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E-ROSETTE FORMATION OF LYMPHOCYTES FROM PATIENTS WITH ATOPIC DERMATITIS

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Abstract. The total number of circulating T lymphocytes in 47 patients with atopic dermatitis was significantly lower than in 98 healthy persons. However, when the T lymphocytes were investigated for their affinity to sheep erythrocytes (E-rosette formation), no difference was found between patients and healthy persons, when using several different techniques varying in length of incubation of sheep erythrocytes and lymphocytes, presence of fetal calf serum, glassware, media, increased mechanical force at resuspension, and different observers. Our findings are discussed with reference to earlier publications of a reduced percentage of E-rosette forming lymphocytes in patients with atopic dermatitis.

Key words: E-rosettes; Atopic dermatitis; T lymphocytes

Many investigators have found that patients with atopic dermatitis have a reduced percentage of T lymphocytes in peripheral blood (for a review, see ref. 1). During a study on subpopulations of T lymphocytes in blood from patients with atopic dermatitis (2), we used four different techniques for the discovery of lymphocytes, which binds sheep erythrocytes (T lymphocytes). We found that the affinity of T lymphocytes from atopic patients towards sheep erythrocytes was similar as found with normal lymphocytes, even when several different rosette assays were used. However, the patients had a slight, but significant T lymphopenia.

PATIENTS AND METHODS

First, 47 patients (22 men and 25 women) with atopic dermatitis were studied. Their ages ranged between 5 and 41 years, mean 26 years. The control group consisted of 98 healthy persons, age range 16 to 60 years, mean 29 years. Most of the patients had increased levels of IgE in serum.

In the following studies on the various technical aspects of the E-rosette assay, a total of 18 patients (4 men and 14 women) and 15 healthy persons (12 men and 3 women) participated. The age range was 9 to 41 (mean 25 years), and 14 to 40 (mean 27 years). All patients had increased IgE in serum, range 535–9450 IU/ml (mean 4340).

Isolation of mononuclear cells

Lymphocytes from heparinized blood were isolated by using Ficoll-Hypaque gradients. Phagocytic cells were removed using either carbonyl iron incubation before separation or incubation of the mononuclear cells in plastic culture flasks. The number of phagocytic cells was reduced to around 1–3%. After separation the lymphocytes were washed three times in Hanks' balanced salt solution (HBSS) with 2% fetal calf serum (FCS) and adjusted to appropriate concentrations.

In the second part of the study the cell washings were performed without any serum added. Also, all rosette studies only employed the E-4 technique (see below). In all tests two observers each read 200 lymphocytes in individual test tubes. There was no significant difference between their results, which are therefore presented as the mean value of the two individual readings.

Enumeration of T lymphocytes

Sheep erythrocytes form rosettes with T lymphocytes under appropriate conditions. We used either AET-treated erythrocytes (E-AET; 2 amino-ethyl-isothio-uronium bromide hydrobromid, Sigma cat. no. A-5879) (3) or erythrocytes, which had just been washed five times in saline (E). Lymphocytes in RPMI-1640 with 20% FCS, previously absorbed with SRBC, and erythrocytes were incubated in a ratio of 1:100 except for the E-active rosette assay, which was done with a ratio of 1:10 and without FCS. A volume of 100 μl lymphocytes (1×10^6 per ml) were incubated with 100 μl sheep erythrocytes at 37°C for 15 min or 5 min (E-active rosette assay). Then the mixture was spun at 150 *g* for 5 min and (*a*) incubated at 4°C in at least one hour (E-AET and E-4), (*b*) resuspended immediately after centrifugation (E-active), and (*c*) incubated for 60 min at 37°C and then resuspended (E-37). The various technical changes in the second part of the study are given in the text or in the tables.

Counting of complement-receptor bearing lymphocytes

The number of B lymphocytes was estimated by the demonstration of cells with receptors for complement using *Sacharomyces cereviseae* yeast cells coated with human serum complement (4).

