a basis for subdividing human lymphocytes into two major groups according to their origin and function: thymus-derived (T) lymphocytes and bursa- or bone marrow-derived (B) lymphocytes. While incisive studies of the function of different lymphocyte subpopulations have been possible in animals through ablation experiments and other manipulations of the immune system, a majority of the functional studies in man have been based on in vitro investigations of lymphocytes from human "experiments-of-nature" where, with rare exception, the primary biologic errors are unknown [3]. Caution should be exercised in drawing conclusions from much of the latter work, since recent studies in immunodeficiency patients have demonstrated disparities between results of tests enumerating blood cells with T- and B-lymphocyte differentiation markers and results of tests of lymphocyte function [5-9]. Nevertheless, the generalization can be made that functions of lymphoid subpopulations in man are probably roughly similar to those in other mammals and in birds. T-lymphocytes mediate delayed type skin reactions, contact hypersensitivity, allograft rejection, and graft-versus-host disease and manifest suppressor activities for other T-lymphocytes and for B-cells; they also cooperate with B-cells in the immune response [10-12]. T-cells perform these functions without any apparent endogeneous synthesis or release of antibody, although they do produce and elaborate the chemical mediators described in detail by Rocklin [13]. With the cooperation of T-lymphocytes and macrophages, B-lymphocytes differentiate into antibody-producing plasma cells that secrete antigen-specific immunoglobulin molecules into body fluids [14-16].

Methods for detecting and enumerating human peripheral blood T- and B-lymphocytes were developed in the early 1970s [17-21], after the discovery of similar methods in lower species [22-24]. These methods are based on certain well-defined membrane characteristics of human T- and B-cells. B-lymphocytes bear easily detectable membrane-associated immunoglobulin molecules that can be demonstrated using fluorescein-conjugated anti-immunoglobulin reagents [18,22]. Although some evidence exists that T-lymphocytes may also bear surface immunoglobulin [25,26], the extreme difficulty in detecting it on these cells makes the technique of membrane immunofluorescence quite useful in distinguishing B-lymphocytes in lymphoid cell populations. B-cells also have surface receptors for C3b and C3d fragments and for the fourth component of complement [2,27,28] and can be detected by rosette formation studies using antibody and complement-coated erythrocytes as indicators [2,23]. Receptors for the Fc portions of IgG molecules are also present on B-lymphocytes, although the question has been raised whether the cells with these receptors represent a distinct third population of lymphoid cells [29,30]. The latter cells lack surface immunoglobulin and C3b receptors, fail to form sheep erythrocyte rosettes, and can be detected by rosette formation studies employing human erythrocytes coated with either human or heterologous IgG (HEA) [31]. Fluorescein-conjugated heat-aggregated IgG has also been used to detect human B-cells [32], but there is some evidence that the B-cell receptor for heat-aggregated IgG may be distinct from the aforementioned Fc receptor detected with the HEA indicator [33]. It has also been shown that some T-cells have receptors for aggregated IgG [34-36], thus putting in question the usefulness of this test for distinguishing the two major subpopulations.

In the mouse, T-cells can be distinguished by the antibodies specific for human T-lymphocytes have been described [37-39]. The latter have been used
in cytotoxicity tests to estimate the number of T-cells present in human peripheral blood and lymphoid tissues. The most useful method for detecting and enumerating human T-lymphocytes is based on the chance observation that sheep erythrocytes form rosettes with human T-cells via a membrane receptor apparently present only on T-cells and probably on all such cells [17-20]. Populations of peripheral blood lymphocytes exhibiting sheep erythrocyte rosette formation and those bearing the complement receptor or easily detectable surface immunoglobulin have been shown to be mutually exclusive [20], although, as stated above, this is not the case for some cells bearing receptors for aggregated IgG [35].

