

Immunological studies in asthmatic children undergoing antigen provocation in the skin, lung and nose

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Summary

In children with perennial asthma, dual (immediate and late) reactions in the skin to *D. pteronyssinus* and Timothy grass pollen were more frequent with high doses of antigen and were associated with large immediate reactions. The frequency of dual bronchial or nasal reactions was not related to the size of the immediate reaction and dual reactions were commonly elicited to the lowest antigen dose which would elicit an immediate reaction. Serum IgG, IgA, IgM or IgE concentrations, IgE or IgG antibodies to the antigens and defective yeast opsonization did not differ in children with dual or immediate only reactions in skin, nose or lung. In five patients undergoing bronchial provocation tests with *D. pteronyssinus* there was no fever, no fine crepitations in the lungs and no significant change in the levels of C3.

Introduction

Children with perennial asthma frequently give both immediate (20 min) and late (4-8 hr) allergic reactions in lung, skin and nose to common, environmental antigens (Van Lookeren Campagne, Knol & de Vries, 1969; Warner 1976; Price, Hey & Soothill, 1982). The immediate (type I) reactions are probably initiated by IgE-mediated degranulation of mast cells (Ishizaka, Soto & Ishizaka, 1973) and have been extensively studied in childhood asthma (Aas, 1975). The mechanism of the late bronchial reaction in children is unknown. We have therefore examined the relationship of the pattern of allergic response in the lung, skin and nose of asthmatic children to the dose of antigen (*D. pteronyssinus*, Timothy grass pollen), serum IgG, IgA, IgM and IgE levels, IgE and IgG antibody and C3 levels and to defective yeast opsonization, a common defect involving the alternative pathway of complement and associated with atopy (Turner *et al.*, 1978).

Patients and methods

Seventy children, including the twenty-one described previously (Price *et al.*, 1982),

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aged 6–14 years with perennial asthma and a positive skin test to the provocation antigens, were studied. Sixty-five of them had a bronchial provocation test and seventeen of them also had a nasal provocation test with *D. pteronyssinus*. Similarly, seventeen children had a bronchial provocation test and fifteen a nasal provocation test with Timothy grass pollen (twelve of these seventeen patients had also been challenged with *D. pteronyssinus*). The methods of skin, bronchial and nasal provocation were as described previously (Price *et al.*, 1982). The initial skin tests were inspected at 15 min and at 6 hr; an immediate reaction was defined as a weal of 3 mm or greater at 15 min, a late reaction as the presence of erythema and palpable induration at 6 hr. None were positive to the control solution. All patients also had skin tests with serial dilutions of the antigens (Fig. 1) which were used for bronchial and nasal challenge and the 15-min reactions were recorded. Six-hour reactions to these antigen dilutions were recorded in the seventeen children tested with Timothy grass pollen, but in only twenty of those tested with *D. pteronyssinus*.

An immediate bronchial reaction was defined as a fall in peak expiratory flow rate (PEFR) of greater than 20% within 20 min, and a late bronchial reaction as a fall in PEFR of at least 30% within 4 to 8 hr of bronchial provocation. Nasal provocation tests were classified as positive for ratios of stimulated to baseline values of effective nasal diameter (D_2/D_1) less than 0.8 within 20 min of provocation for immediate tests and within 4 to 8 hr of provocation for late reactions (Hey & Price, 1982).

All patients were examined clinically before bronchial provocation and during the immediate and late reactions which followed. Oral temperature was taken before provocation and during late reactions.

