

Prediction of late asthmatic responses to inhaled allergen

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Summary

Relationships between cutaneous and bronchial responses to allergen were examined in nineteen atopic asthmatics. Allergen inhalation tests elicited an isolated early asthmatic response (EAR) in ten subjects and a dual asthmatic response (DAR) in nine subjects. Ragweed IgE RAST, performed with the sera of those patients tested with ragweed antigen, yielded higher values in all but one patient who experienced DAR than any of the patients with EAR. In one patient with annual symptoms in the ragweed season, positive skin tests with ragweed antigen and DAR to inhaled ragweed extracts, the IgE RAST was entirely negative and the serum IgE concentration was low. Dilutions of the allergen used in each individual for inhalation were also used in skin-prick tests. Early cutaneous allergic response (ECAR) mean wheal diameters were obtained at 10 min and late cutaneous allergic response (LCAR) mean diameters at 6–8 hr. Early asthmatic response (EAR) subjects differed modestly from DAR subjects in the relationships between ECAR and LCAR; in the EAR group, a significantly larger wheal diameter ($P < 0.01$) was required before an LCAR ensued, however there was some overlap. Once LCAR developed, there was no difference between EAR and DAR groups in the magnitude of the LCAR. There was a trend (not significant) towards a requirement for a higher antigen concentration in the EAR group to elicit an LCAR.

In conclusion, correlates of an isolated EAR from inhaled antigen include: (i) a low positive IgE RAST result with the antigen, (ii) ECAR 6 mm or greater with the antigen which does not proceed to a LCAR, and (iii) a high concentration of antigen is needed in skin tests to elicit an LCAR. Correlates of a DAR include: (i) a high IgE RAST result with the antigen, (ii) an ECAR wheal diameter less than 5 mm with the antigen

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proceeds to a LCAR, and (iii) a low antigen concentration in skin tests elicits an LCAR.

The observed correspondence between the tendency to late skin and late airway responses is evidence of a common immunologic basis.

Introduction

In sensitized individuals, allergen can elicit late responses in different organs including the skin (Dolovich *et al.*, 1973; Solley *et al.*, 1976), nasal mucosa (Pelikan, 1978; Dvoracek *et al.*, 1979) and bronchi (Herxheimer, 1952; Robertson *et al.*, 1974). The skin responds with a wheal and flare maximal at about 10–15 min. If it is sufficiently large, it does not entirely disappear in the next hour or so; at the test site there appears an enlarging zone of erythema and swelling which is usually maximal at 6–12 hr and disappears by 24–28 hr. Clinically, the LCAR is probably responsible for many of the local reactions to insect stings and allergen injections and comparable processes are probably involved in urticaria and angioedema. Following allergen inhalation, an EAR may be followed by a LAR and the latter is more prolonged and often more intense than the corresponding LAR. Moreover, a DAR, but not an EAR, is followed by increased non-specific bronchial responsiveness from allergen inhalation under natural (Boulet *et al.*, 1983) or laboratory (Cartier *et al.*, 1982) conditions.

The relationship between late skin and late asthmatic responses has received relatively little attention. Richardson *et al.* (1979) did not find any relationship between skin and late nasal responses. However, Robertson *et al.* (1974) noted that skin-prick tests with ragweed extract in a group of subjects with DAR elicited larger wheal diameters than in a group with isolated EAR. In the latter study, serum IgE antibodies to ragweed antigen, measured at that time by a relatively insensitive method, were detected in subjects with DAR but not EAR and an involvement of these IgE antibodies in the pathogenesis of the late asthmatic responses was postulated.

In nineteen atopic asthmatics, we have now examined relationships between airway responses and serum IgE antibody levels, cutaneous responses and antigen doses. A practical objective was to determine whether it would be possible to identify correlates which might permit a predication of the occurrence of a LAR to inhaled antigen.

Materials and methods

Subjects

Nineteen atopic asthmatics attending the Firestone Regional Chest and Allergy Clinic at St Joseph's Hospital in Hamilton volunteered to take part in the study (Table 1). All had a past history of episodic dyspnea with wheezing or chest tightness on exposure to one or more airborne allergens and demonstrated FEV₁ variability of greater than 20%. All were atopic as indicated by positive results to at least one common airborne allergen on skin-prick testing with sixteen common allergens. A skin-test index was calculated by taking the sum of the grades (0–4) of positive skin tests. None of the subjects had received allergen injection treatment. At the time of the allergen challenge, the asthma was stable in each case with little or no requirement for medication.

