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T-cell Subsets in Patients with Mild and Severe Atopic Dermatitis

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Mononuclear cells separated from peripheral blood from 34 patients with mild and 19 patients with severe atopic dermatitis, and 24 controls, were stained with OKT3, OKT4 and OKT8, using immunofluorescence technique and counted. In patients with severe atopic dermatitis a highly significant decrease in OKT3, OKT4- and OKT8-positive lymphocytes was found, as compared with controls, as well as an increased OKT4/OKT8 ratio. Patients with mild atopic dermatitis demonstrated a significant decrease in OKT3-positive lymphocytes, whereas no statistically significant difference was found for OKT4, OKT8 or the OKT4/OKT8 ratio. These observations indicate that changes in T cell subpopulations in peripheral blood from patients with atopic dermatitis are related to disease severity.

The impaired cell-mediated immunity in atopic dermatitis, with the increased susceptibility to infections and the high IgE values found in most of these patients, have led many groups to concentrate on T lymphocytes. Over the past 10 years, with the development of immunological techniques, a number of publications have dealt with T lymphocytes in patients with atopic dermatitis (1, 2, 3, 4, 5, 6). Although there have been some conflicting results, most groups found fewer T cells in peripheral blood, as counted by the E-rosetting method. The dermal perivascular infiltrate consists predominantly of T lymphocytes (2).

The development of new monoclonal antisera directed against T-cell subpopulations has enabled us to examine these subpopulations more closely and in recent publications a decreased number of OKT8-positive T cells and an increased T4/T8 ratio have been demonstrated (7, 8, 9, 10, 11).

In order to investigate whether the observed changes in T cell subpopulations are related to disease severity, we have counted OKT3-, OKT4- and OKT8-positive lymphocytes in Lymphoprep-isolated mononuclear cells from peripheral blood of patients with mild and with the severest atopic dermatitis and compared them with an age-matched group of healthy non-atopic controls.

MATERIALS AND METHODS

Patient material

Thirty-four patients, mean age 27 years (range 8–54) with mild, and 19 patients, mean age 25 years (range 9–53) with severe atopic dermatitis were included. Twenty-four healthy non-atopic individuals, mean age 30 years (range 10–51) served as controls. None had received systemic steroid therapy during the last 2 months. Antihistamines and weak topical steroids were used regularly by most of the patients.

Separation of peripheral blood mononuclear cells (PBM)

Cells were separated by the Ficoll-Isopaque method from defibrinated blood by means of Lymphoprep (Nyegaard & Co., Oslo, Norway).

Staining of cells with OKT3, OKT4 and OKT8 antisera

PBM were incubated with the various monoclonal antibodies at 4°C for 30 min and then, after two washes, incubated at 4°C another 30 min with fluorescein-conjugated rabbit anti-mouse antibody

(DAKO). To prevent capping, 0.3% sodium azide was added during the incubations. Cells were also incubated at 4°C for 30 min with only fluorescein-conjugated rabbit anti-mouse antibody. Counting was performed using a fluorescence microscope, and more than 200 cells were counted in each preparation.

Statistical analysis

Student's t-test was used.

RESULTS

Staining with only fluorescein-conjugated rabbit anti-mouse antibody demonstrated less than 1% positive cells. Table I shows the mean percentages of peripheral blood mononuclear cells staining with OKT3. Patients with both mild and severe atopic dermatitis demonstrated a decreased percentage, which was statistically significant, $p<0.001$.

Table II gives the mean percentages for staining with OKT4. Severe atopic dermatitis patients demonstrated a significant decrease ($p<0.001$) vis-à-vis controls, but there was no difference between controls and mild atopic dermatitis patients.

In Table III it can be seen that severe atopic dermatitis patients demonstrated a significantly decreased mean percentage ($p<0.001$) of OKT8-positive cells as compared with controls, while there was no difference between patients with mild atopic dermatitis and controls.

The mean OKT4/OKT8 ratios are shown in Table IV. Patients with severe atopic dermatitis demonstrated an increased ratio ($p<0.001$) as compared with controls. No difference was found between patients with mild atopic dermatitis and controls.

DISCUSSION

The present study demonstrates that changes in T-cell subpopulations in peripheral blood from patients with atopic dermatitis are related to disease severity. In patients with mild atopic dermatitis a significantly decreased mean percentage of 64% OKT3-positive T cells was found, versus controls 69%. The decrease was more profound, however, in patients with severe atopic dermatitis, with 49% OKT3-positive T cells. The other T-cell subpopulations and the T4/T8 ratio did not differ between controls and patients with mild atopic dermatitis, while the patients with severe atopic dermatitis demonstrated significant differences versus controls.

