The Journal of Immunology

RESEARCH ARTICLE | APRIL 01 1980

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D L Schuster; ... et. al

J Immunol (1980) 124 (4): 1662–1667.
https://doi.org/10.4049/jimmunol.124.4.1662

0022-1767/80/1244-1662$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 124, No. 4, April 1980
Printed in U.S.A.

SELECTIVE DEFICIENCY OF A T CELL SUBPOPULATION IN ACTIVE ATOPIC DERMATITIS

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The T cell subpopulation bearing a receptor for the Fc fragment of IgM (Tμ) or the Fc fragment of IgG (Tγ) were studied in 12 patients with active atopic dermatitis (AD) and elevated IgE (5678 I.U./ml ± 1382) and compared with a control group with normal IgE (33 ± 17).

Values for the total lymphocytes and for absolute and relative numbers of total T and Tμ cells were comparable in both groups. However, AD patients had markedly reduced relative Tγ levels, 1.8% ± 0.4%, compared with normals, 10.5% ± 0.7% ($p<0.001$). The patients also had reduced absolute Tγ levels, 29 ± 7, compared with 181 ± 36 ($p<0.0005$). This abnormality was not indicative of allergic disease in general, nor did it appear to be caused directly by serum factors. The Tγ cell deficit did reflect disease activity and correlated inversely with the levels of serum IgE in the AD patients. Thus, patients with active AD and elevated IgE have a selective reduction in circulating Tγ cells.

Patients with atopic dermatitis (AD)[2] demonstrate a variety of immunologic defects. *In vivo* and *in vitro* cell-mediated immune responses are frequently impaired (1–7). Circulating levels of thymus-dependent cells (T cells) have been reported to be markedly depressed by some (1, 2, 6, 8–11) but normal by others (12–15). In addition, impaired cell-mediated immunity is often accompanied by elevated serum levels of IgE (1–3, 5, 16, 17).

Although the relationship between these immunologic abnormalities is unclear, experimental animal studies suggest that hyperimmunoglobulinemia E may occur secondarily to a defect in regulatory T cell function (18–27).

In man, immunoregulatory sets of T cells have been identified by virtue of the Fc receptor for immunoglobulin that they express (28–30). T cells bearing a receptor for the Fc fragment of IgM (Tμ) have been shown in certain *in vitro* assays to function as helper or suppressor cells (28, 29). By contrast, T cells bearing a receptor for the Fc fragment of IgG (Tγ) appear to express predominantly suppressor activity (29). In order to further characterize the nature of T cell dysfunction in AD, we now report an analysis of T cell subsets in the blood of patients with this disease.

MATERIALS AND METHODS

Subjects. The study populations consisted of 1) 12 patients with active AD, ranging in age from 5 to 40 years, and 2) five patients with inactive AD ranging in age from 9 to 35 years. Controls included 1) 12 healthy individuals ranging in age from 4 to 50 years, and 2) four patients with allergic rhinitis and/or asthma ranging in age from 13 to 40 years.

The patients had not been on oral corticosteroids for at least 1 year before the study. In addition, they had received no antihistamines or other systemic therapy for 48 to 72 hr and had utilized no topical corticosteroids for at least 2 weeks before the time of study.

The diagnosis of AD was based on the following two criteria: a) a focal or generalized maculopapular lichenified pruritic skin rash that showed a predilection for the flexural areas of the extremities and/or face and neck; b) a personal or family history of allergic disease.

IgE levels. IgE concentrations were measured in patient and control sera by a radioimmunoabsorbent assay (Phadebas, Pharmacia Laboratories, Piscataway, N. J.).

