

Intrinsic and extrinsic asthma, a shared lymphocyte abnormality

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Summary

We have examined *in vitro* cell-mediated lymphocyte responses to Concanavalin A, (Con. A), and the effects of histamine and indomethacin upon these responses, in normal subjects, and patients with extrinsic and intrinsic asthma, and chronic bronchitis. Lymphocytes from both intrinsic and extrinsic asthmatics are particularly sensitive to histamine-induced suppression of their response to Con. A, and this increased sensitivity was reversed by indomethacin. In these respects, lymphocytes from intrinsic and extrinsic asthmatics behave in an identical fashion, but differ significantly from lymphocytes from both normal subjects and patients with fixed airways obstruction (chronic bronchitis). It is suggested that there is a common immunological mechanism in extrinsic asthma and intrinsic asthma.

Introduction

Rackemann (1947) suggested that as a working classification, asthmatics be divided into extrinsic and intrinsic groups. He noted that causative external allergens could not be demonstrated in the intrinsic group. There is, however, much circumstantial evidence suggesting that, as with extrinsic asthma, allergic factors may be relevant to the pathogenesis of intrinsic asthma.

A family history of asthma can be obtained in 40% of patients with intrinsic asthma, and there is an increased family history of atopy in their relations (Turner-Warwick, 1971). It has also been shown that 80% of patients with intrinsic asthma are homozygous for HLA W6—suggesting that intrinsic asthma may be a disease with recessive inheritance (Brostoff *et al.*, 1976).

Sputum from both groups of asthmatics frequently contains numerous eosinophils, and may contain mediators of hypersensitivity, such as histamine and SRS-A (Turnbull *et al.*, 1977). It has also been reported that basophils from both groups are

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coated with increased amounts of IgE, and to a lesser extent, IgG, than normal subjects (Assem *et al.*, 1971). In contrast, however, is the observation of normal levels of total circulating IgE in intrinsic asthmatics—a finding which, in the presence of sputum with eosinophils, suggests that a local, bronchial hypersensitivity may occur in intrinsic asthma. Postulated antigens, however, remain undefined.

Histamine has been shown to moderate cell-mediated immune responses, both *in vivo* (Pepys, 1955), and *in vitro* (Boot, Hudspith & Brostoff, 1980). We have previously shown (1) that lymphocytes from atopic patients, both with and without asthma, have increased histamine inhibition of their Concanavalin A response, and (2) that this increased sensitivity to histamine appears to be prostaglandin mediated, in as much as the cyclo-oxygenase inhibitor, indomethacin, reduces this increased suppression to levels seen in normal subjects (Boot, Hudspith & Brostoff, 1980; Hall, Hudspith & Brostoff, 1982).

This study was undertaken to compare the *in vitro* cell-mediated lymphocyte responses in normal subjects, intrinsic and extrinsic asthmatics, and patients with chronic bronchitis, in order to investigate whether both groups of asthmatics share a similar pattern of *in-vitro* immunological responsiveness, and whether they could be differentiated from the chronic bronchitics. Further, if this were to be the case, it may be possible to develop an *in-vitro* laboratory test to avoid the necessity of performing steroid trials to identify those patients with asthma, who are refractory to bronchodilators.

Materials and methods

We studied ten normal subjects (mean age 51.3, range 23–68), ten intrinsic asthmatics (mean age 61.4, range 33–71), ten extrinsic asthmatics (mean age 41.9, range 28–60) and ten patients with chronic bronchitis (mean age 64.0, range 56–75). Patients were selected from the Out-Patient Department of the Middlesex Hospital. On the day of investigation, patients were venesected, after which they underwent skin-prick testing to ten common allergens. They also had full lung-function testing, including measuring the response to inhaled Salbutamol. Drug therapy was noted (Table 1).

Asthma was defined as an improvement in forced expiratory volume in one second (FEV₁), of greater than 20% after inhalation of Salbutamol. Subjects with asthma were divided into extrinsic or intrinsic, according to their response to skin-prick testing to ten common allergens. Those patients who showed no evidence of reversibility of their airways obstruction after Salbutamol constituted the group with fixed airways obstruction. All the patients in this latter group fulfilled the MRC criteria for the diagnosis of chronic bronchitis.

Twenty ml of venous blood diluted 1:1 with balanced salt solution was layered

Table 1. Patient drug treatment

Patient group	<i>n</i>	Inhaled sympathomimetic	Oral sympathomimetic or xanthine	Inhaled steroid	Oral steroid
Chronic bronchitics	10	10	5	7	2
Intrinsic asthmatics	10	10	4	9	2
Atopic asthmatics	10	9	4	6	3

onto Ficoll-isopaque gradients, centrifuged at 400 g for 35 min at 18°C, and then the mononuclear cells at the interface washed three times with RPMI 1640, containing 2 mM glutamine and 10% heat-inactivated foetal calf serum. The cells were then resuspended in RPMI 1640, containing 2 mM glutamine and 20% heat-inactivated foetal calf serum at a concentration of 2×10^6 cells ml⁻¹. 100 µl of cell suspension was used for each experiment, and all experiments were done in triplicate, in microtitre trays.

