

Suppressor T-lymphocyte activity in wheezy children with and without treatment by hyposensitization

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Summary

The suppressor cell activity of peripheral blood lymphocytes was investigated in wheezy children by determining the ability of concanavalin A induced suppressor cells to inhibit a mitogen provoked proliferative response. The activity was compared with that of eighteen healthy controls. Of ten infants aged from 5 months to 4½ years, with recurrent wheezy bronchitis, three had much reduced suppressor cell activity (2 s.d. or more below that of controls). Of twelve asthmatic children aged from 4½ to 12 years, seven had much reduced suppressor cell activity. The remaining five asthmatic children with normal suppressor cell activity had all received hyposensitization therapy. We speculate that immune hyposensitization therapy might act by stimulating lymphocyte suppressor activity.

Introduction

Human peripheral blood lymphocytes are composed of a number of subsets. Among these the role of the suppressor cell subset is to decrease the immune response. This subset has been shown to exhibit diminished activity in eczema, active lupus erythaematosus and inflammatory bowel disease (Ogdan, Krueger & Hill, 1979; Miller & Schwartz, 1979; Hodgson, Wands & Isselbacher, 1978). Based on experiments in animals a correlation has been found between T suppressor cells and the IgE antibody response (Tada, 1974; Tada, Taniguchi & Takemori, 1975; Okumara & Tada, 1975). Circumstantial evidence such as elevated IgE levels in anergic patients with the Wiskott–Aldrich syndrome (Waldman *et al.*, 1972) and patients with the Di George syndrome (Kikkawa *et al.*, 1973) support the concept of a correlation between suppressor T cells and the reaginic response. In bronchial asthma Goldman *et al.* (1974) have shown reduced numbers of T cells together with eosinophilia and increased IgE levels, and Strannegård, Lindholm & Strannegård (1976) found decreased T-cell function in children with this disease.

A recent paper by Martinez *et al.* (1979) stated that no differences were detected

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between allergic subjects and healthy controls with respect to non-specific suppressor-cell function. This was shown by the ability of concanavalin A (con A) induced suppressor cells to depress polyvalent immunoglobulin synthesis, IgE secretion or mitogen induced proliferative responses. On the other hand, using the Fc receptor for IgG (Tg) as a marker for T suppressor cells, Canonica *et al.* (1979) have shown reduced Tg cells in blood samples of patients with respiratory allergic disease. After specific hyposensitization, the Tg cell subset reached normal values coinciding with an improvement of symptoms. Because of the divergent results we decided to study the suppressor cell activity of peripheral blood lymphocytes from patients suffering from recurrent bronchitis or asthma, by analysing the activity of the non-specific con A induced suppressor cells to depress a mitogen induced proliferative response.

Patients

The first group of patients comprised ten children with recurrent wheezy bronchitis who had suffered from more than three attacks of wheezing cough, shortness of breath and prolonged expiration without temperature. Some of these children were on prophylactic treatment with aminophylline. The children in this group were aged between 5 months and 4½ years.

The second group comprised twelve children aged from 4½ to 12 years suffering from typical recurrent attacks of asthma with reversible airways obstruction demonstrated by lung function testing. Within this group, patients with chronic perennial asthma who had a significant positive skin test and bronchial challenge to a specific allergen, received hyposensitization therapy.

All these patients received salbutamol when needed. All except one received prophylactic treatment: four received aminophylline, four cromolyn sodium, three steroid inhalations and one alternate day oral steroids.

A third group of eighteen normal non-atopic individuals served as a control. They were in the same range as the previous groups.

Methods

Mononuclear cell fractionation

Peripheral blood mononuclear cells were obtained by the standard Ficoll-Hypaque density gradient separation (Boyum, 1968). The cells were collected from the interface and washed three times with phosphate-buffered saline, pH 7.2. Following the last wash, the pellet was resuspended in tissue culture medium RPMI-1640 supplemented with 10% heat inactivated foetal calf serum, 5×10^{-5} 2-mercaptoethanol and 2 mM glutamine.

