

Lymphocyte Responses to an Aqueous Extract from Cow Hair and Dander

Investigations of Asthmatics and Normal Controls with Lymphocyte Transformation Tests

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The proliferative responsiveness to cow dander antigens of lymphocytes from 23 asthmatic patients with allergy to cow hair and dander, from 10 patients with allergic asthma to other antigens and from 31 non-atopic subjects was investigated. Lymphocytes from patients with allergic asthma due to cow epithelium antigens showed a statistically significant decreased responsiveness to an extract from cow dander, compared to that seen with lymphocytes from newborn infants, lymphocytes from normal controls and from asthmatic patients without allergy to cow. The response to the cow dander extract seemed to be an accessory cell dependent T lymphocyte response.

Key words: antigen specificity; cow dander; lymphocyte transformation.

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CLINICAL ASPECTS

T-cells play an important role in the regulation of the immunological response to antigens. To evaluate cell-mediated immune-responsiveness to cow dander antigens in atopic subjects we have studied the deoxyribonucleic acid (DNA) synthesis responses of their cultured lymphocytes to these antigens.

In rodents T lymphocytes seem to play an important role in the regulation of IgE antibody response to specific antigens (16, 21). During the last 10 years several studies have been published evaluating the responsiveness of human lymphocytes to pollen antigens. Although the results have been conflicting most of the studies found an overall greater responsiveness of lymphocytes from atopic pollen sensitive persons than of those from non-atopic controls (2, 3, 10, 14, 20, 22, 24). In some studies lymphocytes from desensitized patients showed less responsiveness to pollen antigens than did lymphocytes from untreated patients (8, 9, 15). In other papers immunotherapy was reported to have no effect (24). However, still

other reports indicated responses of lymphocytes from normal subjects to pollen antigens and even lymphocytes from human umbilical cord blood or from patients with agammaglobulinemia have been shown to give vigorous DNA synthesis response to pollen antigens (1, 4).

In the reports published until now only pollen antigens have been used as stimulating agents, and the results appear to be contradictory. No studies have been undertaken to explore the responsiveness of human lymphocytes to other atopic antigens, such as those in extracts from mammalian hair and dander. We therefore carried out a series of experiments to evaluate the responsiveness of lymphocytes

from atopic and non-atopic individuals to an extract from cow hair and dander. The present report summarizes the results of these studies.

MATERIAL AND METHODS

Subjects

Lymphocytes from 23 asthmatic patients with allergy to cow hair and dander, from 10 patients with allergic asthma to other antigens (pollen, mite and animal dander) and from 31 healthy normal non-atopic controls have been studied at 68 different occasions. The diagnosis of allergic asthma was based upon "prick-test", RAST or histamine liberation test, bronchial provocation test and disease history (25). None of the patients had been admitted to hospital as a result of asthma within the last year, and none had been on steroids for the last 6 months. Judged from disease history the severity of the asthma was equal between the two groups of patients. All medication was stopped 24 h before the blood samples were taken. None of the patients had been given immunotherapy.

Cell suspensions

Mononuclear cells containing less than 2% granulocytes were isolated from heparinized blood by gradient centrifugation on Ficoll-Isoopaque (Lymphoprep, Nyegaard, Oslo), as described by Bøyum (5). The cells were washed twice and resuspended in culture medium: HEPES-buffer RPMI 1640 (Grand Island Biological Co., N.Y.), supplemented with penicillin (500 U/ml, LEO, Copenhagen), streptomycin (500 µg/ml, NOVO, Copenhagen), L-glutamine (1.5 µM/ml, Flow Labs., Irvine, Scotland) and 15% pooled human serum from non-transfused male blood.