A blood sample was collected from most of the children before the provocation tests and serum was stored at -70° . Total IgG, IgA, IgM and C3 were measured by single radial diffusion (Fahey & McKelvey, 1965) and total IgE by a double antibody radioimmunoassay (Nye *et al.*, 1975). IgE antibodies to *D. pteronyssinus* and Timothy grass pollen were measured by the RAST procedure using allergen coated paper discs (Wide, Bennich & Johansson, 1968; Ceska, Eriksson & Varga, 1972). IgG antibodies to the F₄P₁ antigen of *D. pteronyssinus* were measured in forty-two children with positive bronchial provocation tests by a double antibody antigen-binding radioimmunoassay as described by Chapman and Platts-Mills (1978). Antibody levels were measured at two dilutions; mean values were calculated and results expressed in arbitrary binding units. In fifty children yeast opsonization was measured using the microscopic assay described by Soothill and Harvey (1976).

Results

The frequencies of positive, immediate and dual reactions in skin, lung and nose to *D. pteronyssinus* and Timothy grass pollen (Table 1) were similar to the results of a previous smaller series which included some of these patients (Price *et al.*, 1982). No patient gave a late reaction alone, but the method of nasal and bronchial testing limits our ability to detect them.

During immediate and late reactions, wheezing was heard at some time in all children; we never heard fine crepitations at the lung bases. All children had oral temperature $<37.5^\circ\text{C}$ before provocation and none had a rise in temperature $>0.5^\circ\text{C}$ within 8 hr after provocation. The mean temperature during late reactions (36.9°C) did not differ significantly from that before provocation (37.1°C).

Increasing doses of antigen applied to the skin elicited progressively larger

Table 1. Frequencies of immediate and dual reactions in three organs following challenge with two different allergens in seventy children with perennial asthma

		Negative	Immediate only	Dual	Total
<i>D. pteronyssinus</i>	Skin	0	16	49	65
	Lung	6	12	47	65
	Nose	5	9	3	17
Timothy grass pollen	Skin	0	5	12	17
	Lung	0	6	11	17
	Nose	1	5	9	15

immediate reactions and more frequent late reactions. No late reaction was seen if the immediate skin weal was less than 3 mm diameter regardless of antigen concentration (Fig. 1).

Late skin reactions were more frequent if the immediate weal to *D. pteronyssinus* or Timothy grass pollen was > 5 mm diameter ($P=0.019$ and $P=0.029$ respectively, Fisher's exact test). However, the size of the immediate reaction in the lung and in the nose gave no indication of whether a late reaction would follow (Fig. 2).

The method of bronchial and nasal testing did not permit us to analyse the effect of antigen dose, but late bronchial responses were often seen at the lowest concentrations of antigen which elicited immediate reactions.

Late bronchial reactions to *D. pteronyssinus* were not significantly more frequent in patients who gave large (> 5 mm) immediate skin weals ($P>0.05$ Fisher's exact test). (Table 2). The findings were similar for the smaller number of patients tested with Timothy grass pollen.

Serum IgG, IgA, IgM and IgE levels in children giving dual reactions in the skin, lung or nose to either of the antigens tested were not significantly different from those giving immediate reactions only ($P>0.1$ Wilcoxon rank sum). Serum IgE and IgG antibody levels to *D. pteronyssinus* and IgE antibody to Timothy grass pollen were not significantly higher ($P>0.05$, Wilcoxon rank sum) in those giving dual responses compared with those giving only immediate reactions. (Fig. 3 and Table 3).

Serum C3 was measured from five asthmatic children before bronchial provocation and 6 hr afterwards during a late bronchial reaction. All were within the normal range and there was no consistent change in the serum levels during the late reactions (Table 4).

Yeast opsonization was defective (< 2.5 yeasts per polymorph) in three of eleven who gave immediate reactions only with *D. pteronyssinus* and five of the thirty-seven who gave dual responses ($P>0.05$, Wilcoxon rank sum) two patients with the defect had a negative bronchial challenge.