Study design

All subjects had allergen inhalation tests in the preceding year. Each attended the laboratory during the winter of 1982 for provision of a serum specimen after which skin tests with serially diluted antigen were done.

Allergen extracts

Defatted powder extracts of ragweed pollen, grass pollen, and cat epidermis were purchased from Greer Laboratories, Lenoir, North Carolina. They were dissolved in phosphate-buffered saline and after dialysis, the protein content was determined to be 2.6 mg/ml for grass pollen, 0.41 mg/ml for cat antigen and 3.5 mg/ml for ragweed antigen. The ragweed antigen E content was determined to be 167 μ g/ml by the single-radial-diffusion method performed with a monospecific antiserum produced locally and a reference whole ragweed extract with specified IgE content provided by the Bureau of Biologics of the N.I.H. of the U.S.A. Human serum albumin 0.03%, was added as stabilizer and these solutions were stored in aliquots at -20°C . On the eve of the skin tests, 10-fold dilutions were made in phosphate-buffered saline and stored at 4°C until used.

Allergen inhalation tests

Control and allergen inhalation tests were performed by the method described by Cartier *et al.* (1982). Aerosols were continuously generated by a Wright nebulizer using air at 50 psi and 7 l/min giving an output of 0.13 ml/min. They were delivered directly into a mask held loosely over the mouth and clipped nose. During tidal breathing, increasing doses of allergen were administered for 30 sec at intervals of 10 min. After observation of a fall of 20% in FEV₁ post-allergen inhalation, FEV₁ measurements were repeated at 20, 30, 40, 50 and 60 min and then hourly for 8 hr to note any occurrence of LAR, defined as a fall in FEV₁ of 15% or greater from the baseline at 3–8 hr after the challenge.

Skin tests

A series of skin-prick tests was performed on each subject with 10-fold dilutions of the same allergen used for the inhalation test. The intent was to elicit reactions with a series of ECAR wheal diameters from 0 to 10 mm. The site of injection was randomized by the method of Latin squares, always in the middle part of the forearm with a distance of at least 3 cm between each test site. No skin tests had been performed on the arms of the subjects in the preceding 6 months. The early skin responses were read at exactly 10 min. Two perpendicular diameters were measured and the mean wheal diameter was calculated. Skin-test sites were examined at 6–8 hr by an observer who was uninformed of the arrangement of the skin-test sites, the exact concentrations of antigen required and the type of asthmatic response experienced by the subject. The results represent the mean values of two duplicate skin tests performed at the same time.

Immunologic tests

Serum IgE antibodies to ragweed were quantitated by the use of cellulose discs to which were conjugated locally prepared ragweed pollen extract (see above) and ¹²⁵I-labelled anti-IgE purchased from Pharmacia (Canada) Ltd. Antibody levels were expressed in relative units based on a reference serum and a standard provided by Pharmacia (Canada) Ltd.

Total serum IgE concentrations were measured by the Phadebas IgE PRIST method with kits purchased from Pharmacia (Canada) Ltd.

Analyses

Early and late cutaneous responses, antigen concentrations required in skin and inhalation tests, magnitude of fall of FEV₁ in EAR and LAR and the PC₂₀ allergen in