T and B lymphocyte suspensions

Phagocytic cells were removed prior to the E-rosette formation by means of carbonyl iron powder, as described by Lundgren et al. (13). Lymphocyte suspensions containing less than 1% phagocytic cells were adjusted to 6×10^6

cells per ml medium and mixed with equal amounts of a 1% dilution of carefully washed and packed SRBC in medium containing 20% human AB serum; the serum was previously inactivated and absorbed (30 min at 37°C and 60 min at 4°C) with equal volumes of washed and packed SRBC. The lymphocyte SRBC mixture was now incubated for 10 min at 37°C, then pelleted in 6 ml aliquots in plastic tubes (NUNC, Denmark) and kept overnight at 4°C. The following day, the rosette mixture was gently but carefully resuspended and layered on a new Ficoll-Isoopaque gradient and centrifuged for 30 min; during the first 10 min the speed was slowly accelerated to 600 g. The interphase layer was then removed and after two washings with medium used directly as B lymphocyte-enriched suspension.

The sediments containing E-rosettes were pooled, resuspended in medium with a concentration of 100 µl packed SRBC/ml, and incubated for 10 min with 4 volumes of 0.83 w/v % NH Cl (Merck) in water at 4°C to lyse the red cells. The T lymphocytes were then washed twice with medium.

Cultures

Cultures of 2.0×10^5 cells/0.5 ml medium with 15% pooled human serum in plastic tubes (Nunc, Denmark) were stimulated with the following agents (the final concentrations in the tubes are indicated): a) Phytohaemagglutinin P (PHA, Difco, Detroit – 40 and 4 µg/ml), b) Pokeweed mitogen (PWM, Gibco, New York – 100 µg/ml), c) concanavalin A (con A, Pharmacia, Uppsala, Sweden – 5 and 1 µg/ml), d) purified tuberculo-protein derivative (PPD, State Serum Institute, Copenhagen – 1 µg/ml), e) an aqueous dialyzed extract of hair and dander from black and white cattle, containing major and minor allergens, produced according to Prahl et al. (19) (St. C-75, – 200, 20, 2 and 0.2 µg/ml, – optimal concentration: 200 µg/ml). All cultures were incubated in a humidified atmosphere of 5% CO₂ in air at 37°C for 96 h (PHA, con A and PWM), 120 h (PPD, *C. albicans*, *S. aureus* and *E. coli*) and 144 h (St. C-75). During the final 24 h of culture 0.1 µCi of

$2\text{-}^{14}\text{C}$ thymidine was added and the cells were collected on glass-fiber filters (Skatron, Norway). The filters were transferred to tubes containing 1.5 ml Instagel (Packard Instrument Co., Downer's Grove, 111), and the activity was measured in a Beckman liquid scintillation counter. All cultures were made in triplicate, and unless otherwise indicated the results are given as Icpm, i.e. the median cpm of triplicates minus the median cpm of unstimulated controls of the same suspension. Significance tests were made by the non-parametric Wilcoxon method.

Total serum IgE was measured by the PRIST assay (a double antibody assay) using the Pharmacia kit (7, 12).

Specific serum IgE was estimated according to Wide et al. (23) with an aqueous extract from cow hair and dander (St. C-75) (19) coupled to the discs (6).

RESULTS

With all of the stimulating agents the peak proliferative response occurred at the same time with lymphocytes from patients allergic to cow and from normal controls (Fig. 1). With St. C-75 no peak response was seen earlier than day 6 of culture. Consequently 6 days was chosen as standard incubation period for this stimulating agent. Fig. 2 shows that response to optimal concentrations of PHA (40 $\mu\text{g}/\text{ml}$) was at the same level for lymphocytes from all three groups of subjects. This was also the case with optimal concentrations of con A and PWM (results not shown). In contrast to this, the response of lymphocytes from the group of cow-allergic asthmatics to the optimal concentration of St. C-75 ($P < 0.01$) and the sub-optimal concentration of PHA was significantly lower than that of lymphocytes from the asthmatic and non-asthmatic controls ($P < 0.01$). Lymphocytes from patients with allergies other than cow allergy did not differ from the normal controls. With all of the stimulating agents used the doses eliciting maximum response were the same for all three groups. The unstimulated