Discussion

We have confirmed previous reports (Warner, 1976; Price *et al.*, 1982) that the dual reaction, particularly to *D. pteronyssinus*, is the characteristic bronchial response of children with moderately severe perennial asthma. Although the immediate component of this reaction can be explained by degranulation of mast cells, the mechanism

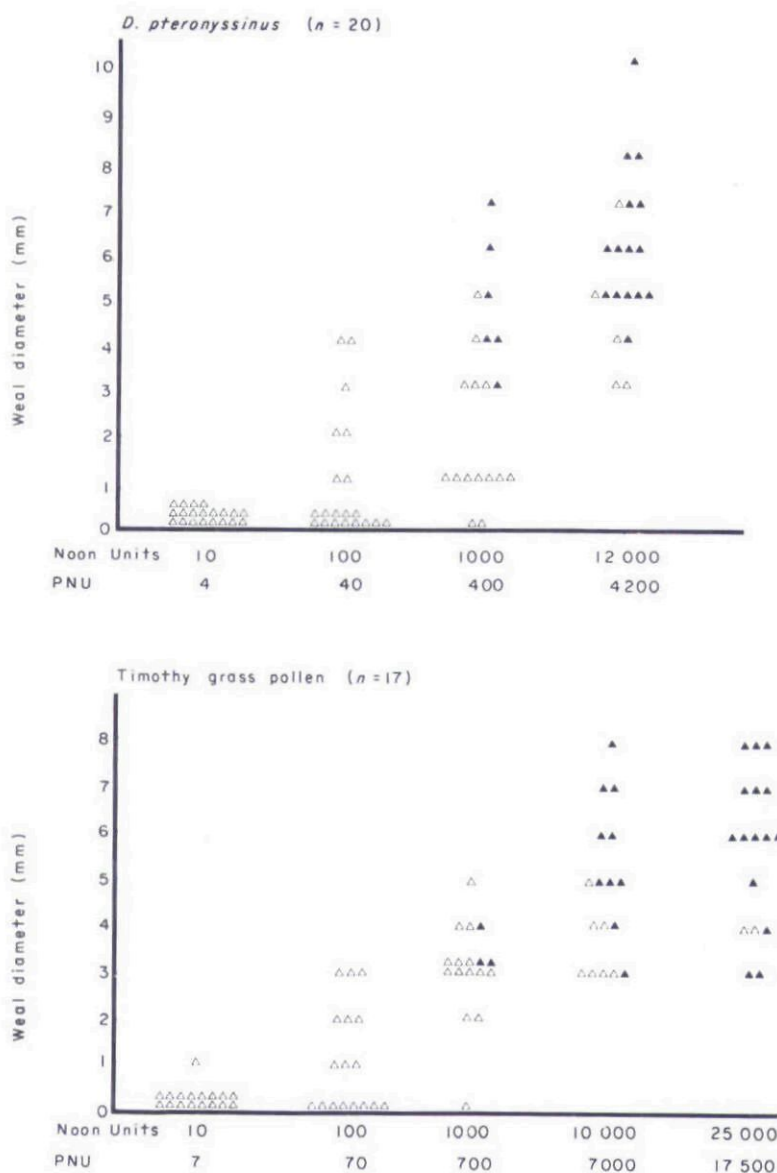


Fig. 1. Immediate skin responses in 20 patients tested with four dilutions of *D. pteronyssinus* and in seventeen patients tested with five dilutions of Timothy grass pollen. Skin test sites subsequently manifesting late responses are indicated by the closed symbols.

of the late component remains obscure. In patients with allergic bronchopulmonary aspergillosis, late asthmatic responses are associated with clinical signs suggestive of a type III reaction, i.e. fever, leukocytosis and crepitations in the lungs (McCarthy & Pepys, 1974). Deposits of immunoglobulin and complement have been identified in biopsies of late skin reactions to *Aspergillus fumigatus* (Pepys et al., 1968). In the children we studied we detected no consistent fall in C3 which might be expected to accompany a type III reaction, nor did we observe a rise in temperature or fine

