

Natural killer and interleukin-2 induced cytotoxicity in asthmatics

I. Effect of acute antigen-specific challenge

M. JIRA, E. ANTOSOVA, V. VONDRA, J. STREJCEK, H. MAZAKOVA & J. PRAZAKOVA

Dept. of Medicine, Faculty of Medicine and Hygiene, Charles University, District Allergology Unit, Dept. of Internal Medicine, and Research Institute for Tuberculosis and Respiratory Disease, Prague, Czechoslovakia

Natural killer (NK) cell activity and interleukin-2 (IL-2) induced cytotoxicity to NK-resistant targets T24 in a group of patients with grass pollen seasonal asthma were investigated. Acute bronchial provocation with this antigen was also performed and blood samples were taken before and 15 min after the maximal clinical response. NK activity was elevated in this group but there was a significant decrease after acute challenge. IL-2 induced cytotoxicity was also increased and declined after antigen challenge. Limiting dilution analysis for minimal frequencies of precursors of the IL-2 induced killer cells was introduced and showed increased numbers of lymphokine-activated killing precursors in this group of patients. These data might indicate the aberrations of the IL-2 system in immediate type allergy but the clinical significance of these findings remains to be determined.

Key words: acute antigen challenge; grass pollen allergy; IL-2 induced killer cells; natural killer cells.

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Blood circulatory leukocytes with the potential for *in vitro* cellular cytotoxicity in the absence of active prior sensitization are termed natural killer (NK) cells (10). Recently, another cytotoxic system has been recognized and termed lymphokine-activated killing (LAK). Cytotoxicity of LAK cells has been suggested to include allogeneic, autologous tumor cells and hapten-modified autologous cells (6, 16, 19). Activation of LAK cells seems to be mediated by interleukin-2 (IL-2) (3, 13, 14, 15). Because of the evident discrepancy of data on NK activity in IgE-mediated allergies in the literature (11, 12, 20) and the lack of information on LAK activity in this study we investigated these indices. A group of asthmatics with symptoms of grass pollen allergy of immediate type was selected in order to study NK activity and activity of IL-2 induced killer

cells before and after acute antigen-specific bronchial provocation.

MATERIAL AND METHODS

Subjects with seasonal asthma related to grass pollen antigens were selected. This group comprised 13 men and five women, aged 22 to 42 years (mean 31.2 years) where diagnosis was confirmed by classical clinical picture of seasonal symptoms and positive intradermal tests for grass pollen antigens (wheals > 5 mm in diameter after mixed grass pollen antigen of concentration 100 PNU). Before previous season all patients had reversible bronchial obstruction when challenged with grass pollen antigen. The reversibility of bronchial obstruction was defined as the normalization of predicted values of ventilatory parameters. The reversibility was

thus confirmed by circadian measurements in symptom/symptom-free periods of the disease. These patients had previously undergone courses of hyposensitization (Pollinex Bencard, or Pollen Antigen, Institute of Sera and Vaccines, Prague, Czechoslovakia), but not in the last 2 years.

Thirty-three healthy subjects matched for age and sex served as controls. These subjects had no clinical evidence of allergic or pulmonary diseases, and predicted pulmonary function values were comparable with those of the patient group in a symptom-free interval.

Bronchial challenge was performed before season, in February. Lung function was tested by Pulmoscreen Jaeger. Grass pollen doses were divided into 3-min inhalation periods and the total protein dose inhaled was equal to that to which the individual patient had significantly responded a year ago ($> 15\%$ reduction of FEV_1). Patients inhaled from 10 to 1000 PNU of antigen (mean 514 PNU). After antigen inhalation lung function characteristics were measured under standard conditions for at least a further 30 min (21). Reduction in FEV_1 varied from 0.1 to 58.8% (mean 21.7%). Peripheral blood samples for lymphocyte cytotoxicity were taken before the test and 15 min after the maximal clinical response to inhaled antigen.

Cytotoxicity assays

Heparinized samples were isolated by standard density gradient centrifugation. The peripheral blood mononuclear cells (PBMC) were washed three times in Eagle's minimal essential medium (E-MEM, Institute of Sera and Vaccines) and were resuspended at a concentration of 2×10^6 cells/ml in E-MEM containing 10% fetal calf serum, 50 IU/ml penicillin, 50 μ g/ml streptomycin, non-essential amino acid mixture for tissue culture and 0.3 g/l glutamine. These were the test's effector cells. The target cells for NK assay were K562 cells (10). These cells were labelled with 5 MBq of chromium-51 (Rotop, Berlin, GDR) for 60 min at 37°C; they were then washed three times in E-MEM until most of the release background activity was elimi-

nated. The cells were then resuspended in complete medium at a concentration of 10^5 /ml. The effector cells to be tested (0.1 ml) were added to 0.1 ml chromium-51-labelled target cells in V-shaped microtiter plates (Flow Laboratories Ltd., Irvine, UK). An effector/target (E/T) ratio of 20:1 was achieved, and all tests were performed in duplicate. The plates were incubated for 4 h at 37°C in a humidified atmosphere of 5% carbon dioxide in air; 100 μ l of supernatant was then harvested from each well and radioactivity measured in a Packard gammacounter. Spontaneous release (SR) counts were obtained from target cells incubated alone, and total release (MR) data were obtained from target cells incubated with Triton X-100 at a final concentration of 0.2%. The percentage of specific chromium-51 release from target cells exposed to effector cells was calculated according to the following formula:

$$CI = \frac{ER-SR}{MR-SR} \times 100 (\%)$$

Where ER is experimental release of individual sample and CI cytotoxicity index.