RESULTS

Patients with atopic dermatitis had a small, but significant decrease in the total number of T lymphocytes in blood (Table 1). However, the lymphocytes

Table I. *Total number of rosette-forming lymphocytes in blood from patients with atopic dermatitis and healthy persons*

The E-AET technique gives the total number of T lymphocytes, the other techniques give a fraction of the T lymphocyte population. The size of each fraction is expressed as a percentage of the total number of T lymphocytes (E-AET rosette forming lymphocytes). The rosette assays were done in RPMI-1640 with 20% FCS, except for the E-active assay which was done in medium alone. Number of persons is indicated in parentheses. Mean values ±1 S.D. are given

	Atopic patients	Control persons	Percentage of total no. of lymphocytes	
E-AET	1 858±530 (44)[a]	2 089±682 (87)	100%	100%
E-4	1 464±365 (16)	1 611±496 (20)	74%	74%
E-active	1 021±344 (42)	1 179±433 (84)	56%	57%
E-37	305± 92 (8)	319±103 (6)	15%	20%

[a] The total number of lymphocytes (E-AET) was significantly lower in patients with atopic dermatitis ($p<0.05$, Student's t-test).

present in blood did not show a diminished E-rosette formation even when four different rosette techniques were used (Table II). Thus, the T lymphocytes from patients exhibited the same affinity for sheep erythrocytes as healthy persons.

Because many previous investigations have shown that the E-rosette formation of lymphocytes from patients with atopic dermatitis is reduced (1), we looked at various technical aspects of the E-4 rosette technique, which has been used in most of the previous investigations (1).

We found that incubating the pellet of sheep erythrocytes and lymphocytes for 1 or 18 hours did not give any difference in the percentage of rosettes formed (atopics ($n=6$) 1 hour: 61±8%; 18 hours: 65±6%; healthy persons ($n=4$) 1 hour: 56±7%; 18 hours: 61±7%). When fetal calf serum was omitted from the medium, a significant reduction occurred in the percentage of rosettes, but this applied to both patients and healthy persons (Table III). Also, no difference occurred when the rosette assay was performed in siliconized glass tubes instead of the usual plastic tubes, whether with fetal calf serum present, without serum, or without serum and without centrifugation of erythrocytes and lymphocytes.

The rosette formation was better in an enriched medium as RPMI 1640 than in HBSS, but no difference was observed between patients and healthy persons (atopics ($n=8$): RPMI: 65±13%, HBSS: 54±8%; healthy persons ($n=3$): RPMI: 55±4%; HBSS: 50±3%).

One highly crucial technical detail in E-rosette studies is the force used to resuspend the cells after the formation of rosettes. In Fig. 1 it is shown that we found no difference between patients and healthy persons, when the rosettes were resuspended with different forces.

The number of complement-receptor bearing lymphocytes was 9.2±2.1% in 17 patients with atopic dermatitis and 11.4±1.4% in 10 healthy persons.

DISCUSSION

A decrease in the percentage of T lymphocytes in blood indicates either a disappearance of cells or a decrease in sheep erythrocyte receptor affinity.

Table II. *Percentage of rosette-forming lymphocytes in blood from patients with atopic dermatitis and healthy persons*

The E-AET technique gives the total number of T lymphocytes, the other techniques give a fraction of the T lymphocyte population. The size of each fraction is expressed as a percentage of the total number of T lymphocytes (E-AET rosette forming lymphocytes). The rosette assays were done in RPMI-1640 with 20% FCS, except for the E-active assay, which was done in medium alone. Number of persons is indicated in parentheses. Mean values ±1 S.D. are given

	Atopic patients	Control persons	Percentage of total no. of lymphocytes	
E-AET	80.5±6.12 (47)	80.5±5.44 (98)	100%	100%
E-4	59.4±6.74 (18)	59.6±4.38 (22)	79%	77%
E-active	45.0±8.00 (45)	45.9±9.06 (95)	55%	56%
E-37	11.8±2.39 (9)	16.1±3.80 (7)	16%	15%

Table III. *Rosette formation in glass/plastic tubes, with/without serum*
Mean values ±1 S.D. are given

	Plastic tubes		Siliconized glass tubes	
	With 20% FCS	Without serum	With 20% FCS	With 20% FCS without centrifugation
Patients with atopic dermatitis	60%±10%[a] (n=22)	51%±9%[a] (n=22)	70%±7% (n=9)	49%±17% (n=9)
Persons without atopy	57%±7%[a] (n=23)	47%±9%[a] (n=23)	66%±5% (n=8)	42%±12% (n=8)

[a] $p<0.02$; Wilcoxon's test of pair differences.

