

Detection of decreased cytotoxic effector CD8+ T lymphocytes in atopic dermatitis by flow cytometry

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Atopic dermatitis (AD) is a common dermatologic disease of unknown etiology. Patients are admitted with a chronically recurring inflammation of the dermis and epidermis characterized by severe pruritus.^{1, 2} Studies to date have demonstrated immune anomalies associated with AD. A frequently reported observation in patients with AD is a reduction in the peripheral blood of CD8+ T lymphocytes,² whereas the percentage of CD4+ cells are generally not altered, and altered CD4/CD8 ratios are usually credited to the reduction in CD8+ cells. A previous report by Leung et al.³ has demonstrated that a decrease in peripheral blood CD8+ lymphocytes is associated with diminished in vitro cytotoxic activity. Unfortunately, the distinction between reduced cytolytic activity caused by decreased numbers of cytotoxic effector cells versus impaired cytolytic abilities of the cells could not be made. Recently a monoclonal antibody (designated S6F1) has been produced that can distinguish between cytotoxic effector and suppressor effector CD8+ lymphocyte subsets.⁴ This antibody recognizes a novel epitope of the LFA-1 antigen, which is located on the surface membrane of CD8+ cells. CD8+, S6F1^{bright} cells exhibit cytotoxic activity, whereas CD8+, S6F1^{dim} lymphocytes manifest suppressor function.

In the current study we used flow cytometric immunophenotyping to study lymphocyte subsets, particularly CD8+ subsets defined by the S6F1 antibody and CD4 subsets defined by CD29, in peripheral blood of adult patients with AD. These subsets were examined to better define the immune lesions in AD and

to correlate this immunophenotyping with severity of disease.

METHODS AND MATERIAL

Patient selection and severity scoring

Twenty-nine adult (>18 years old) patients with active, moderate to severe AD were clinically evaluated. All satisfied the diagnostic criteria for AD and had a history of chronic AD ranging from 0.5 to 59.1 years.¹ At the time of evaluation each patient was being treated with topical triamcinolone acetone cream 0.1% and an oral H₁ antihistamine. The extent of an individual patient's AD was determined by establishing the AD severity index (ADSI). The ADSI was calculated by multiplying the percentage of body surface involvement, by use of the scale of nines,⁵ by the sum of the severity scores for pruritus, erythema, and induration with a 0 to 3 scale (0, none; 1, mild; 2, moderate; 3, severe) times 100. All patients were evaluated by the same investigator (M.D.T.). Patients with ADSI scores greater than 300 were empirically defined as having severe disease. Before participating in the study, patients were informed of the nature of the study according to the guidelines of the Institutional Review Board for Biomedical Research at the University of Pittsburgh.

Collection and preparation of peripheral blood

Peripheral blood was obtained by aseptic venipuncture from 29 patients with AD and 26 healthy volunteers and analyzed for serum IgE antibody levels, absolute leukocyte counts with three-part differentials, and lymphoid subsets by immunophenotyping. The peripheral blood specimens were stained for immunophenotyping by use of a standard whole blood preparation method. Monoclonal antibodies, directly labeled with fluorochromes, were added to the test tubes as per manufacturers' instructions. Antibodies used in this study were directly conjugated antibodies. The S6F1 monoclonal antibody was obtained from Coulter Immunology (Hialeah, Fla.). Stained samples were analyzed with use of either a FACStar or FACStar-plus flow cytometer (Becton Dickinson, San Jose, Calif.) equipped with either the Consort 30 or Consort 40 (Becton Dickinson, San Jose, Calif.) data acquisition and analysis systems. CD8+ /S6F1-bright and dim were identified by observations of clustered staining on healthy controls as reported in the original description of this antibody.⁴

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Statistical analyses

Student's *t* tests were performed to compare the immunophenotyping data from the patients with severe AD with either the patients with moderate AD or controls, and to compare the patients with moderate AD with controls. The Student's *t* tests were performed with StatWorks Software (Cricket Software, Philadelphia, Pa.) on a MacIntosh IIe (Apple Computer, Inc., Cupertino, Calif.) computer. A one-way analysis of variance (ANOVA) was performed to determine any differences among the groups for CD8+ /S6F1^{bright} staining.

RESULTS

Eleven of the 29 patients with AD were classified as having severe disease, whereas 18 were considered to have moderate skin disease. Although both groups of patients with AD demonstrated similar total leukocyte counts ($X = 7000 \pm 2756$ for the severe group and $X = 7033 \pm 2100$ for the moderate group), the patients with severe AD demonstrated a statistically significant decrease ($X = 1429 \pm 394$) in absolute lymphocyte count in comparison with patients with moderate disease ($X = 1944 \pm 601$) and healthy controls (2180 ± 740) ($p = 0.002$). No statistically significant changes in the number of neutrophils or monocytes were found in the groups with AD in comparison with each other or controls (data not shown).