Isolation and enumeration of T lymphocytes. Peripheral blood mononuclear cells (PBM) were obtained from the blood of active AD, inactive AD, allergic rhinitis and/or asthmatic patients, and healthy volunteers, and numbers of T lymphocytes and Tμ and Tγ subpopulations were enumerated by previously described techniques (28). Briefly, PBM were separated on a Ficoll-Hypaque gradient (31). Adherent cells were depleted by incubation of PBM on plastic tissue culture plates at 37°C for 60 min. Relative numbers of T lymphocytes were determined by rosetting the nonadherent cells with neuraminidase-treated sheep erythrocytes (SRBC). Incubation mixtures were maintained at 37°C in a humidified 5% CO_2 incubator for 15 min and then centrifuged in the cold at 1000 rpm. After a 1-hr incubation at 4°C, the pellet was resuspended, and the suspension was transferred to a hematocytometer for enumeration of rosetting T cells. Rosette-forming cells were identified by at least three adherent SRBC.

Purified T cell fractions were obtained by rosetting nonadherent cells with neuraminidase-treated SRBC followed by centrifugation on a Ficoll-Hypaque density gradient.

This procedure routinely provided a suspension containing

Received for publication September 19, 1979.
Accepted for publication December 17, 1979.
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[2] Abbreviations used in this paper: AD, atopic dermatitis; T cells, thymus-dependent cells; Tμ, T cells bearing a receptor for the Fc fragment of IgM; Tγ, T cells bearing a receptor for the Fc fragment of IgG; PBM, peripheral blood mononuclear cells.

95 to 98% T lymphocytes. T lymphocytes were freed of SRBC by hypotonic lysis (32).

Preparation of ox-erythrocyte-anti-ox erythrocyte complexes. Rabbit anti-ox erythrocyte antiserum was prepared by repeated injection of 10% ox erythrocytes emulsified in Freund's complete adjuvant. The IgM-rich antiserum was prepared by injecting rabbits every 3 days for a total of three doses and bleeding 3 days after the last injection. To prepare IgG-rich antiserum, animals were injected weekly for a total of four injections and exsanguinated 7 days after the last dose. Antisera from three rabbits were pooled, dialyzed against 0.01 M Tris buffer, pH 8.0, and applied to a DEAE column equilibrated with 0.01 M Tris buffer, pH 8.0. The breakthrough peak was collected and pooled; pH was adjusted to 7.0 and concentrated by precipitation with 50% $(NH_4)_2$ SO_4. The precipitate was dissolved and exhaustively dialyzed in phosphate-buffered saline, pH 7.4. The purity of the fraction was determined by immunoelectrophoretic analysis with sheep anti-rabbit IgM, IgG, IgA (kindly provided by Dr. Rose Mage). Purified rabbit IgG and monospecific goat anti-rabbit IgG (both heavy chain specific) were used to establish identity with the isolated IgG fraction. IgM-rich antisera were pooled, and the globulin was precipitated with 33% $(NH_4)_2$ SO_4. The precipitate was dissolved and dialyzed against 0.05 M Tris buffer, pH 8.0, containing 0.15 M NaCl. The IgM fraction was obtained by separation of a number of small aliquots on a G-200 Sephadex column equilibrated with 0.05 M Tris buffer, pH 8.0, containing 0.15 M NaCl. The ascending portions of the breakthrough peak of each separation were concentrated in an Amicon filtration apparatus with UM 10 filter. The pooled material was rechromatographed, and the ascending portion of the breakthrough peak was collected, pooled, concentrated, and dialyzed against phosphate-buffered saline, pH 7.0. The purity of the fraction was established by immunoelectrophoresis with polyspecific and monospecific anti-rabbit immunoglobulins. Ox erythrocytes were washed three times and then mixed with purified IgM anti-ox (1:20) antibody or IgG anti-ox (1:20) antibody. After incubation at 37°C for 30 min, the coated ox erythrocytes were washed and resuspended to a concentration of 1% for subsequent identification of Tγ and Tμ cells.

