Acta Dermatovener (Stockholm) Suppl. 92: 48–51, 1980

NATURAL KILLER CELLS AND INTERFERON PRODUCTION IN ATOPIC DERMATITIS

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Abstract. Natural killer (NK) cells, cytotoxic for Burkitt lymphoma cells, were found to be more active in atopic than in non-atopic children. Upon stimulation of lymphocyte cultures with Sendai virus, less interferon was produced by cells from atopic than from non-atopic individuals. Increased NK activity and decreased interferon production may both be consequences of a T lymphocyte deficiency which has been suggested to be causally related to atopic disease.

Key words: Natural killer cells; Sendai virus; Interferon; Atopic disease

Recently, evidence has accumulated that atopic allergy is associated with a T lymphocyte deficiency (reviewed in ref. 11). Although it has not been definitely excluded that this deficiency is secondary to treatment or to manifestations of the disease, this seems unlikely for two reasons; first, low T cell counts and decreased lymphocyte responsiveness to mitogens and antigens are obvious even in patients without overt disease and without treatment (5) and second, low T cell counts are present in babies of atopic parents in early life, before atopic disease and elevated levels of IgE develop (7).

The consequence of a T cell deficiency is certainly dependent on the degree and quality of depression of various T cell functions. In atopy there is some evidence that suppressor T cell function is abnormally low (10) and that suppressor cells regulating, in particular, IgE synthesis are deficient (1). Thus one of the consequences of the T cell deficiency may be hyperproduction of IgE. However, the findings of depressed delayed hypersensitivity reactions to various antigens and the increased susceptibility of atopics to severe infections with certain viral and fungal agents (reviewed in ref. 2) certainly suggest that several different T cell functions are disturbed in atopy.

In the present paper we suggest two mechanisms by which the cellular defect in atopy may lead to disease. First, several immunological functions are subject to regulation by suppressor cells. For instance, the activity of cytotoxic, so-called natural killer (NK) cells may possibly be regulated by suppressor cells (9) and failing suppressor function might therefore lead to hyperactivity of NK cells and hence disease. Second, several kinds of immunocompetent cells appear to have the ability to produce interferon (12). A faulty function of some particular kind of lymphoid cell might therefore, directly or indirectly, result in depression of interferon (IF) production and hence to increased susceptibility to viral infections. In this paper we present data on NK cell activity and IF production in children with atopic disease.

PATIENTS AND METHODS

Patients. Blood specimens were obtained from children with asthma, rhinoconjunctivitis and/or atopic dermatitis, who visited the outpatient clinic at the Childrens Hospital, Göteborg. To be included in the study the children had to have positive skin tests and elevated serum levels of specific and/or total IgE. Control specimens were obtained from healthy children without history or signs of atopy.

Assay of natural killer cell activity. The target cells used (Burkitt lymphoma cells of the P3HR1 line) were labelled with Cr^{51} for 4 hours, then washed and placed in microtrays. Lymphocytes, prepared by centrifugation in Metrizoate-Ficoll, were then added to the target cells at a ratio of 25:1. After 18 hours' incubation at 37°C, aliquots of the supernatants of the cultures were collected, and the radioactivity determined in a gamma counter. Specific lysis was calculated as:

$$\frac{\text{cpm in test sample} - \text{cpm in medium control}}{\text{cpm in totally lysed sample} - \text{cpm in medium control}} \cdot 100$$

Assay of interferon production. Metrizoate-Ficoll separated lymphocytes were cultured in microtrays at a cell concentration of 1.5×10^6/ml. Sendai virus at a final concentration of 300 hemagglutinating units per ml was added to the cultures. After incubation for 4 days the culture supernatants were frozen and then titrated for IF content essentially according to the method of Havell & Vilcek (3). The cell used for titration were of bovine origin (MDBK cells) and as challenge virus vesicular stomatitis virus was used.

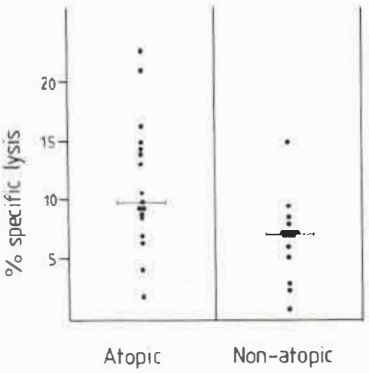

Fig. 1. Natural killer cell activity againts P3HR1 cells in atopic and non-atopic children. Studies performed on 17 atopic (mean age 11.7) years and 16 non-atopic (mean age 12.2 years) 9–17-year-old children. Effector : target cell ratio was 25 : 1. Horizontal bars indicate median values. The difference between the two groups is statistically significant (Wilcoxon test, $p < 0.01$).

The titres obtained were calibrated against an international reference IF preparation.

RESULTS

Natural killer cell activity. The NK activity against P3HR1 cells was tested in 51 1–17-year-old children (19 controls and 32 with various atopic diseases). There was no evident age dependency of the activity. Thus the median activity (% specific lysis) in the group of atopic 1–8-year-old children was 10.2% and the median activity in atopic 9–17-year-old children 9.7%. In the whole material NK cell activity was significantly higher in atopic than in non-atopic children (Wilcoxon test, $p < 0.01$). The same significant difference ($p < 0.01$) was found when only the age group 9–17 years (17 atopic and 16 non-atopic children) was considered (Fig. 1). The number of non-atopic children in the age group 1–8 years was too small to allow statistical calculations.