The term “null” has been used to describe those lymphocytes that cannot be detected by any of the above-mentioned techniques. Whether such cells actually exist or are a reflection of insensitivity of methods for detecting and enumerating T- and B-cells is a controversial issue; moreover, if they do exist, the question has arisen as to whether they represent undifferentiated pluripotent “stem” cells or just less mature versions of cells already destined to be T- or B-lymphocytes. In favor of the latter are studies by Chess et al [40] who found that a population of lymphocytes bearing neither surface immunoglobulin nor receptors for sheep erythrocytes, but containing cells with the C3b receptor, developed surface immunoglobulin after 2 to 3 days in culture.

Another means of distinguishing lymphocyte subpopulations is through their in vitro responsiveness to certain mitogenic agents or to allogeneic cells, through their activities in non-antibody-dependent or antibody-dependent lymphocyte cytotoxicity assays, and through immunoglobulin biosynthetic studies. Soluble phytohemagglutinin (PHA) and concanavalin A (Con-A) both have been considered to stimulate predominantly T-cells [41], although new evidence has been presented that casts some doubt on this conclusion [42]. In addition, recent studies in the mouse [43] and in man [44] indicate that PHA and Con-A may stimulate different subpopulations of T-cells. Pokeweed mitogen (PWM) has been shown to stimulate both human T- and B-cells, although low doses appear to stimulate B-cells preferentially [45]. In the in vitro response to allogenic cells, only T-cells have been found to act as responders [46], whereas it appears that both T- and B-cells can stimulate [47]. T-cells have been shown to be killer cells in non-antibody-dependent cytotoxicity tests in vitro [48,49], whereas non-immunoglobulin-bearing but Fc receptor-bearing (K) cells have been shown to be the effector cells in similar but antibody-dependent in vitro assays [30,50-52]. Finally, in in vitro immunoglobulin biosynthetic studies of mixed populations of human T- and B-cells cultured in the presence of PWM, only B-lymphocytes differentiate into immunoglobulin synthesizing and secreting cells [14,15].

It can be seen from the above that the evaluation of numbers and functions of human lymphocyte subpopulations is complex; the results of such studies have been confusing, and conflicting data have appeared in the many published reports dealing with such studies. Technical difficulties and variations account to a large extent for these discrepant findings. In this paper the various methods noted above will be described only briefly, but the major technical pitfalls will be noted for each. More detailed information can be found in the paper by Aiuti et al [21].

**DETECTION AND ENUMERATION OF B-CELLS**

A major problem with all tests quantifying peripheral blood B-lymphocytes in mixed cell populations arises from the fact that monocytes also have both Fc and C3b receptors. Although methods have been described for monocyte depletion [53,54] or their more precise identification [55,56], the cell yields are often low in the former situation, resulting in selective losses of either T- or B-cells, and few of the latter identification methods ensure detection of promonocytes. The most promising of these appears to be the peroxidase method [56].

**Membrane Immunofluorescence Studies**

These studies are carried out by mixing purified peripheral blood lymphocytes with fluorescein-isothiocyanate conjugated (FITC) antisera specific for the heavy chains of the different immunoglobulin classes or for either kappa or lambda light chains, or with an anti-FAB reagent that detects both types of light chains [18]. It is important that these studies be carried out at 4°C on living cells and in the presence of 0.02% sodium azide in order to prevent capping of surface immunoglobulin. Relatively high fluorescein to protein molar ratios (i.e., 2:1 to 4:1) and an epi-illuminated microscope give the clearest membrane fluorescence, since positive cells usually show a finely granular staining pattern all over the cell surface. Winchester et al [35] have recently demonstrated important technical problems in the use of this assay. These occur primarily due to the presence of Fc receptor-bearing lymphocytes, monocytes, and/or promonocytes in the cell populations being tested; such cells may have IgG antibody-antigen complexes bound to the Fc receptors and/or aggregated IgG in the fluoresceinated reagent may bind, giving false positive results. In studies where lymphocytes were treated with proteolytic enzymes to remove surface immunoglobulin and the cells allowed to resynthesize immunoglobulin, only IgM- and IgD-bearing cells could be detected. In addition, only surface IgM and IgD were found if F(ab')2 anti-heavy-chain reagents were employed in the routine assay. It has been shown that most (75-86%) peripheral blood lymphocytes bearing IgD also bear IgM, i.e., are double producers [57]. The mean percentage of all immunoglobulin-bearing cells detected by Win-
chester et al [35] by a mixed F(ab')₂ anti-IgM and anti-IgD reagent in 8 normal subjects was 5.9%, far less than the normal means of total immunoglobulin-bearing cells cited in most published reports, which range from 20 to 30% [21].