Identification of Tγ and Tμ cells. To identify Tγ cells, 0.1 ml of 1×10^6 purified T lymphocytes suspended in RPMI 1640 media (Grand Island Biological Co.) supplemented with L-glutamine (2%), penicillin and streptomycin (final concentration 100 units/ml and 100 μg/ml, respectively), and heat-inactivated 10% FCS (v/v) (from a single lot, Microbiological Associates, Bethesda, Md.) were mixed with 0.1 ml of 1% ox erythrocytes coated with IgG rabbit anti-ox-erythrocyte antibody. The mixture was incubated at 37°C in a humidified 5% CO_2 incubator for 5 min and then centrifuged at 4°C at 1000 rpm. After a further incubation for 30 min at 37°C, the pellet was gently resuspended, and percentage of rosettes was counted. Lymphocytes were identified as Tγ cells. Values represent the means of duplicate determinations with a minimum of 200 cells counted.

To identify Tμ cells, an aliquot of purified T cells was cultured overnight in a humidified 5% CO_2 incubator at 37°C. The cells were resuspended at a concentration of 2×10^6/ml, and 0.1 ml was added to an equal volume of 1% ox erythrocytes coated with IgG rabbit anti-ox erythrocyte antibody. The mixture was incubated at 37°C for 15 min in a humidified 5% CO_2 incubator and then centrifuged at 4°C at 1000 RPM. After a further incubation on ice for 60 min, pelleted cells were gently resuspended, and the percentage of rosettes was determined.

Assays for immune complexes. Immune complexes were kindly performed on sera from our patients by Drs. Thomas Lawley and Russell Hall, National Institutes of Health. Immune complex determinations were made by a modification of the radiolabeled C1q assay (33) and the Raji cell assay (34).

Mechanism of reduced T levels. Purified T cells at a concentration of 10×10^6 cells/ml were cultured overnight in RPMI 1640 supplemented with 10% heat-inactivated FCS (v/v), penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2%) at 37°C in an attempt to elute any serum factors bound to the cells. After incubation, Tγ cell numbers were determined as described above.

In additional experiments, purified T cells from normal individuals were cultured for 1 hr at 37°C in the presence of sera from active AD patients containing high amounts of IgE. After this 1-hr incubation, Tγ cells were again enumerated as previously described.

Statistical evaluation of data. Group data were analyzed by using Student's *t*-test.

RESULTS

IgE levels. Patients with active AD had mean ± S.E.M. serum IgE concentrations of 5678 ± 1382 I.U./ml with a range of 350 to 14,800 I.U./ml. Patients with inactive AD had IgE levels of 291 ± 141 with a range of 22 to 500 I.U./ml. Normal subjects had mean IgE concentration of 33 ± 17 I.U./ml with a range of <0.5 to 121 I.U./ml, whereas the atopic control group without AD had mean IgE levels of 1263 ± 974 I.U./ml with a range of 110 to 3200 I.U./ml.

Characterization of lymphocyte populations in AD. Absolute numbers of lymphocytes were comparable in the patient and normal control group, ($p>0.1$). Also, both groups had comparable relative and absolute numbers of T lymphocytes ($p>0.4$) (Fig. 1). Analysis of T cell subpopulations revealed similar values for relative and absolute numbers of Tμ cells, ($p>0.1$) (Fig. 2). However, active AD patients had markedly depressed

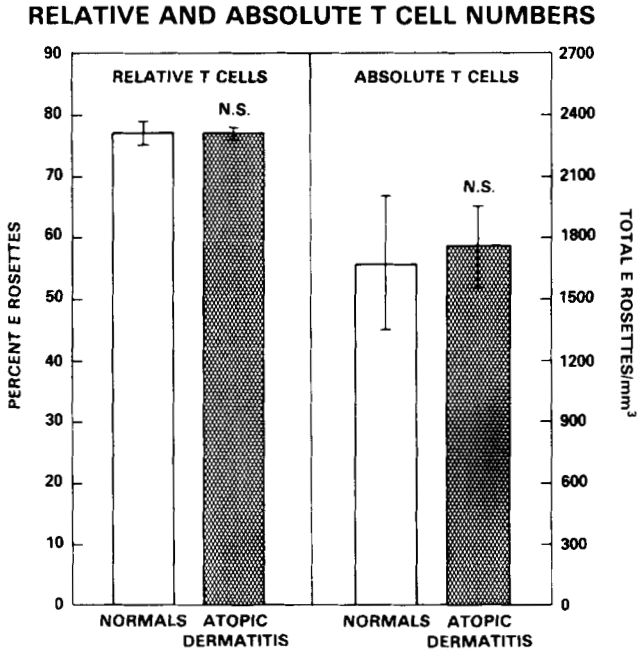

Figure 1. Comparison of relative and absolute numbers of cells forming rosettes with SRBC in 12 patients with AD and 12 normal individuals. Data expressed as mean ± S.E.M. The differences are not statistically significant ($p>0.4$).