Analysis of lymphocyte subsets in the peripheral blood revealed no statistically significant alterations in the percentage of CD3+ (T-cell receptor+) or CD19+ (pan B cell) cells in the groups with severe AD or moderate AD in comparison with each other or with healthy control values. Patients who had severe AD but not moderate AD had a statistically significant ($p < 0.01$) increase in the percentage of circulating CD4+ lymphocytes when they were compared with controls (49.3% in patients with severe AD vs 40.9% in controls). No statistically significant difference in CD4+ cells was observed between the two AD groups (49.3% in the group with severe AD vs 45.5% in the group with moderate AD). An analysis of CD4 subsets by use of the CD29 (4B4) antibody revealed no statistically significant differences among the groups with AD in comparison with each other or in comparison with the control group. Thus the increase in CD4+ lymphocytes that was observed in the patients with severe AD was not associated with a selective increase in helper-inducer (CD29+) cells.

A statistically significant decrease in the percentage of CD8+ (suppressor/cytotoxic) lymphocytes was observed in both the patients with moderate AD and those with severe AD in comparison with the healthy controls ($p < 0.01$ in each instance), but no signifi-

cant difference was observed between the groups with moderate and severe AD. Further evaluation of the CD8+ cells with use of the S6F1 monoclonal antibody revealed two populations of CD8+ cells—S6F1^{bright} and S6F1^{dim} cells (Fig. 1, A). No significant differences were seen in the percentage of CD8+ /S6F1^{dim} cells among the population with AD and controls. A one-way ANOVA comparing the percentage of CD8+ /S6F1^{bright} cells among the three groups yielded $0.01 < p < 0.05$. By use of the Student's *t* test, a statistically significant decrease in CD8+ /S6F1^{bright} cells was observed in the patients with severe AD in comparison with either the group with moderate AD ($p < 0.02$) or the healthy controls ($p < 0.05$) (Fig. 1, B). No correlation was observed between the percentage of CD8+ /S6F1^{bright} cells and either IgE levels or incidence of infections.

The decreased absolute lymphocyte count in severe AD in comparison with moderate AD yields a net decrease in the absolute number of all of the lymphocyte subsets examined. The most pronounced decrease was found among the CD8+ /S6F1+ cells.

DISCUSSION

Numerous studies have documented a decrease in CD8+ lymphocytes associated with AD. Leung et al.³ demonstrated a decrease in *in vitro* cytolytic activity of CD8+ cells, but they could not distinguish whether this diminished activity was due to a decrease in the number of cytotoxic effector CD8+ cells or impaired function of normal numbers of these cells. In the current study we have extended the findings of these investigators by using a recently defined monoclonal antibody (S6F1) that distinguishes between cytotoxic effector CD8+ cells and suppressor inducer CD8+ cells. Our flow cytometric analyses of CD8 and S6F1 have demonstrated that patients with severe AD have a statistically significant decrease in the percentage and absolute number of CD8+ /S6F1^{bright} cells. This would strongly suggest that the decrease in cytolytic activity in patients with AD observed by Leung et al.³ was due in large part to reduced numbers of cytotoxic effector cells. It is also possible that the remaining CD8 cytotoxic effector cells in these patients are not fully functional. The current observation, in conjunction with the observation of Leung et al.,³ fits well with the hypothesis that increased incidence of bacterial and viral infections observed in severe AD is linked to impaired immune responses in these patients. We propose that examining cytotoxic effector CD8+ lymphocytes in patients with AD may be of use in monitoring susceptibility to infection and in assessing response to therapy.