**Rosette Formation with Complement-Coated Erythrocytes**

In most reports these tests have been carried out with sheep erythrocytes coated with rabbit antibody and mouse complement [2, 23]. A rosette is usually defined as a lymphocyte binding three or more erythrocytes. The percentage of normal peripheral blood complement receptor lymphocytes (CRL) detected by this assay in most laboratories approaches 15% [21]. Caution must be taken to carry out the incubation at 37°C when sheep erythrocytes are used, however, to avoid falsely high percentages, since coating sheep cells with antibody and complement does not cover determinants that bind to human T-lymphocytes when the test is conducted at lower temperatures [20]. Alternatively, the problem can be circumvented by using human erythrocytes coated with a rabbit IgM antihuman erythrocyte antibody and/or a human IgM cold agglutinin and human or mouse complement (HEAC). It should be recognized, however, that use of human complement results in red blood cell binding of predominantly the C₃b fragment [28], whereas use of mouse complement results in red blood cell binding of mainly the C₃d fragment.

**Tests for Aggregated IgG (Agg-IgG) Binding Cells**

These tests are carried out with Cohn fraction II IgG that is fluorescein-conjugated, heated to 63°C for 15 min, and centrifuged at high speed to remove large aggregates [32]. A World Health Organization Committee examining technical aspects of human T- and B-cell enumeration found the mean percentage from seven laboratories of peripheral blood lymphocytes binding FITC Agg-IgG to be 17.2%. Although cells binding FITC Agg-IgG were initially thought to be the same as cells binding erythrocyte-bound IgG, as noted above recent evidence indicates that they are two distinct subpopulations [31] and some cells forming sheep erythrocyte rosettes have also been noted to bind FITC Agg-IgG [35].

**Rosette Formation with IgG-Coated Human Erythrocyte Indicators**

In these studies, human erythrocytes are coated with either human or rabbit IgG antibodies directed to red cell antigens; they are then mixed with macrophage-depleted purified lymphocyte suspensions and examined for rosette formation as defined above. Using such assays, the percentages of peripheral blood lymphocytes detected in several laboratories ranged from 5 to 10% when human red blood cells coated with Ripley incomplete anti-D antibody were used [21] and were 6.23 ± 4.07% when human red blood cells coated with rabbit antibody were used (Gilbertsen and Metzgar, unpublished data). The large differences between percentages of cells detected with these indicators and the percentage obtained with FITC Agg-IgG reagents are further evidence for the likely involvement of separate receptors in these two types of assays.

**DETECTION AND ENUMERATION OF T-CELLS**

**Spontaneous Sheep Erythrocyte (E) Rosette Studies**

In this assay, sheep erythrocytes are mixed with purified populations of human peripheral blood lymphocytes in a ratio of 50–100:1, centrifuged at 200 g at room temperature, then incubated for 1 hr at room temperature before being gently resuspended and examined for rosette formation [20]. Using this method, greater than 90% of human thymocytes and 50 to 60% of peripheral blood lymphocytes form rosettes. The addition of protein to the medium increases the stability of the rosettes. Care must be taken to avoid extreme incubation temperatures, as the rosettes dissociate at 0° or 37°C [20]. More recently, this assay has been modified to increase both the stability and the percentage of T-lymphocytes detected by an average of 20% more (i.e., to 75%) by pretreatment of the sheep erythrocytes with neuraminidase (E₃) [58]. That the additional cells detected are T-cells has been shown by CRL rosette depletion studies that failed to alter the absolute number of E₃ binding cells. As already mentioned, however, some E₃ rosette-forming cells do bind FITC Agg-IgG; in addition, a small percentage (2-3%) of E-rosetting cells in normal subjects have been found to have surface immunoglobulin by FITC whole anti-immunoglobulin reagents [34] or to bear the complement receptor [59]. Another modification of the E rosette test, employing a shorter incubation time, is claimed to identify only “active” rosette-forming T-cells [60]; the significance of these cells remains to be established.