relative and absolute numbers of Tγ cells compared with normal subjects ($p<0.0005$) (Fig. 3).

To determine whether decreased levels of Tγ cells were related to the activity of AD and/or the atopic state in general, Tγ cells were determined in patients with inactive AD and patients with allergic rhinitis and/or asthma but not AD. There was no significant decrease in the relative or absolute numbers of Tγ cells in either inactive AD patients or in patients with allergic rhinitis and/or asthma compared with normals ($p>0.1$) (Fig. 4). Of further interest, sequential studies in one patient

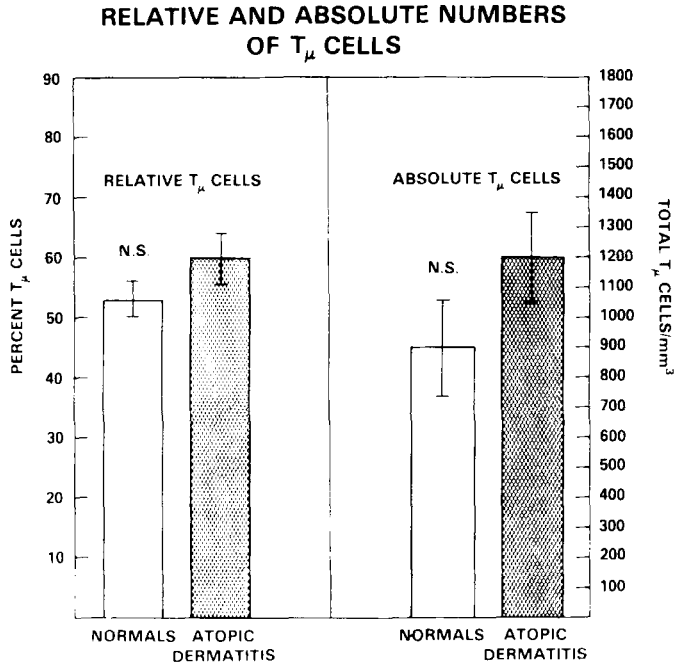

Figure 2. Comparison of relative and absolute numbers of Tμ cells in 12 patients with AD and 12 normal individuals. Data expressed as mean ± S.E.M. The differences are not statistically significant ($p>0.1$).

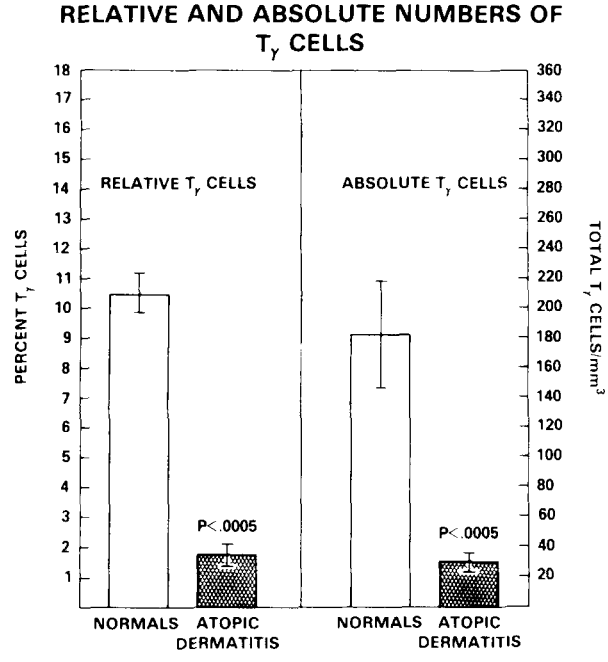

Figure 3. Comparison of relative and absolute numbers of T cells in 12 patients with AD and 12 normal individuals. Data expressed as mean ± S.E.M. The differences are statistically significant ($p<0.0005$).