Table I. *Percentage of peripheral blood lymphocytes staining with OKT3*

	Mild atopic dermatitis		Controls		Severe atopic dermatitis
Mean±SD	60.63±9.45		69.41±7.66		48.89±5.4
Test for difference		$p<0.001$		$p<0.001$	

Table II. *Percentage of peripheral blood lymphocytes staining with OKT4*

	Mild atopic dermatitis		Controls		Severe atopic dermatitis
Mean±SD	47.12±6.76		49.34±7.47		35.23±4.25
Test for difference		$p>0.1$		$p<0.001$	

Table III. *Percentage of peripheral blood lymphocytes staining with OKT8*

	Mild atopic dermatitis		Controls		Severe atopic dermatitis
Mean±SD	32.46±3.41		34.85±5.72		17.19±3.15
Test for difference		$0.1>p>0.05$		$p<0.001$	

Table IV. *Ratio T4/T8*

	Mild atopic dermatitis		Controls		Severe atopic dermatitis
Mean±SD	1.45±0.24		1.46±0.31		2.05±0.45
Test for difference		$p>0.5$		$p<0.001$	

We tried to perform the study with patients who had been off antihistamines and topical steroids for at least 72 h, but despite promises, the patients admitted using antihistamines and group 2 topical steroids. It can therefore not be entirely excluded that the observed changes in the T cell subpopulations may be caused in part by topical steroids being absorbed through the skin.

It is tempting to speculate that the impaired cell-mediated immunity observed in atopic dermatitis is caused by the observed changes in T cell supopulations, i.e. that the changes are of etiological importance; but they may also very well be secondary phenomena. An attractive hypothesis would be that a decreased number of OKT8-positive T cells secretes too few suppressor molecules upon antigen stimulation, which then leads to the high IgE production in many, but not all, patients with atopic dermatitis.

We did not, however, find any correlation between a low percentage of OKT8-positive T cells and high IgE values (data not shown). Others have also been unable to show such a correlation (7).

There is now firm evidence indicating that additional functional heterogeneities exist within the OKT4- and OKT8-positive subsets. OKT8-positive cells may be subdivided further into cytotoxic and suppressor T-cell subpopulations and OKT4-positive cells into helper, inducer, and killer subpopulations (12). At T-cell expressing OKT4 or OKT8 markers could possess one of several functions associated with the two subsets. To assume that OKT8-positive cells are suppressor T cells might be incorrect—they may well be killer T cells against class I HLA antigen-bearing cells. Likewise, OKT4-positive cells may not be helper T cells, since they could be killer T cells against class II HLA antigen-bearing cells.

In conclusion, delineation of T cell subsets by monoclonal antibodies is an important means for the study of cellular changes in patients with atopic dermatitis, and the use of future monoclonal antibodies will undoubtedly give new insight into cellular changes in this intriguing disease.

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REFERENCES

1. Braathen LR, Førre Ø, Natvig JB, Rajka G. Lymphocyte subpopulations, serum immunoglobulins and complement factors in patients with atopic dermatitis. Br J Dermatol 1978; 98: 521.
2. Braathen LR, Førre Ø, Natvig JB, Eeg Larsen T. Predominance of T lymphocytes in the dermal infiltrate of atopic dermatitis. Br J Dermatol 1979; 100: 521.
3. Luckasen JR, Sabad A, Goltz RW, Kersey JH. T and B lymphocytes in atopic eczema. Arch Dermatol 1974; 110: 375.
4. Carapeto FJ, Winkelmann RK, Jordon RE. T and B lymphocytes in contact and atopic dermatitis. Arch Dermatol 1976; 112: 1095.
5. Strannegård IL, Lindholm L, Strannegård Ø. T lymphocytes in atopic children. Int Arch Allergy Appl Immunol 1976; 50: 684.
6. Thestrup-Pedersen K, Ellegaard J, Thulin H, Zachariae H. PPD and mitogen responsiveness of lymphocytes from patients with atopic dermatitis. Clin Exp Immunol 1977; 27: 118.
7. Zachary CB, MacDonald DM. Quantitative analysis of T-lymphocyte subsets in atopic eczema, using monoclonal antibodies and flow cytofluorimetri. Br J Dermatol 1983; 108: 411.
8. Kang K, Cooper KD, Vanderbark A, Strannegård I-L, Strannegård Ø, Hanifin JM. Immunoregulation in atopic dermatitis: T-lymphocyte subsets defined by monoclonal antibodies. Semin Dermatol 1983; 2: 20.
9. Chandra RK, Baker M. Numerical and functional deficiency of suppressor T cells precedes development of atopic eczema. Lancet 1983; ii: 1393.
10. Faure MR, Gaucherand MA, Thivolet J, Czernielewski JM, Nicolas JF. Decreased levels of T-cells and cells with suppressor T-cell phenotype as defined by specific monoclonal antibodies in patients with atopic dermatitis. Clin Exp Dermatol 1982; 7: 513.
11. De Maubeuge J, Delespesse G, Achten G, Servais G, De Vetter L, Gaillaert N. Détermination des sous-populations de lymphocytes circulants dans la dermatite atopique a l'aide d'anticorps monoclonaux. Dermatologica 1984; 169: 6.
12. Kung PC, Berger CL, Estabrook A, Edelson R. Monoclonal antibodies for clinical investigation of human T lymphocytes. Int J Dermatol 1983: 22: 67.

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