The pharmacological agents used were added for the duration of the culture. Cultures were performed with the mitogen Concanavalin A, at a concentration of 2 µg ml⁻¹, and a parallel set of experiments was run as controls without mitogen. The drugs under investigation, histamine and indomethacin, were used at a final concentration of 10^{-4} M and 1 µg ml⁻¹ respectively.

Cultures were made with no added agents, with 20 µl histamine, with 20 µl indomethacin, and with 20 µl of both histamine and indomethacin, to give the concentrations stated above. Where appropriate, 20 µl of Concanavalin A was added. The volume of each culture was adjusted to 200 µl using RPMI 1640.

The cells were incubated at 37°C for 48 h in an atmosphere of 5% CO₂ in air, after which tritiated thymidine (0.2 µCi well⁻¹) was added, and the cultures incubated for a further 18 h. The cells were then harvested on to paper discs, using an Ilacon harvester, dried, and the incorporation of radiolabel into the cells determined by liquid scintillation counting.

Calculations

Results are shown as counts per minute (CPM) or as a percentage of control values, where the control value is for Con. A stimulated cells alone.

Calculations were made as follows:

$$\text{Histamine suppression} = \frac{\text{CPM (mitogen and histamine)} \times 100}{\text{CPM mitogen alone}}$$

Indomethacin reversal of histamine suppression =

$$\frac{\text{CPM (mitogen and histamine and indomethacin)} \times 100}{\text{CPM (mitogen and indomethacin)}}$$

All appropriate background counts from unstimulated cultures were subtracted.

Statistics

Comparisons between groups were made using analysis of variance, and Student's *t*-test for unpaired data.

Results

Effect of Concanavalin A, and Concanavalin A plus indomethacin, on lymphocyte transformation (Table 2)

There was no significant difference in responses between normal subjects, and both groups of asthmatics. The group with chronic bronchitis however, showed significantly lower transformation ($P < 0.001$) in response to Con. A when compared to all the other groups. However, this group showed a significantly greater indomethacin-

Table 2. Effect of Concanavalin A, and Concanavalin A plus indomethacin, on lymphocyte transformation in patients with airways obstruction

Patient group	n	Effect of Con. A alone.		Effect of Con. A and indomethacin.	
		CPM, mean $\pm$ s.e.m.	P vs. normals	CPM, mean $\pm$ s.e.m.	P vs. Con. A alone
Normal subjects	10	7453 $\pm$ 679		7898 $\pm$ 649	ns
Extrinsic asthmatics	10	7304 $\pm$ 766	ns	7642 $\pm$ 793	ns
Intrinsic asthmatics	10	6837 $\pm$ 610	ns	7233 $\pm$ 923	ns
Chronic bronchitics	10	4261 $\pm$ 396	$P < 0.001$	5434 $\pm$ 466	$P < 0.05$

ns, not significant.

induced enhancement of the Con. A response ($P < 0.05$) than the other groups, normal subjects and both groups of asthmatics producing a slight, but insignificant, enhancement of response.

Effect of histamine alone, and histamine plus indomethacin, on Concanavalin A response (Fig. 1)

Both extrinsic and intrinsic asthmatic groups showed significantly greater sensitivity to histamine-induced suppression of Con. A response when compared to normal subjects ($P < 0.001$, $P < 0.01$ respectively). There was no significant difference between the two asthmatic groups. This increased suppression was reversed to that seen in normal subjects, by indomethacin, to the same degree in both asthmatic groups.

Lymphocytes from patients with chronic bronchitis also showed significantly greater histamine-induced suppression of their Con. A response than normal persons ($P < 0.05$), but no reversal of this suppression was seen with indomethacin.

