

Cellular immunity and IgE levels in asthmatic children

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Summary

Several reports have suggested that asthmatic children may be particularly vulnerable to viral respiratory infections, in addition to this, abnormal cellular regulation has been suggested as an explanation for the increased IgE levels in atopy. Despite such evidence there have been few studies evaluating cellular immune function in asthmatic children. This study has examined *in vivo* and *in vitro* aspects of cellular immune function in twenty-six atopic asthmatic children and compared them with nineteen age-matched non-atopic control subjects. *In vivo* cellular immunity was assessed by quantitating the cutaneous delayed hypersensitivity (CDH) response to four ubiquitous antigens. Lymphocyte responsiveness to three polyclonal mitogens was measured *in vitro*. The serum IgE level was determined in all study subjects. The results indicate that CDH to *Candida* antigen is significantly diminished among the asthmatic subjects and lessened to streptokinase/streptodornase. Lymphocyte mitogen response did not differ significantly between the study groups. No correlation could be shown between *in vitro* lymphocyte responsiveness and IgE level. These studies suggest that a subtle cellular immune defect may exist in asthmatic children and support the need for further studies of this question.

Introduction

The integrity of the cellular immune system in asthmatic subjects has been questioned by observations from at least two separate points of inquiry. A number of studies of viral infection in asthmatics have suggested that as a group asthmatics may be particularly susceptible to such infections. Minor *et al.* (1974) have reported that a group of asthmatic children had significantly more viral infections than did their non-asthmatic siblings. Horn, Reed and Taylor (1979) likewise reported that children with asthmatic bronchitis developed recurrent viral infections with unusual frequency.

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Since host defence against primary viral infections is known to be mediated by cell-mediated immune (CMI) mechanisms, it is appropriate to question whether CMI is intact in the asthmatic. In addition to these observations, it has been suggested that atopic persons may produce excess IgE because of a failure of their thymus derived lymphocytes (T cells) to mediate suppressor cell activity as suggested by Tada, Taniguchi and Okumura (1971). It is of interest that the T cell is also the lymphocytic mediator of CMI. Grove *et al.* (1975) have examined T cell function in a group of adult asthmatics and reported a defect to be present. Previous studies from this laboratory examining T-cell numbers in the peripheral blood found asthmatic and normal children to be similar (Saraclar, McGeady & Mansmann, 1977). The present study examines *in vivo* and *in vitro* aspects of CMI in a group of twenty-six asthmatic children with demonstrated atopy, comparing them with age matched non-atopic control subjects. Attempt is made to correlate mitogen responsiveness with serum IgE levels.

Materials and methods

Study subjects

Twenty-six asthmatic children ranging in age from 1 to 15 years (mean age 8.5 years) were studied. All subjects demonstrated positive immediate hypersensitivity skin tests to several pollen and/or inhalant allergens. Ten of the group had a history of atopic dermatitis, however, only minimal flexural lichenification was present at the time of the study. Fourteen of the group received 5–25 mg prednisone in a single 8 a.m. dose. Thirteen of the group were receiving weekly hyposensitization injections. All study subjects received theophylline, antihistamines and some received catecholamines as well. Blood for *in vitro* studies was always drawn at 8 a.m. prior to medications being given.

Control subjects were nineteen children of mean age 7.5 years, hospitalized for child abuse, trauma, or orthopedic problems. No control subject was receiving medications and none were atopic or asthmatic as determined by history or physical examination. Written informed consent was obtained from the parent or legal guardian of each subject.

Serum IgE levels

The serum concentration of IgE was measured with the commercially available Phadebas IgE test kit.

Cutaneous delayed hypersensitivity (CDH)

Cell-mediated immunity was evaluated in all subjects by intracutaneous skin testing with a battery of ubiquitous antigens. These included *Candida albicans* (Hollister-Steir, 1:10 w/v in buffered saline), streptokinase/streptodornase (SK/SD) (Varidase, Lederle), trichophyton (Hollister-Steir, 1:10 w/v in buffered saline), and PPD (Connaught) intermediate strength. Testing with *Candida albicans*, SK/SD, and trichophyton consisted of an intracutaneous injection of 0.1 ml of the most dilute concentration of the antigen. The injection site was observed at 48 and 72 hr and the diameter of induration recorded. Positive response was considered to be greater than 5 mm of induration. If a negative response was noted, the test was repeated using more concentrated antigen. Concentrations of antigen employed included candida 1:100 and 1:10 (i.e. undiluted), trichophyton 1:1000 and 1:100, and SK/SD 100/25 units and

1000/250 units. Fresh SK/SD was prepared every 2 weeks to ensure stability. Only intermediate strength PPD was tested in these subjects.