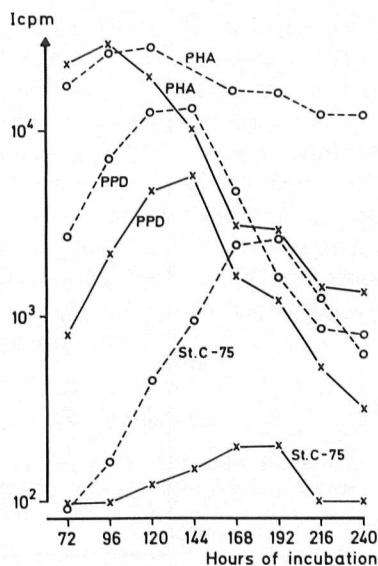


Fig. 1. Proliferative responses obtained with lymphocytes from a cow allergic asthmatic (x—x) and a non-atopic control (O—O) after incubation for different periods of time in the presence of St. C-75 (200 $\mu\text{g}/\text{ml}$), PHA (40 $\mu\text{g}/\text{ml}$), con A (5 $\mu\text{g}/\text{ml}$) and PPD (1 $\mu\text{g}/\text{ml}$).

cultures showed no differences among the three groups.

Antigen specificity of St. C-75

The findings of stimulation of DNA synthesis in lymphocytes from normal non-atopics by St. C-75 led us to study the response by lymphocytes from individuals who would not be expected to respond to cow dander allergens, i.e., newborn infants. Lymphocytes from three out of four umbilical cord blood samples showed an even greater response to St. C-75 than the three other groups of subjects. The lack of specificity of the proliferative response was further illustrated in experiments where two major allergens (Ag 1 and Ag 3) from cow hair and dander, purified according to Prahl (18) were used over a wide range of concentrations (Ag 1 from 540 to 2 $\mu\text{g}/\text{ml}$ and Ag 3 from 300 to 1 $\mu\text{g}/\text{ml}$). Neither Ag 1 nor Ag 3 stimulated DNA synthesis in lymphocytes from cow-allergic patients and non-atopic controls, nor did they cause any inhibition.

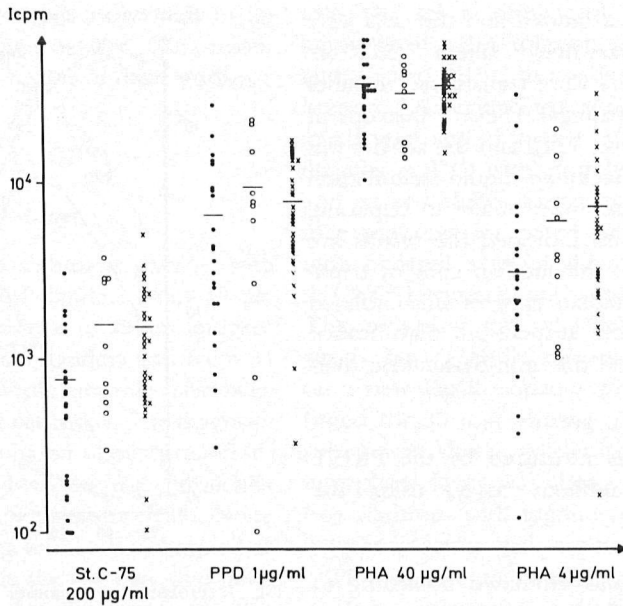
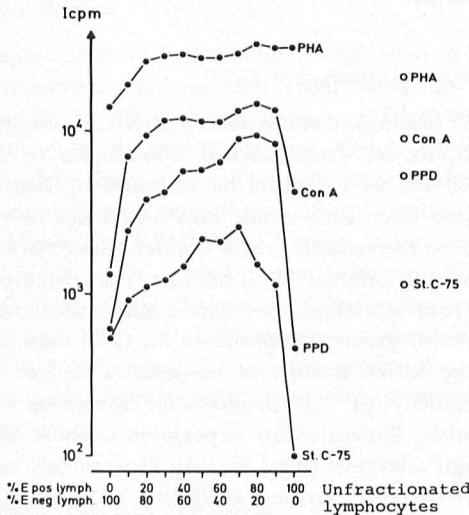


