

Immediate and late phase allergic cutaneous reactions are not inducers of unspecific or specific local hyperreactivity

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The present study evaluates the possibility of allergen-induced unspecific and specific dermal hyperreactivity with special reference to the presence of late cutaneous reactions and allergen-induced nasal hyperreactivity. Twenty-six patients with strictly seasonal allergic rhinitis participated. All had a positive skin prick test for birch (*Betula verrucosa*) and/or timothy (*Phleum pratense*). Ten patients had previously displayed an allergen-induced nasal hyperreactivity and six patients a late cutaneous reaction. An initial skin prick test with a relevant pollen allergen was done in triplicate. The immediate skin reactions were recorded after 15 min and any late-phase reaction after 6 h. Twenty-four hours later the patients were retested. The same pollen allergen was sited in the first flare reaction from the previous day. A histamine prick test was sited in the weal as well as in the third reaction from day 1. A histamine control was also performed in a previously unaffected area. The allergen-induced weal reactions decreased significantly at rechallenge compared with the results from the previous day ($P < 0.05$). The histamine tests resulted in similar skin reactions regardless of whether or not they were done on a previous allergen test site. This was true for both specific and unspecific reactions when the subgroups of patients with previously demonstrated allergen-induced nasal hyperreactivity or late-phase skin reactions were evaluated separately. These results indicate that allergen-induced hyperreactivity is not a general feature of allergic inflammation but is a phenomenon restricted to specific sites, such as the airway mucosa.

Key words: allergic rhinitis; flare; hyperreactivity; late cutaneous reaction; skin test; weal.

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When patients with allergic asthma inhale a relevant allergen they develop a rapid increase in airway resistance which constitutes the early response, peaking within 15-20 min and disappearing within 1 or 2 h. After a quiescent period some of them may develop a prolonged increase in airway resistance which peaks after 6-8 h and continues for more than 12 h (17, 18, 21). Reactions similar to those in airway challenge can be demonstrated in the allergen skin test (17). The early allergic skin reaction is observed as a central weal with a surrounding erythematous flare which develops within 15 min and disappears within 1-2 h. The size of the reaction is related to the amount of allergen

presented, the number of mediator cells available, the degree of sensitization of these cells, and the reactivity to released mediators from the mast cells, and to histamine in particular (17). The late cutaneous reaction is recognized as an edematous cellular infiltrate, and has been suggested as the dermal equivalent of a similarly timed late pulmonary reaction after bronchial antigen provocation (21, 23). The late asthmatic response is closely associated with a prolonged increase in airway responsiveness (7, 8), a feature which can also be induced in the upper airways in the majority of hay fever patients (1).

In contrast to these findings in allergen chal-

lenges of the airway mucosa, no increase in the allergic weal and flare response was noted when patients with allergic rhinitis were re-prick-tested after 24 h in the area of the initial allergen-induced weal (2). The lack of increase in reactivity in the skin test procedure may actually be a falsely masked positive finding, due to differences in the relationship between mediator cell and allergens. In the skin test a very high allergen concentration is given in a very small tissue volume, possibly depleting the mast cells of available mediators (12, 14), a phenomenon which is not apparent when more diluted allergen solutions are deposited over a wide area, such as in airway challenges. Such a difference in the mediator cell/allergen relationship would not affect any induced unspecific hyperreactivity which would be independent of mast cell activation. The aim of the present study was to further study the possibility of allergen-induced specific or unspecific hyperreactivity in the skin with special reference to the presence of dermal late phase reactions and allergen-induced hyperreactivity in the airways.

PATIENTS AND METHODS

Study design

The study was designed as a 2-day procedure. On the first day allergen skin prick tests using one relevant pollen allergen were performed in triplicate. Twenty-four hours later a re-prick test with the same allergen was sited in the flare area of the prick test of one of the three allergen tests. A histamine test was performed in triplicate on the second day: one in the weal of the second allergen test from the previous day and one in the corresponding flare area. The third histamine test was performed in an area not involved in any previous allergen testing and the result of this test thus served as a control for the other two histamine tests.

Patients

Twenty six patients participated in the study (6 women and 20 men; and age range 21 to 37

years (mean 25.2). All had a history of allergic rhinitis with no bronchial or dermal manifestations of their allergic disease. They all had positive skin prick test for birch pollen allergen (*Betula verrucosa*) and/or timothy pollen allergen (*Phleum pratense*). Ten of these patients had previously displayed a nasal allergen-induced hyperresponsiveness using a nasal challenge model previously described (1). No medication was allowed for at least 3 weeks prior to and during the study. The investigation was performed outside the Scandinavian pollen season and was approved by the Ethics Committee at the University of Lund.