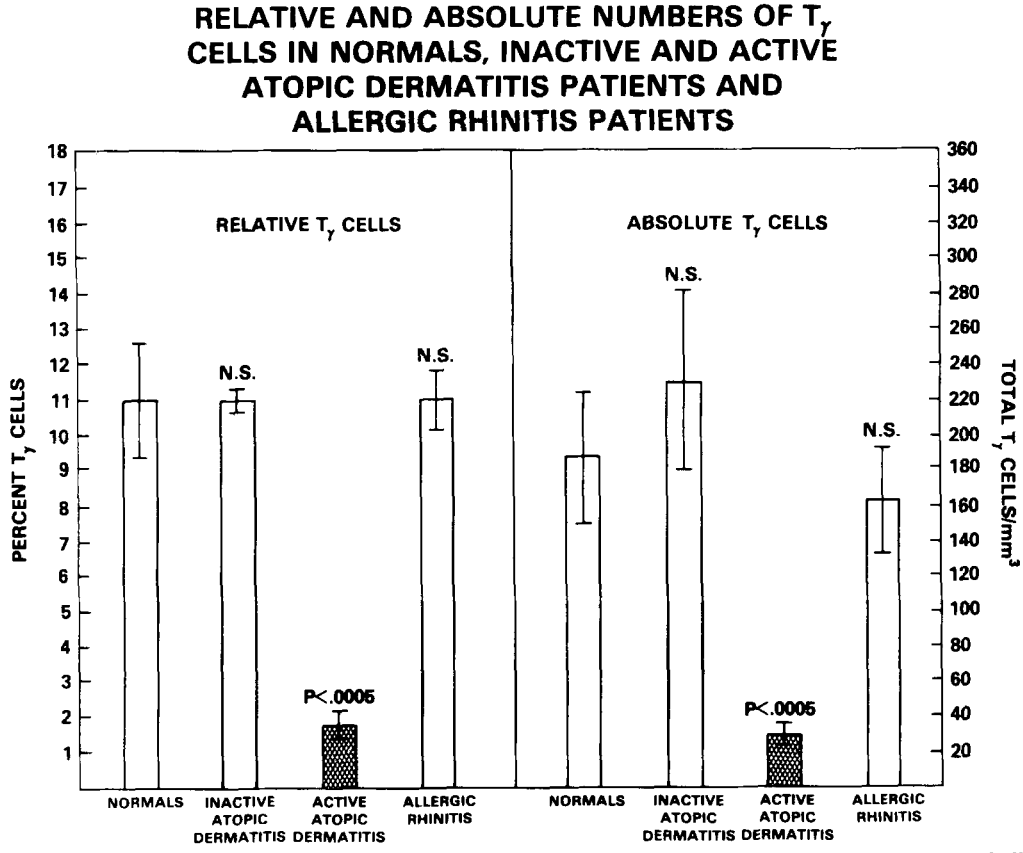

Figure 4. Comparison of the relative and absolute numbers of Tγ cells in normals, inactive, and active AD patients and allergic rhinitis, and/or asthmatic patients. Data expressed as mean ± S.E.M. The differences between either inactive AD patients or patients with allergic rhinitis and/or asthma, and normal controls are not statistically significant ($p>0.1$).

with active AD showed normalization of Tγ levels from 3 to 9% together with clinical improvement (Fig. 5).

The relationship between IgE and the percentage of Tγ cells in normal, active AD, and allergic rhinitis and/or asthmatic patients is shown in Figure 5. Twelve of the 12 patients with active AD had elevated IgE levels with decreased numbers of Tγ cells. However, one patient with active AD and decreased Tγ cells displayed a relatively low IgE level (320 I.U./ml), and another patient with allergic rhinitis and/or asthma was noted to have a normal percentage of Tγ cells even though she had significantly elevated IgE (3200 I.U./ml).