Fig. 2. Proliferative responses shown by lymphocytes from 23 asthmatic patients with allergy to cow (●), 10 asthmatics without allergy to cow (○) and 31 non-atopic controls (×) after incubation with St. C-75 (200 µg/ml), PHA (40 and 4 µg/ml) and PPD (1 µg/ml). Mean values are indicated.



Stimulation of lymphocyte subpopulations with St. C-75

To determine which population of lymphocytes St. C-75 stimulated, subpopulations of lymphocytes from normal controls were studied. The responsiveness of T- and non-T cells to St. C-75 was investigated with different concentrations of the cells mentioned (Fig. 3). The fraction of non-T cells varied from 0 to 100% and vice versa for T cells. The cells were stimulated with four concentrations of St. C-75 and incubated for 5, 6 and 7 days respectively. Pure E-positive lymphocytes showed no response. Addition of 10% E-negative lymphocytes completely restored the responsiveness of the T cell-enriched suspensions. The response peaked at 80% E-positive and 20% E-negative lymphocytes. Compared to unfractionated cells the E-negative cells showed decreased (50%) responsiveness.

Fig. 3. Proliferative responses obtained with different amounts of E-positive and E-negative cells from a non-atopic control after incubation for 144 h with St. C-75 (200 µg/ml), PHA (40 µg/ml), con A (5 µg/ml) and PPD (1 µg/ml) (●). The responses shown by unfractionated cells are indicated to the right (○).

*Correlation with IgE antibodies
(specific and unspecific)*

The individual responsiveness to St. C-75 was not correlated to the level of cow dander-specific IgE antibodies or to the total amount of IgE antibodies.

DISCUSSION

We have investigated the proliferative response to mitogens and antigens of lymphocytes from asthmatics with and without allergy to cow dander and from normal controls. The lymphocytes were also stimulated with an extract from cow hair and dander. The kinetics of proliferation did not differ among the three groups of subjects. All of the lymphocyte cultures showed a normal proliferative response to optimal concentrations of standard stimulating agents, such as PHA, con A, PWM and PPD, and no severe impairment of the responsiveness would therefore be expected.

The results obtained from experiments with varying quantities of T- and non-T cells and from the responsiveness of lymphocytes from human cord blood and atopic and non-atopic controls indicate St. C-75 to be a non-specific T cell mitogen. Purified major allergens from St. C-75 failed to stimulate DNA synthesis.

Lymphocytes from patients with allergic asthma to cow dander responded significantly less to an optimal concentration of the extract from cow hair and dander and to a suboptimal concentration of PHA than lymphocytes from healthy controls. We have no explanation for this phenomenon. It might be caused by a T cell defect, but since lymphocytes from all of the cow allergics gave a normal response to mitogen and microbial antigens in optimal concentration a general T cell defect is hardly to be expected. However, a defect restricted to a specific subpopulation of T cells is possible. The responsiveness to suboptimal concentrations of a given mitogen seems to be more dependent on accessory cell function than that of the optimal concentration of the same mitogen (17). So the decreased responsiveness of the cow allergic pa-

tients' lymphocytes could be explained by a defect in accessory cell function. Inhibitory substances produced by accessory cells might also influence the proliferative responses.

As suggested by the present study the proliferative response to St. C-75 seems to be non-specific, and since the pronounced IgE antibody production seen in cow allergic patients is antigen specific, the two reactions may represent two completely different parts of the immunoreactivity.

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