Prick test procedure

All skin prick tests were performed with PhazetTM (Pharmacia, Uppsala, Sweden), a preloaded standardized skin prick test needle (16). On day 1 the pollen allergen prick test were done in triplicate on the volar aspect of the forearms. The allergen (birch or timothy) previously producing the largest weal reaction was chosen for the present study. The area of the weal and flare reaction was determined with a technique previously described (3, 19). In brief: 15 min after the prick test the contours of the induced weal and flare reactions were outlined with a felt pen and transferred with the aid of transparent tape onto paper. The size of the weal and flare reactions was measured with a digitizer served by a microcomputer. Any possible late phase cutaneous reactions were recorded after 6 h. After 24 h the patients were prick tested again. By keeping the outlined contours of the weal and flare reactions from the initial day, the re-prick tests could be set at desired skin sites. An allergen test using the same pollen allergen as the day before was sited in the flare reaction from the first day. Three histamine tests (according to the manufacturer, equilibrated to histamine HCl 1 mg/ml–10 mg/ml) were done; one in the second allergen weal, one in the flare reaction of the third allergen test, and a third in a previously unaffected site, thus serving as a control. The size of the weal and flare reactions obtained after 15 min was measured in the same way as on the first day.

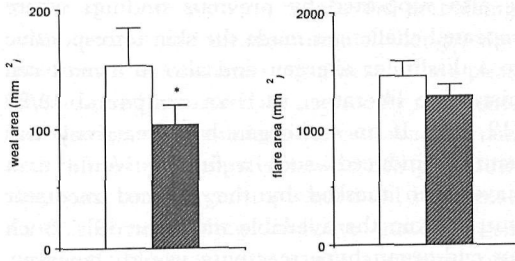


Fig. 1. Mean (+ SEM) size of the weal and flare induced by an allergen skin test ($n = 26$). Initial allergen challenge (□) and a rechallenge 24 h later (■) in the flare of the skin prick test from the previous day. Reduction in weal size is statistically significant.

Statistics

Statistical evaluation was performed on a micro-computer (Apple Macintosh, Cupertino, CA, U.S.A.) using a statistical software package (Statview 512+, Brainpower Inc. Calebasas, CA, U.S.A.). For the allergen tests the results from day 1 were compared with the results from day 2 using Student's *t*-test for paired data. The results of the three histamine tests were analysed with an analysis of variance. If a statistically significant difference emerged, further analyses was performed using Student's *t*-test. *P* values of < 0.05 were considered as significant.

RESULTS

Fifteen of the 26 patients were tested with timothy and 11 with birch pollen allergen. Six

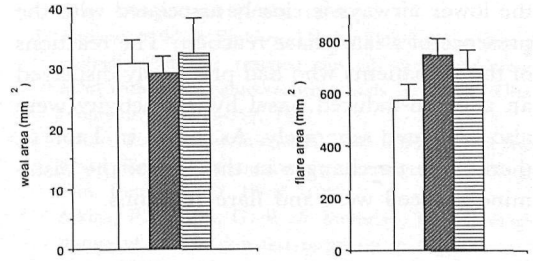


Fig. 2. Mean (+ SEM) size of the weal and flare induced by histamine at three sites (□): control histamine skin test in an area previously unaffected; (■): test performed at skin site of an allergen-induced weal 24 h previously; (▨): test performed at a skin site of an allergen-induced flare 24 h previously. Differences are not significant.

patients displayed a late cutaneous reaction after the initial allergen challenge. The mean size of the allergen-induced weal and flare reactions on days 1 and 2 for the whole patient group is shown in Fig. 1. A statistically significant decrease in the weal size from 151 ± 32 (mean + SEM) to 104 ± 16 mm² was noted ($P < 0.05$). No such difference was observed in the subgroups between those with a cutaneous late phase and those with allergen-induced airway hyperreactivity (Table 1). The results of the three histamine challenges for the whole patient group are shown in Fig. 2. An analysis of variance revealed no statistically significant differences between the three observations. As with the allergen-induced reactions, a separate evaluation was made for those with a cutaneous late phase (Table 2), since hyperreactivity in

Table 1

Results from prick-tests with allergen in subgroups of patients with late phases and patients who had previously displayed allergen-induced hyperreactivity. Re-prick-test in flare reaction from the previous day

Patient subgroup	Day 1 (mean ± SEM)	Day 2 (mean ± SEM)	Significant level <i>P</i> -value (<i>t</i> -test)
Late phase ($n = 6$)			
weal size (mm ²)	235 ± 79	154 ± 41	0.13
flare size (mm ²)	1689 ± 248	1284 ± 229	0.05
Airway hyperreactivity ($n = 10$)			
weal size (mm ²)	115 ± 44	95 ± 24	0.49
flare size (mm ²)	1142 ± 18	1300 ± 111	0.36

the lower airways is closely associated with the presence of a late phase reaction. The reactions of the 10 patients who had previously displayed an allergen-induced nasal hyperreactivity were also evaluated separately. As shown in Table 2, there were no changes in the size of the histamine-induced weal and flare reactions.