Mechanism of reduced Tγ levels. Studies were carried out to determine whether factors present in AD serum were responsible for depressed Tγ levels. First, circulating immune complexes were probably not involved, since significant amounts of immune complexes were found in only two patients with AD. Second, other serum factors that might have blocked expression of Fc receptor for IgG on Tγ cells after adsorption *in vivo* were also unlikely mediaries. Similar values of Tγ cells (p>0.4) (Fig. 6) were observed in aliquots of freshly isolated T cells and of T cells cultured overnight. Serum factors adsorbed to the T cell

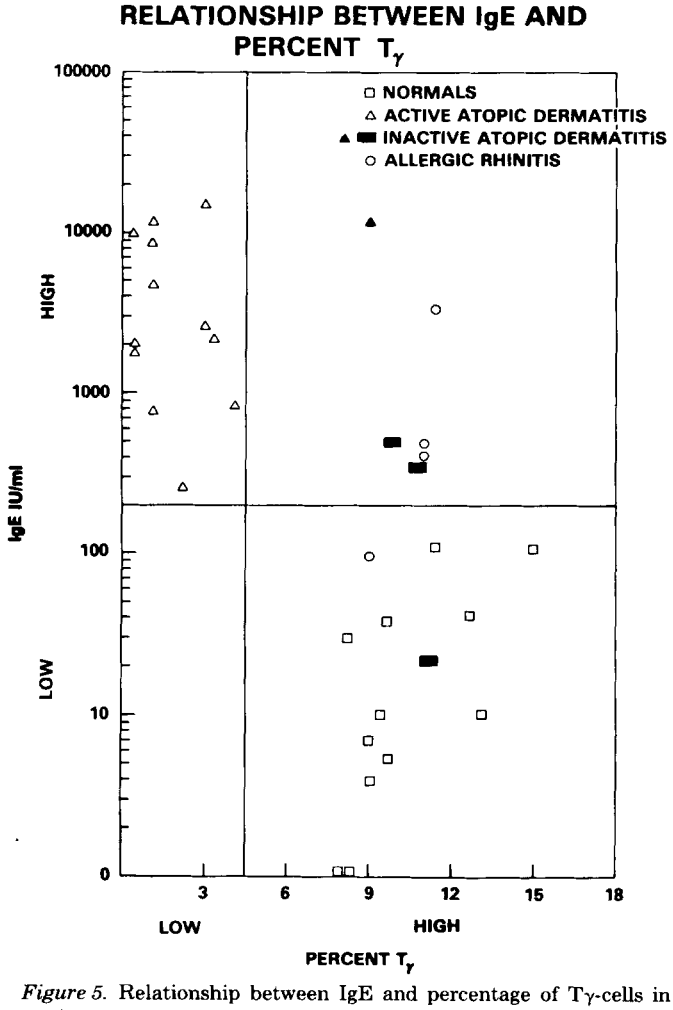

Figure 5. Relationship between IgE and percentage of Tγ-cells in normal, active and inactive AD, and allergic and/or asthmatic patients. *Y axis* represents IgE level (I.U./ml) and X axis represents percentage of Tγ cells. Twelve of the 12 patients with active AD had elevated IgE levels with decreased number of Tγ cells. One patient with active AD and decreased Tγ cells displayed a *relatively* low IgE level (320 I.U./ml), and another patient with allergic rhinitis displayed normal percentage of Tγ cells with a significantly elevated IgE (3200 I.U./ml). One patient with active AD showed normalization of Tγ levels from 3 to 9% together with clinical improvement.