**Cytotoxicity Tests with Anti-T Cell Antisera**

These assays are limited by the specificity of the antisera. Since few reagents have been produced that demonstrate reciprocity with tests to detect and enumerate B-cells [37–39], this method is not widely employed for quantifying T-cells.

**FUNCTIONAL STUDIES**

**Responses of Lymphocytes to Mitogens and to Allogeneic Cells**

These studies are quite useful in distinguishing severe combined immunodeficiency disease (SCID) from other forms of immunodeficiency but help very little in distinguishing the other categories of immune disorders. The primary reason for
this is that there is marked day-to-day and sub-
ject-to-subject variability in results of lymphocyte 
stimulation studies. In addition, there is considera-
ble variability in responsiveness of the lympho-
cytes from the same subject with time and with 
respect to the optimal dose of stimulant and time of 
harvest. We have found it necessary to do 
time-course and dose-response studies in each 
evaluation in order to obtain any meaningful data. 
A microtiter plate modification of these methods 
has made these studies possible even on reasonably 
small volumes of blood and also permits more 
replicates. The several sources of variability lead to 
ble variability in responsiveness of the lympho-
cyte. These studies are carried out by mixing purified 
immune or mixed lymphocyte reaction (MLR)-
stimulated lymphocytes with \(^{51}\)Cr-labeled target 
lymphocytes or monolayers in varying ratios and 
measuring the amount of \(^{51}\)Cr released over that 
spontaneously released in cultures without immune 
lymphocytes [48]. The data are expressed as a per-
centage of the total \(^{51}\)Cr bound to the targets. This 
activity is mediated only by immune T-lymphocytes 
[49].

**Antibody-Dependent Killer Cell Assays**

In these experiments, macrophage-depleted, 
Ficoll-purified, nonimmune human lymphocytes 
are mixed with \(^{51}\)Cr-labeled target lymphocytes, 
erthrocytes, or tumor cells in the presence of heat-
inactivated (56°C for 30 min) antitarget anti-
body. Following incubation the amount of specific 
\(^{51}\)Cr released is determined as above, after sub-
tracting the amount of \(^{51}\)Cr released in cultures 
without effector lymphocytes [61]. This activity 
has been shown to be a property only of lympho-
cytes bearing the Fc receptor [30,50,52] and it does 
not require interaction with C3 [62].

**Immunoglobulin Biosynthesis**

This can be studied in vitro by culturing washed 
human lymphocyte populations with PWM in 
cultures supplemented with fetal calf serum; the 
amount of human immunoglobulin released in the 
culture supernatants 7 to 21 days later is quanti-
fied by double antibody radioimmunoassay [14]. 
Alternatively, unstimulated lymphocytes can be 
cultured in the presence of \(^{3}H\)- or \(^{14}C\)-labeled 
amino acids and the labeled immunoglobulins 
measured by a solid phase radioimmunoassay [16]. 
Methods of these types have been extremely valua-
bly in the study of agammaglobulinemic patients 
with various types of B-cell maturational defects 
[15,63] or with defective immunoglobulin synthesis 
due to excess numbers of T-suppressor cells [14].