Table 1. Subject characteristics and results

Subject No.	Age	Sex	Sixteen allergy skin tests		Serum IgE (U/ml)	Antigen	Ragweed IgE RAST (U/ml)	Antigen provocation test			Largest ECAR not proceeding to LCAR (mm)	Smallest ECAR proceeding to LCAR (mm)	Diameter of LCAR at smallest ECAR, proceeding to LCAR	Lowest antigen conc. to produce LCAR
			No. that were 2 mm or more	skin-test index				% max. fall in FEV ₁ 1st hr	3-8 hr	PC ₂₀ Allergen				
1	36	M	6	18	180	ragweed	4.6	20.7	7.0	0.002	10	>10	0	undiluted
2	38	F	7	17	>1000	ragweed	17	19	0	0.004	5.5	8	47	undiluted
3	55	M	1	4	62	ragweed	11	27.3	13.1	0.01	6.7	9.5	66	1:10
4	36	F	4	14	81	ragweed	2.5	23	13	0.004	4.5	6.0	6.5	1:100
5	19	M	3	14	86	cat		29.4	1.5	1.0	9	>9	0	undiluted
6	25	F	9	25	990	cat		26.5	7.8	0.01	6.75	9.0	11.5	1:10
7	33	F	2	8	60	cat		21.7	2.0	0.6	6.5	6.5	6.5	undiluted
8	20	M	3	13	415	grass		24.5	0	0.01	6.75	11.5	9	undiluted
9	29	M	5	13	58	grass		28.8	8.0	0.01	7.25	8.5	5.0	1:10
10	40	F	3	17	66	grass		31.5	7.0	0.002	4.75	7.0	7.0	1:100
Mean $\pm$ 1 s.d.														
11	38	F	4.3	14.3 $\pm$ 5.1	299.8 $\pm$ 382	ragweed	14.4 $\pm$ 9.0	25.2 $\pm$ 4.1	5.9 $\pm$ 4.9	0.17 $\pm$ 0.3	6.8 $\pm$ 1.7	8.5 $\pm$ 1.7	15.9 $\pm$ 22.2	0.53 $\pm$.5
12	47	F	1	7	156	ragweed	>53	21.2	38	0.006	2.1	4.8	10.5	1:1000
13	19	F	1	7	290	ragweed	>53	32.0	36.0	0.02	5.5	7.2	13	1:100
14	39	F	10	31	460	ragweed	>53	33.8	50.0	0.06	5.25	9.5	51	1:10
15	54	F	1	3	112	ragweed	31.5	27.2	40.7	0.02	3.75	6.5	30	1:100
16	29	F	3	10	105	ragweed	28	30.8	56.44	0.004	4.25	4.75	4.5	1:100 000
17	20	M	5	13	144	ragweed	>53	17.2	36.6	0.02	3.0	5.5	21	1:100
18	29	M	2	7	11.8	ragweed	<0.035	15.7	16.2	1.5	3.5	4.25	7.25	undiluted
19	66	M	9	23	560	grass		35	39.8	0.006	4.8	6.3	9.5	1:100
			3	10	140	grass		32.6	23.9	0.02	6.5	7.5	23	undiluted
Mean $\pm$ 1 s.d.														
			3.9	12.3 $\pm$ 9.0	219.9 $\pm$ 181		38.8 $\pm$ 20.3	27.3 $\pm$ 7.4	37.5 $\pm$ 12.1	0.18 $\pm$ 0.5	4.3 $\pm$ 1.4	6.3 $\pm$ 1.7	18.9 $\pm$ 14.6	0.24 $\pm$ 0.4
P value														
							0.025			0.9	0.003	<0.01	0.48	0.186

the EAR group were compared with the DAR group by Student's *t*-test for unpaired data with double-tailed analysis. Relationships between serum IgE concentration and skin-test responses were tested by linear regression analysis.

Results

The allergen inhalation tests elicited an EAR in ten subjects and DAR in the other nine. Characteristics of the subjects are summarized in Table 1. The number of positive skin tests, the index of skin-test reactivity and serum IgE concentrations are recorded in Table 1. These did not differ significantly between the EAR and LAR groups. Linear regression analyses of the serum IgE concentration with the number of positive skin tests and skin-test index respectively yielded correlation coefficients (*r*) of 0.67 ($P=0.0013$) and 0.58 ($P=0.0059$) respectively. The mean wheal diameters of the largest ECAR which failed to proceed to LCAR were significantly higher for the group with EAR than the DAR group (means of 6.8 and 4.3 respectively). The same significant difference can be seen for the smallest wheal diameters (EAR) followed by an LCAR (mean of 8.5 and 6.8 respectively). Two subjects with EAR failed to develop LCAR despite wheal diameters of 9 and 10 mm respectively. In contrast, the diameters of the LCAR at the sites of the smallest ECAR wheals to be followed by LCAR were the same in the EAR and DAR groups with mean LCAR diameters of 15.9 and 18.9 mm respectively (Table 1). These relationships are expressed graphically in Fig. 1.