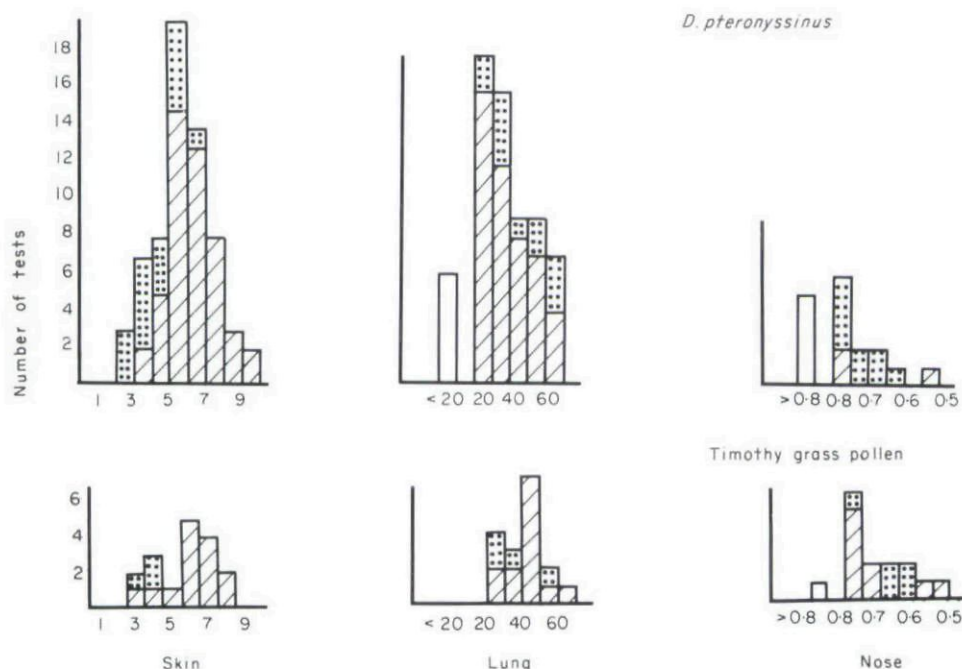


Fig. 2. Relationship of size of immediate reaction after provocation in skin, lung and nose with *D. pteronyssinus* and Timothy grass pollen, to pattern of response. □, negative; ▨, immediate only; ▩, dual.

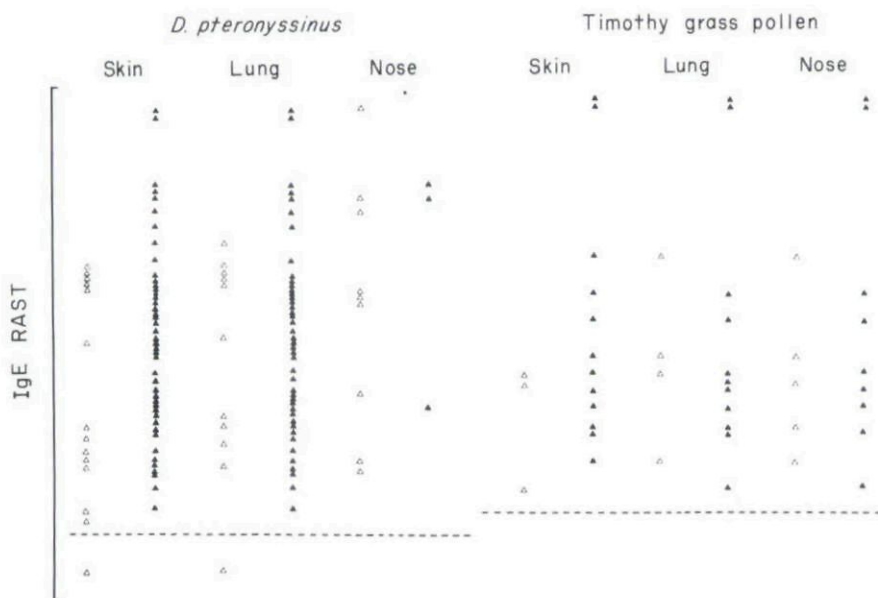


Fig. 3. Serum IgE antibody levels in patients with immediate only (Δ) and dual (▲) responses expressed as a percentage of total radioactive counts bound. The dashed horizontal lines indicate the mean level of binding observed with cord serum in each test.