**STUDIES IN PATIENTS WITH IMMUNODEFICIENCY**

After the above methodologies were developed, 
studies of lymphocytes from large numbers of 
patients with various types of immunodeficiency 
were carried out in many laboratories (see bibiliog-
raphy of Schiff et al [6]). In most of these patients 
varying quantities of lymphocytes have been de-
tected by one or more of the above methods and, 
for the most part, the numbers detected fall within 
or close to the normal range. In addition, the 
responses to mitogens and allogeneic cells are 
usually within the normal range [6]. The excep-
tions to this statement are boys with X-linked 
agammaglobulinemia who have very low or no 
surface immunoglobulin-bearing B-lymphocytes, 
although they do have cells bearing the comple-
ment and Fc receptors [6,64], and infants with 
DiGeorge's syndrome (DiG) [65] and severe com-
bined immunodeficiency, who may have very low 
numbers of E-rosette-forming cells and absent or 
extremely low responses to mitogens and allogene-
ic cells [5].

The most important information has come, how-
ever, from those studies in which T- and B-cells are 
enumerated and their functions evaluated. Through 
such combined studies in our laboratory, we 
have observed unexpected disparities that may 
bear on the primary defects in certain of these 
deficiency states [5]. We have recently evaluated 3 
infants with severe combined immunodeficiency 
disease (SCID) and one with DiG and found 
varying proportions of peripheral blood cells with 
surface markers typical of T- or B-lymphocytes in 
all 4 infants (Tab. I). In 2 of the infants with SCID,
the percentages of B-cells were elevated and the percentages of T-cells very low; in the third, however, both the percentages and absolute numbers of both T- and B-cells were normal or near normal throughout a 4½-month period of study. Despite this, functions mediated by T- or B-cells were either absent or very minimal in all 3 infants with SCID, including cell-mediated responses in vivo; in vitro proliferative response to mitogens, allogeneic cells, and antigens; effector cell function in lymphocyte antibody lymphocytolytic interaction (LALI) assays; and in vitro synthesis of IgG, IgA, and IgM. Co-cultivation experiments with lymphocytes from 2 of these infants (Cases 2 and 3) and unrelated normal control lymphocytes failed to demonstrate suppressor cell activity for immunoglobulin synthesis. A T-helper-cell defect was suggested by results of cocultures with one of the infants' cells and unrelated normal control cells, since immunoglobulin production was augmented 310 to 560% over that expected on the basis of individual culture data. The finding of cells with differentiation markers characteristic of T- and B-lymphocytes in these 3 infants with SCID argues against the concept that this entity represents a stem cell defect. The data are rather more compatible with a block in maturation of cell function or a metabolic defect that prevents normally differentiated cells from carrying out their functions. In contrast to the observations in the SCID patients, the results of functional studies in the DiG patient yielded just the opposite findings. Despite a very low percentage and low number of lymphocytes bearing T-cell differentiation markers, the infant's in vitro lymphocyte response to PHA was completely normal and better than that of the normal control's lymphocytes studied simultaneously; in addition, his cells gave significant though low responses to Con-A and PWM and manifested normal LALI activity, and he had a normal delayed cutaneous response in vivo. Thus, though the defect in DiG resulted in far fewer than normal T-cells, the cells functioned quite normally.

STUDIES IN PATIENTS WITH ATOPIC DERMATITIS

Prompted by recent observations that the thymus exerts an important regulatory influence over IgE antibody production in lower species, we conducted studies of immune function in 21 patients with atopic eczema to seek evidence for a similar relation in man [66]. Paradoxically, while carrier-specific T-helper-cells appear to be necessary for IgE antibapten antibody formation in rodents [67–70], carrier primed thymocytes have also been shown to have a potent inhibitory effect on IgE antibody production [68,71–73]. It is not known whether T-cells serve a similar regulatory function in man. Suggesting such a possibility is the finding that excessive IgE antibody production is associated with impaired cell-mediated immunity in certain human immunodeficiency diseases. Conditions in which this has been observed include the Wiskott–Aldrich, DiG, and Nezelof syndromes, and a recently described syndrome consisting of severe undue susceptibility to infection, exceptionally high serum IgE concentrations, and impaired cellular immunity [74,75]. Serum IgE concentrations are generally higher in patients with allergic eczema than in the other atopic states, hence we felt that the study of eczema patients might offer the greatest likelihood of observing any possible impairment in thymus-dependent immunity that may exist in atopy. The patients in this study were 21 infants, children, and adolescents ranging from 22 months to 17 years of age. In every case a diagnosis of atopic eczema was made on the basis of a focal or generalized macular and papular, lichenified, pruritic skin rash that showed predilection for the flexural areas of the extremities and/or the neck and face [76]. None of the patients was receiving systemic steroids at the time of this study, and only 6 had ever received such therapy. The subjects were divided into three categories based on the severity and distribution of their dermatitis. Group I included 12 patients with such severe generalized eczema that few areas of the head, trunk, or extremities were spared; Group II included 3 children whose eczematous lesions were confined primarily to localized areas of the extremities and face, with little involvement of the trunk; and Group III was composed of 6 subjects with a past history of eczema but in whom only mild xerosis and lichenified patches remained in the flexural areas.