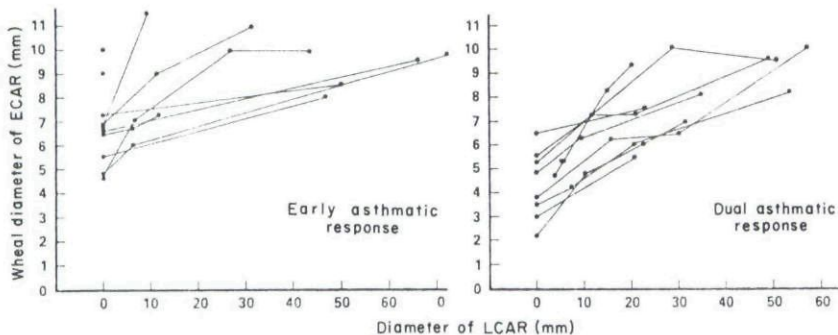


Fig. 1. Relationships between early (wheal) and late skin response diameters: skin response results for subjects with (a) early asthmatic responses (EAR) and (b) dual asthmatic responses (DAR). Wheal diameters are expressed on the vertical axis and late responses on the horizontal axis. Generally in those with EAR, larger wheal diameters were needed before LCAR ensued than was the case for DAR subjects.

A ragweed IgE RAST was performed on the sera of the twelve patients in whom the test antigen was ragweed pollen extract. The numbers are small; nevertheless, all except one subject with DAR had a higher level of IgE antibody to ragweed than the EAR subjects (Table 1). The exception (subject 17) was a patient with annual seasonal hay fever and asthma in the ragweed pollen season who had positive skin and inhalation (DAR) tests with ragweed pollen extract despite a completely negative ragweed IgE RAST and low level of total serum IgE measured at 11.8 U/ml.

Discussion

There was overlap between the EAR and DAR groups in the threshold of ECAR required for LCAR to ensue. Nevertheless, the group of subjects with EAR required

significantly larger ECAR wheal diameters before the reactions proceeded to LCAR than was the case with the DAR group. The demonstration of the relationship between airway and cutaneous responses was accomplished with careful attention to a number of factors. People with recent previous skin tests were not used in order to avoid skin test sites in a refractory phase (Shaikh *et al.*, 1977). Serial dilutions of antigen avoided wide gaps in wheal diameters. The use of a random order of skin-test sites and a 'blind' reading of responses increased the objectivity of the procedure.

Previous reports have cited differing relationships between the minimal required ECAR for the subsequent development of LCAR at test site. Solley *et al.* (1976) did not find any late response at the site of wheal diameters less than 15–20 mm while deShazo (1979) did find LCAR at a wheal size of 9.5 mm. Some studies have showed that LCAR could occur in almost anyone at ECAR responses of 8 mm or greater; the larger the ECAR, the larger the LCAR (Robertson *et al.*, 1974; Umemoto *et al.*, 1976). This latter relationship also applies to airway responses to allergen (Hargreave *et al.*, 1974; Joubert, 1979); deShazo *et al.* (1979) made the additional observation that in some subjects it was impossible to elicit LCAR with ECAR even over 18 mm, even with the simultaneous injection of histamine. The present study indicates that allergen inhalation test in these individuals would stimulate isolated EAR.

The observation of a direct relationship between patterns of cutaneous and of asthmatic responses has implications in terms of pathogenesis. Evidence has accumulated that IgE antibody may be the only immunologic reactant required for the development of both ECAR and LCAR (Dolovich *et al.*, 1973; Solley *et al.*, 1976). It has also been observed that subjects with dual asthmatic responses have tended to have higher serum concentrations of IgE antibody to the inhaled allergen than those with isolated EAR (Robertson *et al.*, 1974; Kurimoto & Baba, 1978; Boulet *et al.*, 1983). This relationship, also observed in the present study, and the observed correspondence between the skin and airways in the pattern of allergic response suggests that IgE antibody may be operative in LAR. Antibodies of the IgG₄ subclass have recently been observed to correlate with the occurrence of LAR and were postulated as a basis for the occurrence of LAR (Gwynn *et al.*, 1982). However, his claim has been contradicted (Lee *et al.*, 1982).

One other question addressed to the present study was the magnitude of LCAR in skin responses in which the wheal diameters exceeded the threshold for the development of LCAR. No relationship between the magnitude of LCAR and the pattern of airway responses was observed. The occurrence of a correspondence of this type between cutaneous and airway responses might have served as a further indication of common origins of skin and airway responses. Processes which have been postulated to underlie late responses include Arthus reactions (Pepys, 1973), fibrin deposition (deShazo *et al.*, 1979), mast-cell granules (Ortel & Kaliner, 1981), and chemotactic factors (Nagy, Lee & Kay, 1982). The information available at the present time does not permit a definitive analysis of the class or classes of antibodies or the mediators which are dominant in the development of late asthmatic responses.

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