Lymphocyte transformation studies

Lymphocyte cultures were performed in microtitre plates using a modification of the method of Hartzman *et al.* (1971) as previously described (McGeady & Buckley, 1975). In brief, lymphocytes were separated from heparinized blood on Ficoll-hypaque gradients, washed and adjusted to a concentration of $1 \times 10^6/\text{ml}$. To each well of the microtitre plate 0.1 ml of the cell suspension was added together with 0.1 ml of the mitogen. Multiple doses of mitogen were employed and parallel cultures were grown in 20% autologous plasma and 20% AB plasma. Cultures were performed in quadruplicate and harvested after time periods shown previously to provide maximum stimulation for the particular mitogen. Before harvesting the cells were pulsed with $2 \mu\text{C}$ of methyl-tritiated thymidine for 6 hr, they were harvested onto filter paper and the tritium incorporation was counted in a Beckman liquid scintillation counter.

Statistical methods

Serum IgE levels between atopic and control subjects were compared by the Kruskal-Wallis test, and CDH responses were compared between atopic and control subjects using two-by-two table comparison with Fisher's Exact Test.

Maximal lymphocytic proliferation response was analysed by logarithmic transformation of the maximal counts per minute (ct/min) to each mitogen. Two-way and three-way analyses of variance were performed to detect possible intra-group differences due to steroid therapy, hyposensitization or associated atopic eczema. The 'f test' was used to determine significance throughout.

Results

The results of IgE determinations are tabulated in Table 1. As expected, non-atopic control subjects demonstrated the lowest levels (Gp A), atopic asthmatics (Gp B) had somewhat higher IgE levels but not significantly higher. Asthmatics with history of atopic dermatitis (Gp C) were found to have the highest IgE levels and these levels were significantly higher than both normal and asthmatic levels. Combination of Gps B & C into a single atopic group, revealed significantly higher IgE levels ($P < 0.01$).

Table 1. Comparison of IgE levels

Group	Group A Control (<i>n</i> = 19)	Group B Asthma alone (<i>n</i> = 16)	Group C Asthma and atopic dermatitis (<i>n</i> = 10)	Total atopic B + C (<i>n</i> = 26)
Serum IgE in ng/ml	Range 47–702 Median = 68	Range 30–1075 Median = 120	Range 90–19 350 Median = 404	Range 30–19 350 Median = 259
Statistical significance by Kruskal- Wallis Test	A : B <i>P</i> = Not significant	B : C <i>P</i> < 0.05	A : C <i>P</i> < 0.001	A : B + C <i>P</i> < 0.01

Table 2. Comparison of CDH responses

Antigen	Subjects		Significance
	Group A Control	Groups B & C Asthmatic	
<i>Candida</i> 1/100	19	15	$P < 0.05$
1/10	ND	2	
No response	0	2	
Not tested	0	7	
SK/SD 100/25 u/ml	9	11	$P = NS$
1,000/250 u/ml	8	5	
No response	0	3	
Not tested	2	7	
Trichophyton 1/1000	1	1	$P = NS$
1/100	8	4	
No response	9	14	
Not tested	1	7	

ND = not done

Cutaneous delayed hypersensitivity (Table 2)

In the atopic group one subject failed to mount CDH to any of the four antigens, while in the control group all subjects had some positive response. Fifteen of the twenty-one asthmatics tested to *Candida* antigen responded to 1:100 dilution and 5/21 to undiluted antigen (i.e. 1:10). All control subjects responded to the 1:100 dilution of *Candida* antigen. The difference between the responses of these groups to *Candida* is significant ($P < 0.05$). Intra-group comparison of the atopic subjects showed that the effects of steroid therapy or atopic dermatitis alone could not explain this difference from the control subjects. The comparison of CDH reactivity to the various strengths of SK/SD and trichophyton revealed no statistically significant differences between atopic and control groups. All study and control subjects were non-reactive to intermediate strength PPD.