Results of skin testing for delayed hypersensitivity to the ubiquitous antigens, Candida and SKSD, are given in Table II. The children with the most severe dermatitis (Group I) exhibited marked delayed cutaneous anergy; delayed cutaneous unresponsiveness was also present in Group II children who had localized eczema. In contrast, the children in Group III, who had nearly healed atopic dermatitis, all had positive delayed skin tests to one or both antigens. Serum IgE concentrations were markedly elevated in most subjects (Tab. III) and a correlation was noted between the extent of the dermatitis and the magnitude of the serum IgE concentration; concentrations of other immunoglobulins were usually normal for age. Surprisingly, despite the markedly elevated serum IgE concentrations, the percentages of IgE-bearing B-lymphocytes were not elevated in these patients; the mean percentage of lymphocytes bearing IgD was, however, significantly greater (p = .0392) than normal. These findings fail to support the observations reported by Cormane [77] and by Cormane et al [78].

Other immunologic abnormalities did not appear related to the severity of the eczema but pertained to the group as a whole. The mean percentage of CRLs (HEAC) was significantly (p = .0324) greater than in the control group (Tab. III). In contrast, the mean percentages of peripheral blood T-cells, as determined by spontaneous sheep erythrocyte formation and by rosette formation
TABLE II. Delayed skin test reactivity* to Candida and SK-SD in 21 patients with atopic eczema

<table>
<thead>
<tr>
<th>Patient</th>
<th>Agee/Race/Sex</th>
<th>Other allergic diagnosesd</th>
<th>Candida: 1:100</th>
<th>1:10</th>
<th>SK-SD: 100/25</th>
<th>1000/250</th>
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<td>Group I—Generalized eczema</td>
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<td>JR</td>
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<td>BM</td>
<td>4/W/M</td>
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<td>CS</td>
<td>5/B/M</td>
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<td>AP</td>
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<td>A, AR, Ad</td>
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<td>KM</td>
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<td>Group III—Xerosis and lichenification only</td>
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<td>AH</td>
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<tr>
<td>SM</td>
<td>17/W/F</td>
<td>A, AR</td>
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</table>

* Denotes no reaction or <5 mm induration at 24, 48, and 72 hr; + denotes reaction of induration 5 mm or greater in diameter at one or more of those times following testing.


Age in years

A = asthma, AR = allergic rhinitis, Ad = angioedema

Concentration per ml

with neuraminidase-treated sheep erythrocytes, were significantly lower than in the normal controls (p = <.0001, Tab. III).

Data from in vitro lymphocyte stimulation studies are presented in Figures 1 and 2. The patient's geometric mean responses were lower than the geometric means of the normal control subjects for each of the three mitogens, PHA, Con-A, and PWM (Fig. 1). The differences between patient and control group means were statistically significant only for Con-A (p = .0013) and PWM (p = .0002), however. In addition, the patient's geometric mean responses to the antigens, Candida albicans extract and tetanus toxoid, were also lower than control group geometric mean responses to these two agents (Fig. 2). This difference was statistically significant only for Candida (p = .0017).