For purposes of IL-2 induced cytotoxicity and limiting dilution analysis, 75 μ l PMNC suspension was added to the first 24 wells of a U-shaped microtitration plate (NUNC AS, Kamstrup, Denmark) with 75 μ l of complete medium. Cultures were 2-fold serially diluted in plates to obtain cultures containing from 75,000 to 586 cells/well. Finally, 75 μ l of complete medium containing 500 μ l of recombinant IL-2 and 75 μ l of complete media was added to control cultures. The plates were incubated for 140 h at 37°C in a humidified carbon dioxide atmosphere. Preliminary experiments had shown that activated cells are responsible for cytotoxicity to T24 (14, 15). Thus, no supernatant had to be removed before adding the target cells. The target cells used were human urinary bladder carcinoma cell line T24 (known to be NK-insensitive) (15). T24 cells were labelled as K562 cells, washed and resuspended in complete medium at a concentration of 1×10^5 /ml. To each well of the microtitration plates 50 μ l of T24 target cells was added to IL-2 stimulated effectors on day 6. Effector/target cell concen-

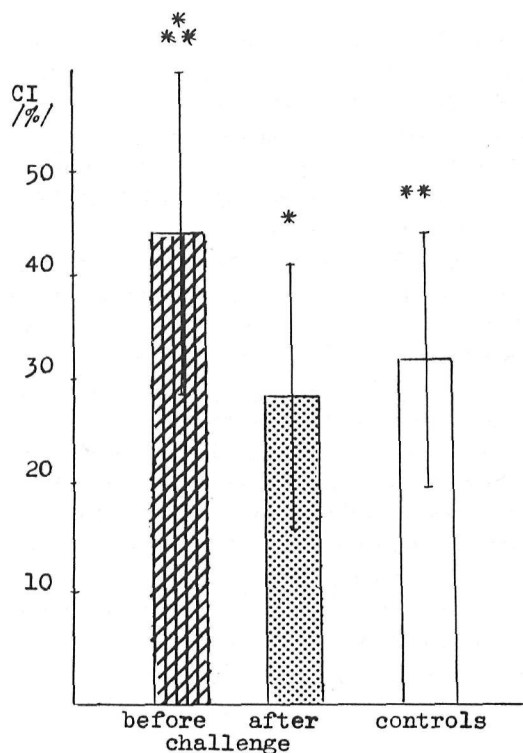


Fig. 1. Natural killer activity of PBMCs in asthmatics before and after acute antigen challenge. E/T ratio is 20:1 (K562 are used as targets). Results in bars are expressed as a mean $\pm$ 1 SD. Statistical significance is indicated by asterisks

* $P < 0.01$ by paired t-test

** $P < 0.05$ by unpaired t-test

trations 15:1, 7.5:1, etc. were obtained, plates were briefly centrifuged and incubated for an additional 5 h, 100 μ l of supernatant was then harvested from each well and radioactivity measured in a Packard gammacounter. Spontaneous release (SR), maximal release (MR) and experimental release (ER) data and calculations of cytotoxicity index (CI) were done as for NK cell assay. Results of IL-2 induced cytotoxicity are presented as the E/T ratio 15:1.

Calculation of minimal frequency of precursors of IL-2 induced killer cells

Minimal estimates of frequencies were obtained by the minimum chi-square method from the Poisson distribution relationship between the responding cell number and the logarithm of the

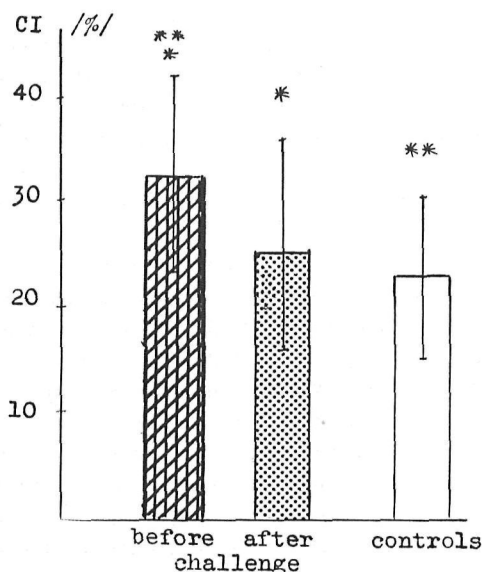


Fig. 2. Interleukin-2 induced cytotoxicity of PBMCs before and after acute antigen challenge, E/T ratio is 15:1 (T24 are used as targets). Results in bars are expressed as a mean $\pm$ 1 SD. Statistical significance is indicated by asterisks

* $P < 0.005$ by paired t-test

** $P < 0.05$ by unpaired t-test

percentage of negative cultures. Special PC software for these purposes was kindly provided by Dr. B. Loveland, Howard Hughes Med. Ctr., Dallas, USA (14).