Suppressor cell activation

Five million cells in 1 ml of tissue culture medium were incubated in the presence of 10 µg con A in tissue culture plates (Costar 3035) in a humidified 37°C, 5% CO₂ incubator. Following 48 hr incubation the cells were collected, washed three times to remove residual con A and treated with mitomycin C to prevent further proliferation. The con A-induced suppressor cells were then cultured together with fresh peripheral blood mononuclear cells, and the phytohaemagglutinin-induced proliferative response was assessed. The cells were cultured in triplicates in microculture plates (Costar 3596) in concentrations of 1×10^5 suppressor and 1×10^5 responder cells per well. The cultures

were incubated for 72 hr in a 37°C humidified incubator in the presence of 5% CO₂. At the end of the incubation the cells were pulsed with 1 μ Ci tritiated thymidine for 6 hr and harvested with a multiple harvester.

The percentage of suppression was calculated by the formula:

$$\left(1 - \frac{\text{ct/min in the presence of suppressor cells}}{\text{ct/min in the absence of suppressor cells}}\right) \times 100.$$

Results

The suppression activity of cells derived from twelve asthmatic children, ten children suffering from wheezy bronchitis and eighteen healthy controls is shown in Fig. 1. The mean suppression in the control group was 56 ± 11 s.d.% and therefore the lower limit of normal was taken as 2 standard deviations below the mean, namely 34%. It was found that seven out of twelve asthmatic children showed suppressor cell activity below this lower limit of normal. The five remaining patients whose suppressor cell activity was within our normal range had all received specific hyposensitization treatment. Only four out of the twelve asthmatics received aminophyllin. There was therefore no relationship between the medications which the patients received and suppressor cell activity.

Seven out of the ten children with wheezy bronchitis showed normal suppressor cell activity while the other three were similar to the asthmatic patients showing reduced activity. Of the five infants below 18 months of age, two showed reduced suppressor cell activity and three were normal.

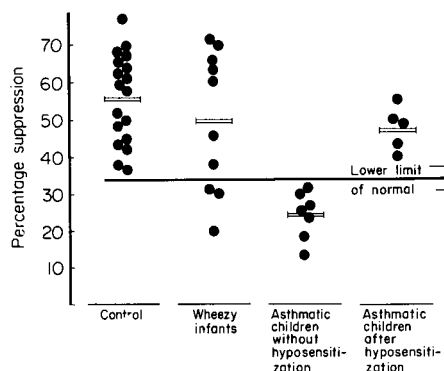


Fig. 1. Suppressor cell activity in wheezy infants and asthmatic children before and after hyposensitization.

Discussion

These studies demonstrate that asthmatic patients often have reduced suppressor cell activity and despite the different methods used confirm the results of Canonica *et al.* (1979). Only those asthmatic patients who had received hyposensitization had normal suppressor cell activity in our studies. Similar results were obtained by Rocklin *et al.* (1980). They have shown in ragweed hay fever that desensitization results in the generation of antigen-specific suppressor cells which may be associated with the efficacy of therapy. In view of the multiplicity of antigens in our patients this methodology was not applicable in our investigations.

We were unable to relate the severity of the asthma to the degree of suppression but the number of patients was small. It is interesting, however, that the two lower levels of suppression in our hyposensitized patients were from the children most recently started

on this type of treatment. In the wheezy bronchitis group we also found both normal and reduced suppressor cell activity. It has been generally recognized that only some 20 to 40% of wheezy infants continue to become childhood asthmatics (Boese, 1953). It is interesting to speculate on the possibility that wheezy infants with low suppressor activity become asthmatic while those with normal activity lose their symptoms at an early age. Clearly we shall need to follow up our patients to see if this occurs.

It has been suggested (Soothill *et al.*, 1976) that a relative immunoglobulin deficiency in early infancy allows the development of atopic disease (eczema) in children. The present study supports the concept of a cellular immune defect in asthma, i.e. the diminished activity of T suppressor lymphocytes and that hyposensitization therapy acts by correcting this deficiency.

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