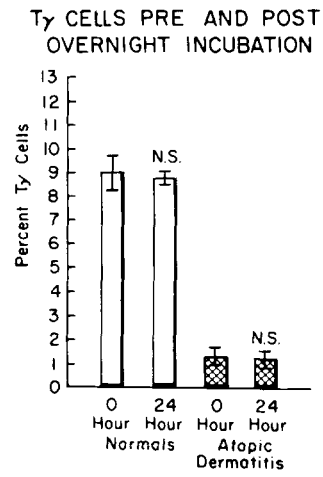

Figure 6. Purified T cells from active AD patients and normal individuals were cultured overnight at 37°C (5% CO_2). Data expressed as mean ± S.E.M. There was no significant change in numbers of Tγ cells in either group. (p>0.1 and p>0.1, respectively.)

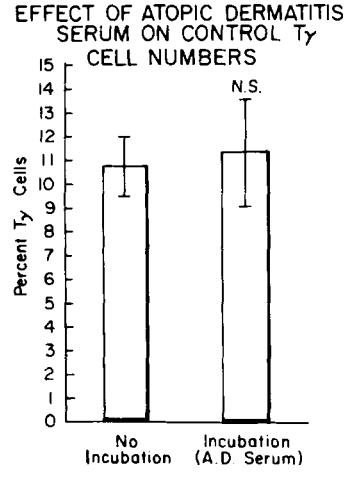

Figure 7. Purified T cells from normal individuals were cultured for 1 h at 37°C in the presence of sera from active AD patients with elevated IgE. Data expressed as mean ± S.E.M. No significant decrease in the normal number of T cells was noted after incubation. (p>0.1.)

membrane would be expected to be eluted under the culture conditions used. In addition, no significant decrease in the number of Tγ cells was noted (p>0.4) when aliquots of normal T cells were incubated with serum from active AD subjects with elevated IgE (Fig. 7).

DISCUSSION

Results of this study are highlighted by the finding of a selective deficiency of Tγ cells in patients with active AD. All 12 patients with active AD studied had markedly reduced values of Tγ cells in blood, whereas numbers of total T cells as well as Tμ cells were normal. The finding of normal total T cell numbers is consistent with reports of some groups (12–15) but not others (1, 2, 6, 8–11). This discrepancy may relate to differences in patient populations studied and methodology employed.

A number of studies were carried out to elucidate the mechanism of Tγ cell deficiency. This abnormality is probably not indicative of allergic disease in general, since Tγ cell deficiency was not found in patients with allergic rhinitis and/or asthma without AD. However, the Tγ cell deficit may correlate with

the activity of the AD, since five patients studied with inactive AD had normal levels of Tγ cells. In addition, as mentioned previously, one of our patients studied sequentially showed normalization of Tγ cell level together with clinical improvement despite maintaining high serum IgE. Serum factors, including immune complexes and increased amounts of IgE, are unlikely as primary mediators of the deficit, since 1) circulating immune complexes were found in only two patients with active AD, 2) Tγ cell number determinations in active AD remained depressed after overnight culture, and 3) AD serum with increased IgE did not inhibit expression of Fc receptors for IgG on normal T cells. In light of the paucity of evidence for circulating immune complexes in these patients, it is unlikely that the depressed Tγ cell levels reflect their conversion to Tμ cells (35).

In general, reduced levels of Tγ cells were associated with high levels of IgE in our active AD patients. In light of a growing body of animal experiments (18–27), it is tempting to speculate that these abnormalities are related in a cause-and-effect fashion. IgE levels ultimately reflect the modulating effect of helper and suppressor T cells (18–27), and Tγ cells subserve suppressor activity in man (29). However, some valid objections temper our willingness to accept this interpretation. First, a role for Tγ cells in the regulations of IgE production in man has yet to be established, although they do appear to suppress other classes of immunoglobulins (29). Second, the dramatic reduction in Tγ cells observed here would be expected to be accompanied by elevations of other classes of immunoglobulins as well. Thus, the basis of the relationship between decreased T cells and increased levels of IgE found in active AD is not clear at this time. We are currently studying our active AD patients sequentially as well as other patients with elevated IgE without AD. We are hopeful, with our studies currently under investigation as well as with the development of sensitive assays for measuring *in vitro* synthesis of IgE (36, 37), that we will gain further insight into the regulation of immune mechanism in AD.

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