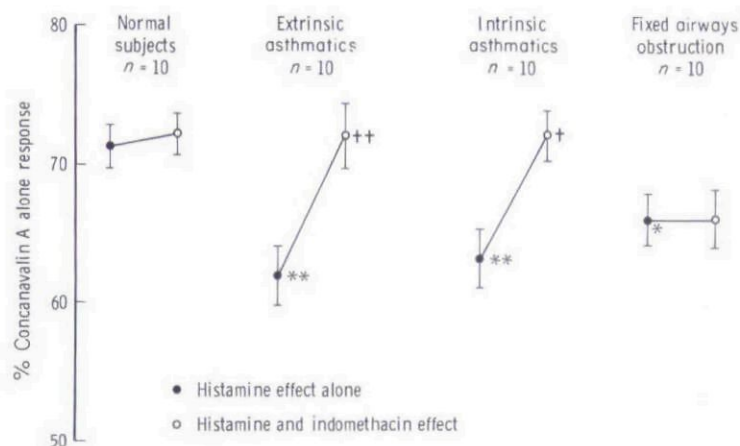


Fig. 1. The effect of histamine alone, and histamine plus indomethacin, on the response of lymphocytes from patients with airways obstruction to Concanavalin A. Mean $\pm$ s.e.m. Histamine effect: P vs. normal subjects: * $P < 0.05$, ** $P < 0.001$. Histamine and indomethacin: P vs. histamine alone: + $P < 0.01$, ++ $P < 0.001$.

Discussion

On examining the responses of lymphocytes from normal subjects, and patients with extrinsic asthma, intrinsic asthma and fixed airways obstruction, we have found no significant difference between the extrinsic and intrinsic asthmatic groups in respect of *in-vitro* histamine-induced suppression of lymphocyte transformation and its reversibility by indomethacin. These asthmatic groups did, however, differ significantly from normal subjects and the group with fixed airways obstruction in this respect.

Concentrations of Concanavalin A ($2 \mu\text{g ml}^{-1}$), and histamine (10^{-4} M), were chosen to produce the maximum suppressive effect in atopics, compatible with non-toxicity, and based upon previous experience (Boot, Hudspith & Brostoff, 1980). The concentration of indomethacin ($1 \mu\text{g ml}^{-1}$) is the standard level shown to cause maximum *in-vitro* suppression of the cyclo-oxygenase system, without effects on other enzyme systems (Hall, Hudspith & Brostoff, 1983).

The immunological significance of the effect of histamine on lymphocyte transformation is not known. It has been proposed that histamine released at the site of Type-I hypersensitivity reaction may suppress transformation of lymphocytes and prevent the development of the more harmful effects of cell-mediated immunity (Boot, Hudspith & Brostoff, 1980). The reversal of the increased effect of histamine seen in both groups of asthmatics by indomethacin, a prostaglandin synthetase inhibitor, suggests that this increased histamine effect is prostaglandin mediated. The group with fixed airways obstruction appear to form a separate group, being relatively anergic to the mitogen used, and showing a greater enhancement of mitogen effect by indomethacin. In this respect, fixed airways obstruction resembles other conditions associated with the presence of activated monocytes and macrophages, for example, sarcoidosis (Johnson *et al.*, 1981).

Although β adrenergic agents and xanthines have been shown to influence *in-vitro* lymphocyte responses, when added to cell cultures (Hirschorn, Grossman & Weissman, 1970), this is unlikely to explain our results. First, the histamine effect has been observed in untreated atopic subjects with rhinitis, and hay fever (Boot, Hudspith & Brostoff, 1980). Secondly, the asthmatic groups differed significantly from the group with chronic bronchitis, despite all three groups receiving comparable treatment (Table 1).

We believe the similarity shown between lymphocyte responses in the two asthmatic groups suggests that extrinsic and intrinsic asthma share a similar immunological abnormality, which is not seen in patients with fixed airways obstruction. This is supported by the finding of Assem *et al.* (1971), the presence of sputum and occasional blood eosinophilia in both asthmatic groups, the genetic data suggesting a link between intrinsic and extrinsic asthma, and the variable, but occasionally beneficial, effect of sodium cromoglycate in intrinsic asthma.

The failure of skin-prick testing to demonstrate hypersensitivity in intrinsic asthma suggests the possibility of local hypersensitivity, restricted to the bronchial tree. The concept of a hypersensitivity reaction being causative receives further support from the frequent failure to obtain evidence of an infecting organism during 'exacerbations' of intrinsic asthma. In one study, infection could only be demonstrated in twelve out of 111 'exacerbations' in fifty-one asthmatics, suggesting other mechanisms may be active (Clark, 1979).

Further research is required to elucidate possible antigens in intrinsic asthma, and their site(s) of action. Lessof (1980) has shown that ingestion of antigens may cause

asthma, so it is also necessary to investigate possible portals of antigen entry, other than the traditionally inhaled route. In addition, the finding that these *in-vitro* responses appear able to clearly separate patients with reversible airways obstruction (asthma), from those with fixed airways obstruction (chronic bronchitis), raises the important possibility that an *in-vitro* test may be able to obviate the necessity of performing high-dosage corticosteroid trials on patients with significant airways obstruction, refractory to broncho-dilator therapy.

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