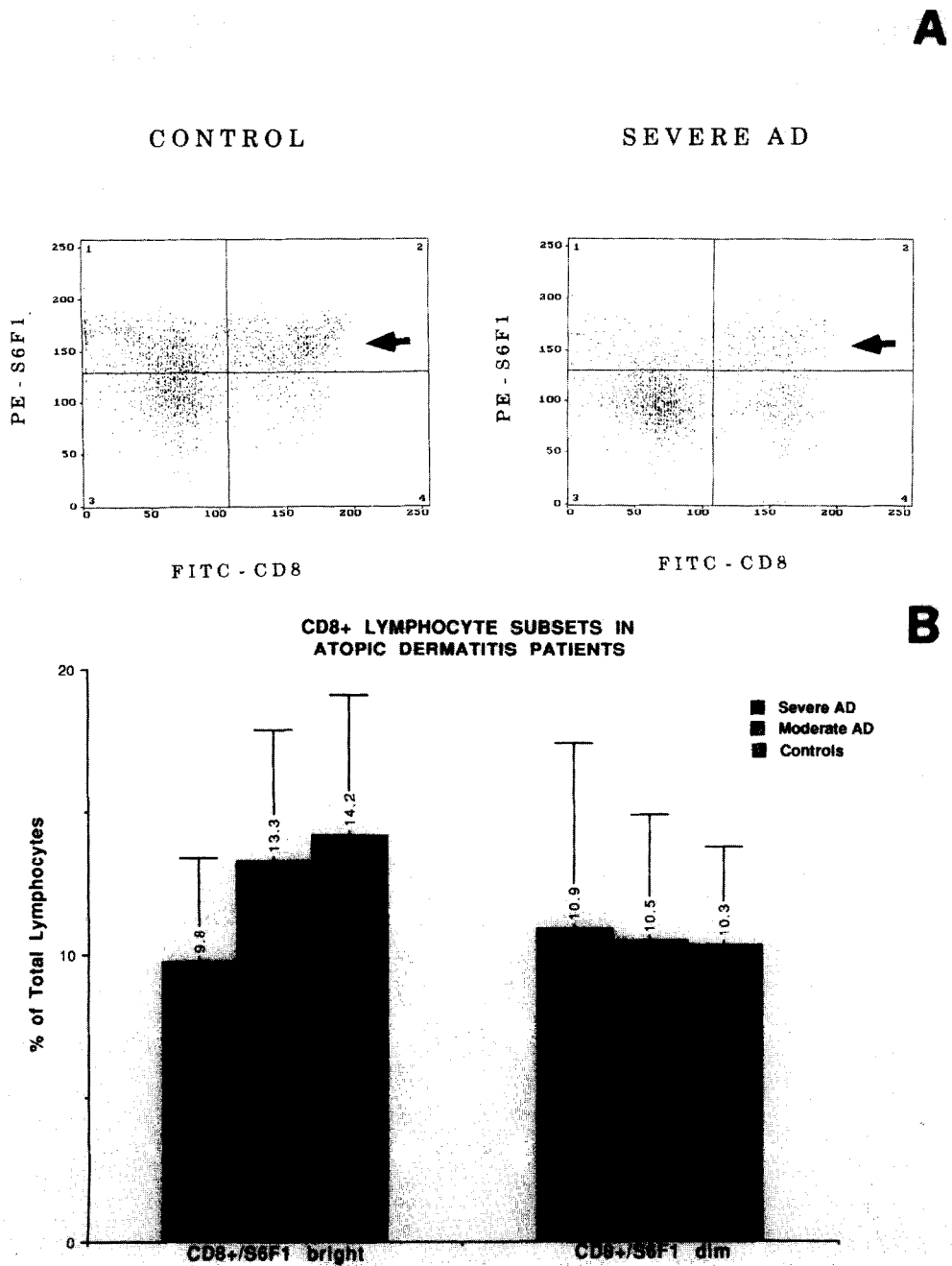


FIG. 1. A, Examples of flow cytometric analyses of peripheral blood stained with CD8 and S6F1 monoclonal antibodies. *Left panel,* blood from a healthy control, *right panel,* blood from a patient with severe AD. CD8+ /S6F1^{bright} cells indicated by *arrow* in each histogram. Percentage of CD8+ /S6F1^{bright} cells (quadrant 2—upper right quadrant in each histogram) are 25.8% (*left*) and 12.5% (*right*). **B,** Mean percentages of CD8+ lymphocyte subsets defined by S6F1, obtained by immunophenotyping of patients with AD (11 severe, 18 mild) and control individuals (26 individuals); standard deviations indicated by *bars*.

REFERENCES

1. Hanifin JM. Atopic dermatitis. *J Am Acad Dermatol* 1982;236:1-13.
2. Leung DYM, Rhodes AR, Geha RS. Atopic dermatitis. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al., eds. *Dermatology and general medicine*. New York: McGraw Hill, 1986:1385-408.
3. Leung DYM, Wood N, Dubey D, Rhodes AR, Geha RS. Cellular basis of defective cell-mediated lympholysis in atopic dermatitis. *J Immunol* 1983;130:1678-82.
4. Morimoto C, Rudd CE, Letvin NL, Schlossman SF. A novel epitope of the LFA-1 antigen which can distinguish killer effector and suppressor cells in human CD8 cells. *Nature* 1987;330:479-82.
5. Evans EI. Fluids and electrolyte requirements in severe burns. *Am J Surg* 1952;135:804.