Until recently, little was known about mechanisms involved in augmented IgE antibody production. Important observations bearing on this were made by Tada and coworkers in 1971 when they showed that rat homocytotropic antibody responses were enhanced by treatment of such animals with small doses of antithymocyte serum, 400-rad whole-body irradiation, adult thymectomy and splenectomy, or by giving various immunosuppressive drugs before or shortly after immunization [79-81]. The common facilitating factor in these treatments was revealed when these investigators found that administration of carrier-specific T-lymphocytes could inhibit on-going hapten-specific homocytotropic antibody formation [71].

The delayed cutaneous anergy observed in these atopic eczema patients is consistent with an abnormality in cellular immunity and is similar to that reported by Lobitz et al [82] who described 2 adults with life-long atopic dermatitis who failed to respond at 48 hr to intradermal injections of a variety of ubiquitous bacterial and fungal antigens and failed to become sensitized to dinitrochlorobenzene (DNCB). The latter finding is in keeping with those of Palacios et al [83] and Jones et al [84], who found that patients with atopic dermatitis were less readily sensitized to DNCB and Rhus extract than were normals, and with those of Rostenberg and Sulzberger [85], who found a very low incidence of positive patch tests among eczema patients. The failure of the patients in the present study to respond to bacterial and
mycotic antigens would appear to be at variance with the results of Palacios et al [83], although, if reactions to Schick and Dick toxins and to mumps antigen are excluded from their results, only 19 of 32 patients had positive delayed reactions; no information was given regarding the severity of the dermatitis in those patients.

The finding of significantly lower percentages of T-lymphocytes in eczema patients in the present study is in keeping with a similar finding by Luckasen et al [86]. Grove et al [87], however, found no differences in absolute numbers of E rosetting or surface immunoglobulin-bearing cells when results in 35 eczema patients (mainly adults) were compared with those in controls, and PHA responses did not differ from controls. On the other hand, as in the present study, Grove and coworkers [87] did find significantly impaired delayed cutaneous responsiveness to bacterial, mycotic, and viral antigens and impaired antibody responsiveness following immunization with tetanus toxoid. These same workers had previously noted similar findings in a group of asthmatics, except that PHA responsiveness was also diminished in those patients [88]. The depressed cell-mediated immunity observed in these eczema patients may have some bearing on their known heightened

<table>
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* Reprinted from J Allergy Clin Immunol 56:393, 1975 [66]  
* Calculated by the Mann-Whitney U test in the case of the IgE data and by Student's t-test in the case of all other data.  
* Eczema patients  
* IgE data expressed as mean ± SD of logarithms of data. Figures in parentheses are geometric means expressed as U/ml.  
* Controls

FIG. 1. Responses of atopic eczema patients' lymphocytes to mitogens. The data presented are the geometric means of results of quadruplicate cultures expressed in counts per minute per 10⁶ lymphocytes and represent the maximum responses obtained for patient or normal control lymphocytes. The geometric means of maximal responses in 129 PHA, 114 Con-A, and 109 PWM determinations on a normal population are shown by the solid horizontal lines, and the antigens of the mean logs ± 1 standard deviation of the logs of the data by the broken horizontal lines. Individual eczema patient values are depicted by the dots and the geometric means of all of the patients' responses are indicated by the horizontal arrows. The differences between the eczema patients' means and the normal Con-A and PWM means are significant at the p = .0013 and p = .0002 levels, respectively.

FIG. 2. Responses of atopic eczema patients' lymphocytes to antigens. See legend to Figure 1 for explanation of data presentation. The geometric means and standard deviations of maximal responses in 20 Candida and 18 tetanus determinations on a normal population are shown by the horizontal solid and broken lines. The difference between the eczema patients' mean Candida response and that of the normals is significant at the p = .0017 level.
susceptibility to certain viral agents, namely vaccinia, herpes simplex, and molluscum contagiosum viruses [76]. Cell-mediated immunity is thought to play a major role in host defense against many viral infections.