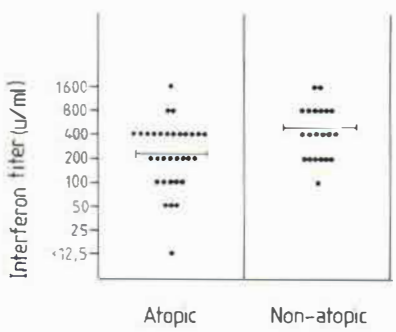

Fig. 2. Interferon production in lymphocyte cultures from atopic and non-atopic children. Studies performed on 32 atopic (mean age 11.8 years) and 21 non-atopic (mean age 12.3 years) 9–17-year old children. Interferon titers were determined after 4 days of culture of Sendai virus-stimulated lymphocytes. The difference between the two groups is statistically significant (Student's *t*-test, $p < 0.01$).

Interferon production. The production of leukocyte IF in lymphocyte cultures stimulated with Sendai virus was studied in 45 atopic and 21 non-atopic 1–17-year-old children. There was a tendency towards age dependency of IF production, since the median values obtained in 1–8-year-old atopic children were somewhat higher than those obtained in the atopic 9–17-year-old individuals. The difference, however, was not statistically significant. When the IF-producing capacity of age-matched children was studied, it was evident (Fig. 2) that the cells from atopic individuals had a depressed capacity to form IF upon stimulation with Sendai virus (Student's *t*-test, $p < 0.01$). One of the atopic children did not produce any detectable IF. This child had a very severe disease, with asthma, eczema, very high serum IgE levels and low T cell counts. Apart from this case there was no obvious correlation between severity of disease and ability to produce IF.

DISCUSSION

During recent years speculation on the pathogenesis of atopic diseases has been focused on the hyperproduction of IgE, which is frequently associated with asthma as well as with atopic dermatitis (6). Many cases of atopic dermatitis have perfectly normal levels of serum IgE, however, and it is hard to envisage the histological picture in the skin of dermatitis patients as exclusively being caused by IgE-mediated release of vasoactive mediators.

The hyperproduction of IgE has earlier been suggested to have a causal relationship to a T cell deficiency and a possible chain of events leading to increased IgE-antibody formation has been proposed (11). If there is, as reported, a more pronounced deficiency in some T cell subsets, notably suppressor cells, than in other cell populations, consequences apart from increased IgE-production may be anticipated. In our present study we have obtained evidence that one of these possible consequences, namely hyperactivity of cytotoxic cells, may in fact occur in atopic disease. Although our experimental set-up did not definitely exclude the possibility that antibody-dependent cellular cyto-

toxicity rather than NK activity was being measured, the results suggest that NK cell activity is increased in atopy. The relevance of this finding for the pathogenesis of atopic dermatitis remains to be elucidated.

Another consequence of defective T lymphocyte function in atopy that can be envisaged is decreased production of various lymphokines. Recently Horsmanheimo et al. (4) have presented evidence that the production of leukocyte migration inhibitory factor is subnormal in atopic patients. In the present study we found that stimulation of lymphocyte cultures with Sendai virus resulted in slightly subnormal IF production in atopic individuals. The experimental procedure was designed to measure production of classical leukocyte IF rather than so-called immune IF and it is therefore unclear to what extent the decreased IF production is related to abnormal T cell function. Studies are in progress to determine the production of immune IF, which is clearly T cell-dependent (8) in atopic individuals.

Since the amount of IF produced was less in atopics than in non-atopics, the present findings may have a bearing on the pathogenesis of atopy. It is well known that some viral infections, e.g. those caused by vaccinia and herpes simplex virus, may run an unusually severe course in atopics and also that warts are very common in atopics (reviewed in ref. 2). It is tempting to speculate that our finding of decreased IF production in the atopic has some bearing on the course of these viral diseases. It is possible, however, that intrinsic skin abnormalities are of greater importance for the spread of these diseases, since there is no evidence that atopics have a generally increased susceptibility to viral infections.

ACKNOWLEDGEMENTS

This study was supported by grant no. 3942 from the Swedish Medical Research Council and by the Ellen, Walter and Lennart Hesselmans Stiftelse. We thank Ms Ingela Carlsson, Ms Birgitta Norén and Ms Liisa Loponen for very skilful technical assistance.

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DISCUSSION

Zachariae (Aarhus). Q: What do you think about prophylaxis, i.e. antigen avoidance, in atopic children?

A: It is well known in several systems that if you give large amounts of a mitogen or an antigen you get an activation preferentially of suppressor cells. On the other hand there are data showing that antigen elimination is of definite value for the prevention of atopic disease so I think it's a tough question.

Hanifin (Portland). Q: How old were the children you investigated and was there a difference between cow's milk versus breast milk-fed?

A: The T cell levels were determined at one month of age and serum IgE levels at 6 months of age. Children with asthmatic fathers had the lowest T cells, and the only definite correlation between low T cell values and high IgE values was obtained in the children that were fed cow's milk.

Saurat (Paris). Q: You saw increase lymphocyte cytotoxicity in atopic dermatitis, was it direct cytotoxicity?

A: Yes this was natural killing. We used cells bearing EB-virus and cannot be absolutely sure that one is not measuring antibody-dependent cellular cytotoxicity.

Giannetti (Pavia). Q: What is your opinion about Tγ and Tμ markers? and did you look at IgE levels?

A: In one investigation the number of Tγ cells was decreased in atopy, but several others did not find any difference between normals and atopics. A defective suppressor function of macrophages should also be considered. We have not studied the effect of suppressor cells on IgE production.

Thestrup-Pedersen (Aarhus): We have investigated 16 adults with atopic dermatitis and found a change in the ratio between the Tμ and Tγ cell, viz. a decrease in Tγ cells and a slight increase in the Tμ cells.