Total serum IgE was also checked by commercial kit (Institute of Sera and Vaccines) and ranged from 22 to 1589 IU/ml (mean 392 IU/ml).

Statistical calculations were made using Students' t-test and the results were expressed as a mean $\pm$ 1 SD. Linear and exponential regression analysis was used for comparison of two separate assays.

RESULTS

Patients with seasonal asthma related to grass pollen antigen had elevated natural killer activity when compared with controls ($P < 0.05$) (Fig. 1). After antigen challenge a decrease in NK activity was observed. The decrease in this group reached statistical significance ($P < 0.01$) but was not related to reduced FEV₁ or the inhaled antigen dose. Compared with the control group, increased IL-2 induced

cytotoxicity in the study group was statistically significant ($P < 0.05$) (Fig. 2). After challenge there was impairment in IL-2 induced cytotoxicity and these differences reached statistical significance ($P < 0.005$).

To clarify whether elevated IL-2 induced cytotoxicity is due to the increased number of precursors of IL-2 activated killer cells, a limiting dilution assay was introduced. It was demonstrated that compared to controls minimal numbers of IL-2 induced precursors are increased but without statistical significance (Table 1). After acute challenge the numbers of precursors decreased but was without statistical significance ($r = 0.291$). NK activity and IL-2 induced cytotoxicity data showed poor correlation, both in asthmatic patients ($r = 0.389$) and control subjects ($r = 0.418$). Moreover, no relationship was found between total serum IgE and cytotoxicity assays.

DISCUSSION

Our results clearly demonstrated elevated NK cytotoxicity of PBMCs in asthmatics with seasonal symptoms related to grass pollen antigens. These observations confirm recent results of Timonen et al. (20). However, IgE-mediated allergy (initially presented as atopic dermatitis) has been shown to have strongly suppressed NK activity (11, 12). These observations suggest that there might be qualitative and/or quantitative differences in these types of allergy in respect to cell-mediated immunity. However, methodological differences should be also considered.

Several factors may contribute to the effect of acute antigen challenge on NK activity. These

include acute stress (1, 5), lymphocyte redistribution (5, 8, 20), and direct or indirect effect of biologically active mediators released during challenge (5, 7, 16, 17, 18). Reduction of Leu-7 positive cells (but not Leu-2a positive) found in another set of patients with the same symptoms (unpublished) supports the hypothesis of Timonen et al. (20) that rapid clearance of NK cells after acute challenge may occur. Recombinant IL-2 or α -interferon was less effective in enhancing NK activity of PBMC in allergic subjects *in vitro* (unpublished).

Recombinant IL-2 induced killer cells were found to exert increased cytotoxic effect on T24 targets. The IL-2 system appears to be important in the immune regulation (3). Recently, IL-2 has been also suggested to contribute to cancer control (13, 19) and to the tolerance phenomenon (14). Increased autologous mixed lymphocyte cultures (AMLC) were demonstrated to be increased in asthmatics (9). As it is known that IL-2 induced killers are generated in AMLC our data are compatible with these observations. IL-2 dependent proliferation was also demonstrated to be elevated in some groups of asthmatic children (8).

An explanation for the decrease of IL-2 induced cytotoxicity after acute challenge can be only speculative and based on observations in other systems. During systemic administration of high doses of IL-2 in human cancer, LAK cells disappeared from circulation within minutes (13). Our own observation of the decrease in precursors of IL-2 induced killer cells in the peripheral blood may support the hypothesis that acute antigen challenge induces redistribution in various lymphoid compartments. However, other factors (e.g. the direct effect of biologically active substances on lymphocyte functions) should also be considered.

The observations of baseline elevated natural and IL-2 induced cytotoxicity and increased number of precursors may be of particular clinical interest.

1) LAK cells are able to kill various cells, including autologous hapten-modified cells (6), and thus this system should also contribute to the pathogenesis of some types of diseases of allergic origin.

Table 1

Minimal frequencies of precursors of interleukin-2 activated killers in peripheral blood before and after acute antigen challenge. Differences are not significant

	Mean	Range
Asthmatics ($n = 18$)		
Before challenge	1/3993	1/1664-1/56008
After challenge	1/5029	1/1596-1/53456
Controls	126031	1/1996-1/70116

2) These cytotoxic phenomena may significantly contribute to the immune surveillance of tumors. In asthmatics, it is believed that the incidence of malignancies is lower than in the general population (2). However, some investigators suggested an elevated incidence of malignancies in atopic dermatitis (4). One might speculate that atopic dermatitis (often accompanied by asthmatic problems) may represent a distinct entity of immediate allergy disease. Further investigations to clarify some of these questions are now in progress.

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Address:

Milan Jira, M.D., PhD.
Immunology Unit
Dept. of Medicine
Faculty of Medicine and Hygiene
Srobarova 50
100 34 Praha 10
Czechoslovakia

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