The reason for the greater impairment of cell-mediated immunity in vivo than in vitro is not clear, although it suggests an abnormality in immune effector function rather than in the T-cell itself. In keeping with this, abnormalities in both polymorphonuclear [89] and mononuclear (Snyderman and Buckley, unpublished) chemotaxis have been observed in eczema patients. Finally, experimental work in lower species points to a deficiency of T-suppressor-cells; it is not likely that definitive proof of abnormal T-cell function can be shown as a possible etiologic factor in the development of atopic diseases.

REFERENCES

noted, however, Grove et al [J Allergy Clin Immunol 55:152, 1975] have also found impaired delayed cutaneous responsiveness to bacterial, mycotic, and viral antigens, diminished PHA responsiveness, and impaired antibody responsiveness following immunization with tetanus toxoid in asthmatics, suggesting that the abnormalities may be present in all atotics.

Rocklin: I have three questions regarding your studies in patients with atopic dermatitis. First, do you have any data concerning the prior skin test reactivity of your patients before the onset of their skin disease? Second, do you have any in vitro evidence for the presence of blocking factors in the serum of these patients? Lastly, what is the nature of the chemotactic defect which you described? Does it involve a response of inflammatory cells to preformed chemotactic stimuli or the production of a lymphocyte-derived chemotactic factor?

Buckley: I have no observations on delayed skin tests in infants or children before the development of clinical atopy; there are studies going on at other centers where infants born to atopic parents are being followed by multiple immunologic parameters from birth onward, but I don't have any of the results as yet.

All of our lymphocyte cultures were conducted in media supplied both with autologous and AB-negative plasma from all male donors and in no instance was any evidence found of plasma blocking factors.

We have looked for possible chemotactic defects in these patients but the findings have been inconsistent. These studies have been carried out by Dr. Ralph Snyderman at our institution. He has found some abnormalities of monocyte chemotaxis, both in the nature of intrinsic hyporesponsiveness of the cells and in the form of plasma inhibitors of monocyte chemotaxis.

Cooper: Although the problem of cytophilic IgG raises the question of the presence of native IgG receptors on B-lymphocytes, an abundance of evidence indicates that B-lymphocyte precursors of IgG-secreting plasma cells may bear native IgG and/or IgM.

The presence of cells with T-cell characteristics in your patient with the DiGeorge syndrome of course could have come from a small ectopic thymus which many such patients have been found to have.

In your patient with nonfunctional T- and B-lymphocytes, could these cells, like those from Kersey's patient, be activated by a calcium ionophore?

Buckley: I also find it difficult to accept the point regarding the binding of fluorescein-conjugated heterologous IgG anti-immunoglobulin to the Fc receptor as being a major source of artifact, since with non-F(ab')2 FITC anti-heavy chain reagents we get no binding to the lymphocytes of patients with X-linked agammaglobulinemia. Yet these patients have normal numbers of cells bearing the Fc receptor.

Dr. Lischner pointed out at the Second Immunodeficiency Workshop that, in reviewing autopsy material from DiGeorge patients at St. Christopher's, he had found small nests of thymic tissue in a majority of the cases and that the tissue appeared normal, i.e., contained both Hassall's corpuscles and thymocytes. I don't believe current textbooks and other literature adequately reflect that this is usually only a partial thymic deficiency. This would certainly explain the spontaneous "cures" that have been noted in such patients.

Our patient died before Dr. Kersey reported his observation on the effect of the calcium ionophore on the lymphocyte responsiveness of his patient. I agree that his patient's lymphocyte counts and subpopulation...
findings were very similar to those in our Case 3. I would like to mention that, at postmortem examination of Case 3, the thymus was extremely small and lacked Hassall’s corpuscles and corticomedullary distinction, and there were no lymph nodes, follicles in the spleen, or gut-associated lymphoid tissue. Therefore, we have wondered where all of the peripheral blood lymphocytes were coming from.

Provost: Will preincubation of the peripheral lymphocytes from atopic dermatitis patients prior to mitogenic stimulation increase their in vitro responsiveness?

Buckley: We have not preincubated the cells but did wash them several times before resuspending them in media supplemented with AB-negative plasma from all male donors. The mitogens and antigens were added at time zero.