Table 2. Skin weal size and nature of bronchial reaction using *D. pteronyssinus* antigen

Skin weal size (mm)	Bronchial reaction			
	Negative	Immediate only	Dual	Total
3	2	1	0	3
4	1	2	4	7
5	2	1	5	8
6	0	5	15	20
7	1	1	12	14
8	0	2	6	8
9	0	0	3	3
10	0	0	2	2
Total	6	12	47	65

Table 3. IgG anti-F4P₁ binding levels in serum and pattern of response to *D. pteronyssinus* in lung and skin in forty-two children with perennial asthma

IgG antibody to F4P ₁ antigen of <i>D. pteronyssinus</i>				
Skin			Lung	
	<i>n</i>	Arithmetic mean (range)	<i>n</i>	Arithmetic mean (Range)
Immediate reaction only	9	1549*(92-4075)	8	1291*(92-4075)
Dual reaction	32	710*(12-4095)	33	798*(12-4095)

* Arbitrary binding units.

Table 4. Serum C3 levels in asthmatic children undergoing bronchial provocation with *D. pteronyssinus*

C3 (% of pooled human serum standard)		
Patient	Pre-challenge	During late reaction
G.C.	100	100
M.R.	88	80
A.B.	96	96
D.A.	80	82
B.W.	76	82

crepitations at the lung bases during late bronchial reactions to house dust mite and Timothy grass pollen. Similarly none of the clinical features of a type III response were observed in adult asthmatics during late bronchial reactions to house dust (Booij-Noord, Orie & de Vries, 1971). There may therefore be more than one type of late asthmatic response.

Our data suggests some pathogenetic link between immediate and late reactions. We saw no isolated late responses (without preceding immediate ones) and increasing doses of antigen applied to the skin elicited not only larger immediate reactions but also more frequent late reactions. This is consistent with previous studies (Robertson *et al.*, 1974; Dolovitch *et al.*, 1973) found that a pepsin digest of anti-human IgE antibody when injected into the skin could induce a late reaction; this preparation would not activate complement so the reactions they observed were presumably complement independent. They suggested that the late response might be due to IgE-dependent mechanisms. Further support for this view was presented by Solley *et al.* (1976) who transferred late reactions by serum, and showed that these could be abolished by heating the serum to 56°C for 4 hr or by removal of IgE with a specific immunosorbent. Recent evidence for a relationship between late reactions and mast cell degranulation comes from studies of chemical mediators. One of these, believed to be associated with mast cells is a large molecular weight substance with neutrophil chemotactic activity and appears in the circulation during both immediate (Atkins *et al.*, 1977) and late (Nagy, Lee & Kay, 1982) asthmatic reactions after antigen provocation.

Unlike others who studied the bronchial response to ragweed pollen in adults (Robertson *et al.*, 1974) we found no association in our children between the dual bronchial reaction and size of immediate skin reactions, or the dose of inhaled antigen which elicited an immediate bronchial reaction. There was also no association of pattern of response in skin, lung or nose with total IgE or IgE antibody levels in the serum. We previously reported that different patterns of reaction could occur in the skin, lung and nose to the same antigen in one individual (Price *et al.*, 1982). If IgE is the critical factor, it is possible that its local concentration influences the nature of the response. The trend for IgG antibody levels to be higher in patients who gave only immediate reactions is against an IgG mediation of the late response, but the difference is too small to suggest a blocking effect. That late reactions in skin, lungs and nose are to some extent independent of the immediate response and of IgE and IgG antibody, is consistent with our observation that following hyposensitization to *D. pteronyssinus* in asthmatic children, clinical improvement was associated with the disappearance of late bronchial reactions (Warner *et al.*, 1978), without significant changes in IgG antibody (Turner *et al.*, 1983).

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