Table 3. Maximum lymphocytic mitogen response

Mitogen	Plasma	Asthmatics ($n = 26$)	Control ($n = 19$)	P value
PHA	auto	127.83	138.41	NS
	AB	124.88	98.90	NS
Con A	auto	55.08	43.41	NS
	AB	14.95	14.70	NS
PWM	auto	23.40	15.38	NS
	AB	9.32	6.10	NS

Geometric mean [^3H] thymidine uptake in $\text{ct/min} \times 10^{-3}$

Table 4. Maximum PHA response

Asthma sub-group	Plasma	Asthmatic group	Control	P value
Steroid Rx (n = 14)	auto AB	154.16 190.16	138.41 98.90	NS < 0.05
Non-steroid Rx (n = 12)	auto AB	102.74 76.47	138.41 98.90	NS NS

Lymphocyte transformation studies

The geometric means of maximal lymphocyte mitogen response are compared between the atopic and control groups in Table 3. No significant differences can be shown. These findings are similar both in autologous and in AB plasma. The sub-groups of asthmatic subjects (i.e. steroid treated, having atopic dermatitis, and hyposensitized) were compared using three-way analysis of variance. Analysis revealed that response to PHA stimulation was greater in steroid treated asthmatics than in the control subjects (Table 4); in AB plasma this difference was statistically significant. Hyposensitization and atopic dermatitis did not appear to influence PHA response. Response to Con A and PWM was not found to be significantly different among these subgroups.

Correlation of lymphocyte response with IgE levels

To test the hypothesis that a suppressor T cell defect might permit the production of increased amounts of IgE, we sought an inverse correlation between maximum response to the T cell mitogens PHA and Con A and the serum IgE level. Analysis revealed that the coefficients of correlation between mitogen response and IgE level for both the atopic and control subjects were not significant. Neither direct nor inverse correlation was demonstrated.

Discussion

The integrity of CMI in the asthmatic patient has been questioned. The postulated deficiency might account for the proneness to viral infection observed among asthmatic children, and might also be related to the excessive IgE production of atopy. This study has examined *in vivo* and *in vitro* components of CMI in a group of atopic asthmatic children, comparing them to age matched non-atopic, non-asthmatic control subjects. Our findings support a subtle CMI defect in the asthmatic group seen *in vivo*. This assertion is based upon the generally diminished CDH responsiveness to ubiquitous antigens in the asthmatic group compared to non-atopic subjects. Diminished CDH responsiveness is statistically significant only for *Candida* antigen, however, the tendency to lessened response could be observed in SK/SD response as well. As noted above, the corticosteroid treatment of one asthmatic subgroup did not account for this diminished response to ubiquitous antigen. The finding of normal *in vitro* CMI correlate (i.e. mitogen response) in the asthmatic group in the face of diminished *in vivo* CMI function is of interest. Since the manifestation of CDH is known to be dependent upon the monocyte-macrophage population in addition to T

cells, it may be that the atopic asthmatic group suffers from a defect occurring subsequent to the T-cell activation event itself. A deficiency of this type might involve inadequate lymphokine production or the unavailability or unresponsiveness of monocyte-macrophage cells to lymphokine. A minor abnormality would be expected, since the atopic asthmatic population clearly do not suffer from severe T-cell dysfunction, but such a defect could explain the increased susceptibility of the asthmatic child to viral infections.

We are unable to show any direct or inverse correlation between responsiveness of lymphocytes to T-cell mitogens and the serum IgE levels. More specific tests of immunoregulatory cell function are now developed and studies employing these testing modalities are currently underway in our laboratory (Haynes & Fauci, 1977). It is also of interest that recent studies in experimental animals have suggested that the macrophage plays a significant role in the regulation of IgE production (Kudo, Sehon & Schwenk, 1981), and describes findings consistent with macrophage dysfunction. The planned studies will attempt to better define macrophage function in the atopic subject.

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