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Reduction of Active Natural Killer Cells in Patients with Atopic Dermatitis Estimated at the Single Cell Level

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The natural killer cell cytotoxicity of peripheral blood lymphocytes from twenty-one patients with moderate to severe atopic dermatitis was studied directly by a single cell assay. Suspensions of conjugated effector cells bound to target cells were examined with a light microscope to evaluate the cell lysis using trypan blue exclusion. The binding to the K 562 target cells and the kinetics of lysis were normal among the patients' lymphocytes, but the percentage of active natural killer cells in the conjugates and the lymphocyte population was found to be significantly reduced. Our results raise the possibility that the number of active natural killer cells may be reduced and/or modulated by enhancing and suppressing signals in patients with atopic dermatitis. *Key words: Atopic dermatitis; Natural killer cells; Single cell assay.*

Patients with atopic dermatitis may have disorders of both humoral and cellular immunity (1). Some patients have elevated levels of serum IgE (5), a reduced number of T lymphocytes and Tγ lymphocytes in blood (2, 4, 12, 15), a low in vitro responsiveness to mitogens and antigens (15), a defective non-specific suppressor cell activity (2, 12), a reduced IgG-antibody-dependent cellular cytotoxicity (6), and a reduced natural killer cell activity (3, 7). The biological significance of these findings is not clearly understood, and it is quite possible that some of the abberations reflect a secondary interference of the disease upon the immune system rather than primary disorders.

Our recent finding of a reduced natural killer cell activity in patients with atopic dermatitis using a ^{51}Cr-release assay has prompted this study of active natural killer cells at the single cell level.

PATIENTS AND METHODS

Twenty-one patients were studied, 15 females and 6 males. Their age range was 13–53 years with a mean of 26.5 years. The degree of skin disease was quantitated on a scale from 1–3 and the extent as the percentage of involved skin. The severity index (SI) of the disease was the product of the two parameters (range 5–300, mean 105). Some of the patients also had asthma and rhinitis. The patients were hospitalized for treatment with tar baths and low-grade topical steroids. None had clinical signs of skin infections. The control persons were 21 heterosexual individuals selected from among a group spontaneously attending the clinic with an unjustified suspicion of a venereal disease. There were 11 females and 10 males, age range 19–53 years, mean 28.0 years. The control individuals were all found to be healthy and had neither family history of atopic disease nor signs of atopic dermatitis.

Isolation of lymphocytes

Twenty milliliters of heparinized blood (20 i.v./ml of heparin) was obtained in the morning from both patients and controls. Phagocytic cells were removed by a 1 h incubation of 0.2% (w/v) carbonyl-iron with the blood, after which lymphocytes were isolated by using a Ficoll-Hypaque gradient centrifugation. The lymphocytes were washed 3 times in Hanks' balanced salt solution with 2.5% fetal calf serum (FCS) and resuspended in RPMI 1640 with 2.5% FCS to a density of 2×10^6 cells/ml. The percentage of monocytes was reduced from 25 to 3 by this treatment.

Single cell assay

The assay conditions were essentially those reported by Targan et al. (13). Target cells were the human myeloid cell line K 562, clone 6, mycoplasma free. The cell line was kindly donated by Susanne Bisballe, Institute of Human Genetics, University of Aarhus. K 562 cells were maintained in RPMI 1640 with 2 mM glutamine, 2.5% FCS, penicillin (100 i.u./ml), streptomycin (100 µg/ml), and gentamycin (1.5 µg/ml) in a 5% CO_2 incubator at 37°C. Effector and target cell conjugates were formed by mixing 2×10^6 lymphocytes 2×10^6 target cells in RPMI 1640 with 10% FCS in a total volume of 1 ml in V-shaped 10 ml centrifuge tubes (Nunc). The cells were kept at 37°C for 5 min and then spun at 1000 r.p.m. for 5 min. All but 0.2 ml supernatant was aspirated and the cells were resuspended by means of a Pasteur pipette with 10 times of mixing. One drop of this suspension was removed for counting of the percentage of conjugated cells. Aliquots of 1%-agarose (Difco) in Earle's balanced salt solution (Flow) were kept at 42°C. The melted agarose was cooled by mixing with a Pasteur pipette and 0.5 ml was added to the suspended conjugated cells and mixed once. The liquid solution of agarose and cells was rapidly spread to form an even larger on a microscope slide and allowed to solidify at room temperature. One milliliter of RPMI 1640 with 10% FCS was over-layered the suspension to prevent dehydration and the slides were placed for 3 hours at 37°C in a 5% CO_2 incubator. The medium was removed and 1 ml of trypan blue was added for 5 min. The stain was aspirated and the agarose was destained by addition of 1 ml of medium for another 5 min. The slides were examined with a light microscope and the number of stained conjugated target cells per 100 conjugates was counted. The background death was determined by counting the percentage of dead target cells on control slides without effector cells. Also, the percentage of spontaneous death among effector cells due to the assay conditions was calculated.