DISCUSSION

The present study was performed in order to find evidence of the existence of allergen-induced hyperreactivity in the human skin. However, no local specific (to allergen) or unspecific (to histamine) allergen-induced hyperreactivity was found in the skin sites of previous allergen-induced weal, flare, or late phase reactions. This was also true for a subgroup of patients who had previously displayed such allergen-induced hyperreactivity in the airways.

These findings are in agreement with a previous study of specific hyperreactivity where the rechallenges were sited in the weal from the previous day (2). The skin prick test device used contains the allergen and histamine freeze-dried on the tip of the skin-prick test needle (16). The local dose of allergen with this method is rather high and might induce a pronounced depletion of the available mast cells at the site tested. They appears to be a reasonable explanation for the slight reduction in allergen-induced weal on rechallenge with allergen as noted by Grönneberg & Strandberg (14). This

is also supported by previous findings where repeated challenges made the skin unresponsive to a dissimilar allergen and also to a mast cell histamine liberator, such as compound 48/80 (12, 22). If an end-organ hyperreactivity was actually induced, such a finding would then have been masked by the reduced mediator output from the available mediator cells. Such an end-organ hyperreactivity would, however, have been disclosed if the challenge had been performed with the actual mediator. Since no increased responsiveness to histamine was noted in the present study either, this indicates that no such end-organ hyperreactivity was present.

In the airway mucosa, notably in the lower airways, the presence of allergen-induced hyperreactivity appears to be linked to the existence of a late phase allergic reaction (8). Such a reaction involves the local infiltration of inflammatory cells into the mucosa. It has been suggested that such blood cells as the eosinophil and basophil contribute to increased airway responsiveness (6, 9); the eosinophil through the activation and subsequent release of cytotoxic proteins facilitating the subsequent penetration of allergen to the mediator cells (13). The basophil, which accumulates at the site of allergic inflammation in the same way as the eosinophil, has been suggested as a potential source of the increased mediator output noted at rechallenge (6). The dermal late phase is also characterized by a local infiltration of inflammatory cells (4, 5) like the airway mucosa. Nevertheless, no increased responsiveness was

Table 2

Results from prick tests with histamine in subgroups of patients with late phases and patients who had previously displayed allergen-induced hyperreactivity. Prick-tests were sited in unaffected area (control) and skin area on the site of a previous (24 h prior) allergen-induced weal and flare reaction

Patient subgroup	Reaction size in mm ² (mean $\pm$ SEM)			P-value (F-test)
	Control	in weal	in flare	
Late phase ($n = 6$)				
weal	21 $\pm$ 5	58 $\pm$ 27	29 $\pm$ 7	0.25
flare	545 $\pm$ 137	540 $\pm$ 97	886 $\pm$ 135	0.07
Airway hyperreactivity ($n = 10$)				
weal	21 $\pm$ 5	22 $\pm$ 5	19 $\pm$ 4	0.65
flare size	611 $\pm$ 68	804 $\pm$ 95	832 $\pm$ 83	0.18

noted in those patients where the rechallenge with allergen and the histamine challenges were sited in an area where such a dermal late-phase reaction had occurred. As previously mentioned no change in histamine-induced reaction size was noted in those patients who had previously displayed an allergen-induced nasal hyperreactivity. These findings indicate that allergen-induced hyperreactivity is not a general phenomenon of the allergic inflammation but rather a phenomenon of the airway mucosa.

A possible explanation for the change in specific responsiveness of the airway mucosa could be that mediator cells, either mast cells or basophils, relocate towards the mucosal surface in response to allergen exposure (6, 11). An alternative explanation is that changes in the permeability of the epithelial lining are responsible for changes in the reactivity of the airways (10, 15). This explanation appears to be supported by the present finding since the skin prick test bypasses the epithelium, the allergens being deposited below the epithelial lining. This contrasts with airway challenges, where the allergens are deposited on the surface of the epithelium of the airway mucosa.

The negative results from the subgroup of patients with allergen-induced nasal hyperresponsiveness further emphasizes the importance of studying the actual organ where the allergic disease is present, since certain properties of the allergen-induced inflammation are apparently restricted to certain localities, such as airways.

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