AET treatment of sheep erythrocytes gives the highest percentage of rosette-forming lymphocytes. At present it is therefore our best assay for estimating the total number of T lymphocytes in peripheral blood. Using this assay, we found that the total number of T lymphocytes was 11% lower in 47 patients than in 98 healthy persons. Thus, patients with atopic dermatitis lack T lymphocytes in their peripheral blood, but this lack of lymphocytes seems not to be related to the affinity of the sheep erythrocyte receptor. It is possible that we loose a certain subpopulation of T lymphocytes during our preparation of our cell suspensions, but our mean figure of 1.858 T lymphocytes per microlitre of blood is the second highest reported in the literature, according to Byrom (1).

We found that the sheep erythrocyte receptors on T lymphocytes from patients with atopic dermatitis had the same affinity as observed in the control group. There was no difference between patients and healthy persons, when difference in length of incubation, presence of serum, glassware, media, increased mechanical influence, and different observers were used.

We will not conclude that our previous observations (5)—as well as those of other investigators—of a decreased percentage of E-rosette forming lymphocytes in patients with atopic dermatitis, were in error. The discrepancy shows that probably even minor changes in technique can lead to differences in the results. Our present techniques apparently do not reveal very subtle differences in the rosette stability of T lymphocytes.

Byrom et al. (1) found a decrease in both percentage and total number of T lymphocytes in atopic dermatitis. They found a subpopulation of T lymphocytes which expressed sheep erythrocyte receptors only after addition of thymosin (6). It is likely that fetal calf serum contains large amounts of thymosin. When we omitted FCS from the medium, we found a significant decrease in the percentage of rosette-forming cells. This is probably in accord with the existence of a fraction of T lymphocytes.

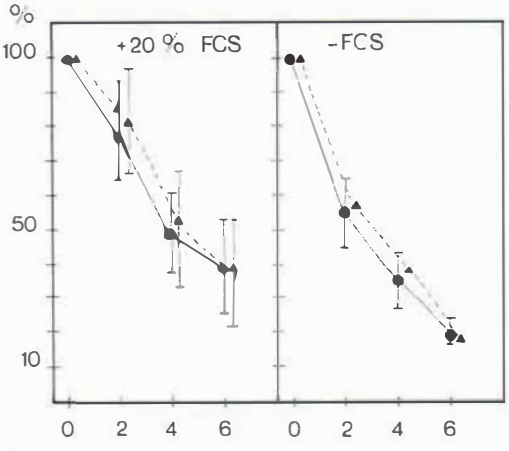

Fig. 1. The figure illustrates the relation between mechanical force and E-rosette stability of lymphocytes from patients with atopic dermatitis (●—●) and healthy persons (▲---▲). The mechanical force was given by a flick of a finger on the tubes, when held in a vertical position. It was applied *after* the E-rosette formation had taken place and the rosettes had been resuspended for ordinary reading. The abscissa indicates the number of flicks given to the tubes. The results from each person are expressed as the percentage of the rosettes found in the tubes that were not 'flicked'. The mean value from each group ±1 S.D. is given in the figure, and results are given with 20% fetal calf serum (FCS) or without serum in the medium. The numbers of persons were 3 (+20% FCS) and 4 (−FCS) patients and 5 (+20% FCS) and 2 (−FCS) healthy persons.

which do not express E-receptors without the presence of thymosin. However, this decrease was the same in patients as in healthy persons. Therefore, it may also reflect the need for protein in the medium to stabilize the rosettes. It may be argued that the addition of thymosin is an addition of protein, which in itself could increase the number of rosettes.

The binding of SRBC to human T lymphocytes is an active process, which has been shown to represent a receptor–ligand interaction rather than a non-specific electrical charge phenomenon (7). Our studies suggest that lymphocytes from patients with atopic dermatitis develop an equal number of these receptors as do cells from healthy persons, when prepared as described in this investigation.

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