The specific killing was calculated as: {percentage of dead targets in conjugates} − {percentage of spontaneous dead targets × the percentage of dead targets in conjugates}. In addition, the percentage of active natural killer cells in the lymphocyte population was calculated by: the percentage of specific killing × percentage of total lymphocytes bound to K 562 cells.

RESULTS

Initially, we found that the kinetics of natural killing was normal among patients with atopic dermatitis (Table I). Both the patients and the controls showed an increase in

Table I. *Kinetics of NK lysis of K 562 cells*

Results indicate mean ± SD of single cell NK lysis of lymphocytes from 3 patients and 3 controls. For details, see Patients and Methods

	% binding		% specific killing		% NK cells	
Time (hours)	Patients	Controls	Patients	Controls	Patients	Controls
½	11.3±3.1	11.0±1.0	2.5±0.5	5.0±0.0	0.27±0.03	0.55±0.05
1	11.0±1.0	11.3±2.1	3.8±1.3	7.0±0.0	0.41±0.10	0.79±0.15
3	11.3±2.1	11.6±1.5	6.3±1.5	9.8±0.3	0.70±0.09	1.15±0.13
4	10.6±1.5	11.3±0.6	6.6±0.6	8.8±0.3	0.71±0.11	0.94±0.05

Table II. *Lysis of K 562 cells by NK effector cells*

Results indicate mean ± SD (number) of single cell NK lysis of lymphocytes from patients and controls. For details, see Patients and Methods

	% binding	% specific killing	% NK cells
Patients	7.61±2.94 (21)	8.19±3.44 (21)**	0.62±0.36 (21)*
Controls	8.71±3.41 (21)	12.28±5.31 (21)	1.13±0.70 (21)

* $p<0.01$, ** $p<0.001$ (Student's *t*-test).

specific single cell killing occurring over a 3–4 hour period after which a maximal number of killer cells was recorded. Thus, we used the end point determination of a 3 h incubation period to measure the frequency of active natural killer cells against K 562.

In the peripheral blood lymphocytes of the patients we could demonstrate the presence of effector cells that could recognize and bind to the target cells (Table II). We found no difference in the frequency of lymphocytes forming conjugates with K 562 in patients and controls (7.61±2.94 vs. 8.71±3.41). Estimation of specific killer cells among conjugate binding cells showed a reduction in the patients compared to the controls (Table II). This relative failure of the lytic mechanism resulted in a reduced frequency of active natural killer cells from the patients by determination in the total lymphocyte population. The calculated fraction of active natural killer cells was 0.62% of lymphocytes from the patients versus 1.13% of lymphocytes from the controls. The numbers of active natural killer cells were equally distributed among patients with both severe and mild atopic dermatitis. In addition, we found no clear relationship to the serum IgE level of the patients.

DISCUSSION

Evidence for a reduced frequency of single active natural killer cells among lymphocytes from patients with atopic dermatitis is presented in this report. The observed reduction was neither due to an abscence of cells that recognized and bound to the target cells, nor to a slowed kinetics of lysis. Apparently, the small fraction of the patients lymphocytes that bound to and lysed the target cells by a single lytic event was reduced.

In a functional assay using the ^{51}Cr-release method we—and others—have recently contended a reduced natural killer cell activity of the lymphocytes from patients with atopic dermatitis (3, 7). However, this finding has no firm general acceptance because conflicting results of both normal (8, 16) as well as enhanced (13) natural killer cell activity has been reported.

With the ^{51}Cr-release assay we found that the natural killer cell activity was correlated to the severity of the skin disease but this could not be clearly established with the use of the single cell assay. However, the possibility remains that these discrepancies could be due to modulations of the lytic efficiency of the natural killer cells as exemplified by a reduced recyclability of natural killer cells from patients with severe atopic dermatitis, a suppression of the lytic mechanism or a failure of recruitment of pre-natural killer cells (9, 11).

Human natural killer cells have been found to be associated with large granular lymphocytes (LGL) (10). However, only a fraction of LGLs bound to the targets actually kill. The mechanism of killing has been proposed to be a stimulus-secretion event in which the appropriate binding stimulates membrane changes and leads to a vesicular secretion with subsequent lysis of the target cell (9).

Although the single cell assay can be used to enumerate the natural killer cells this end point determination must be evaluated in the context of a dynamic equilibrium of enhancing and suppressing signals rather than an ultimate characteristic of a person's natural killer cells. Thus, we have found that the reduced natural killer cell activity in the ^{51}Cr-release assay may be due to loss of natural killer cells, but we have not excluded that the result may be due to modulation of the activity of single natural killer cells.

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