

Allergen-induced hyperreactivity is not a feature of dermal immediate allergic reactions—in contrast to reactions of airways mucosa

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Summary

Allergen challenges of airway mucosa are commonly followed by an increased sensitivity to rechallenge with allergen. In the lower airways this phenomenon has been associated with the late phase of allergic airway reactions, which in turn has been suggested as a link between anaphylaxis and continuous allergic airway disease. The aim of the present investigation was to explore further the phenomenon of allergen-induced hyperreactivity and to see whether it was possible to induce such a reaction in the skin. Twenty-six patients with seasonal allergic rhinitis due to birch and/or grass pollens were studied in the pollen-free winter months. Nine of these patients had previously demonstrated an increased reactivity following allergen challenge in the nose, and nine of the patients had cutaneous allergen-induced late-phase reactions to the allergen tested. Skin-prick tests were performed with pollen allergen, histamine, and a negative control. The areas of the weal-and-flare reactions were measured 15 min after the tests were set. Any late-phase reactions were recorded 6 hr after the skin challenge. The subjects were re-tested with allergen and histamine 24 hr after the initial prick test within the area of the corresponding weal from the previous day. In contrast to previous challenges of human airway mucosa, where the same time interval was used, we found no increased responsiveness to rechallenge as compared with the initial allergen challenge. This was true, even if only the subgroups with previously demonstrated nasal allergen-induced hyperreactivity, or cutaneous late-phase reaction were evaluated. It is, therefore, suggested that allergen-induced hyperreactivity as a feature of allergic airway mucosa may be related to changes in the epithelial barrier function.

Introduction

It has previously been demonstrated that a challenge of airway mucosa with allergen is commonly followed by an increased sensitivity to rechallenge with specific or unspecific stimuli [1–3]. In the upper airways this phenomenon was entitled 'priming' at an early stage and is present in the majority of hay fever patients [2, 4], this expression of allergic airway disease also appears to be present during natural allergen exposure of hay fever patients [5, 6]. In the lower airways the allergen-induced hyperreactivity has been closely associated with the presence of a late-phase reaction following an initial allergen challenge [1]. The late-phase reaction with the reappearance of symptoms and cellular infiltration of the airway mucosa, has also been suggested to be the link between anaphylaxis and continuous allergic disease [7, 8]. Furthermore, bronchial hyperreactivity as a cardinal symptom of asthma has, to some extent, been used as a diagnostic criterium for the diagnosis of asthma [9].

One of the other fundamental tests of the clinical allergy work-up is the skin-prick test where allergen introduced into the skin induces an IgE-dependent immediate hypersensitivity reaction [10]. The two major parts of the allergic skin reaction, the weal and the flare, are thought to be the result of partially different pathophysiological mechanisms. The weal is thought to be the result of the direct action of the released inflammatory mediators on blood vessels, while the flare reaction, at least in part, is thought to be due to a reflex activity, which may be mediated through peptidergic mechanisms [11–14]. As is the case with airways reactions, some patients display a late-phase reaction following the immediate reaction expressed as a red infiltration of the skin with a maximum intensity 6–12 hr after the initial allergic response [15]. As allergen-induced hyperreactivity appears to be an important feature of allergic airways disease, it was of interest to see whether this phenomenon is a general allergic phenomenon and also exists following dermal allergic reactions.

Subjects and methods

Study design

The study was performed as a 2-day procedure. On day 1, skin-prick tests using a relevant pollen allergen, histamine and a negative control were performed. This was followed 24 hr later by a second prick test with allergen and histamine, which was performed within the area of the corresponding weal obtained the day before.

Patients

Twenty-six patients were recruited, sixteen were females and ten males with ages ranging from 17 to 33 years (mean age 23.3 years). They all had a history of allergic rhinitis with no bronchial or dermal manifestations of their allergic disease. They all had a positive skin-prick test for birch pollens (*Betula verrucosa*) and/or timothy pollens (*Phleum pratense*), and they had been free from any medication for at least 3 weeks before entering the study. Nine of these subjects had previously been shown to display nasal allergen-induced hyperreactivity in a nasal challenge experiment performed in the same winter season. These nasal challenge experiments have previously been described in detail [4]. The study was performed in pollen-free autumn and winter months, and was approved by the Ethics Committee of the University of Lund.

Prick-test procedure

At an initial visit a skin-prick test was performed on the volar aspect of the forearm. The preloaded standardized skin-prick test device, Phazet® (Pharmacia AB, Uppsala, Sweden) was used for the tests [16]. Pollen allergens, birch and timothy, and a histamine control were set. The weal-and-flare responses obtained after 15 min were outlined with a felt-pen and transferred onto paper with the aid of an adhesive plastic film. The areas of the skin and flare response were then measured with the aid of a digitizer served by a microcomputer (Hewlett Packard, U.S.A.). Any late-phase reaction was recorded 6 hr after the initial allergen challenge. The patients were prick tested again after 24 hr with the pollen allergen showing the largest initial weal, and the histamine control. The second prick tests were performed within the area of the weal of the corresponding reaction from the previous day. The areas of any late-phase reactions and the second prick tests were transferred onto paper and measured in the same way as the initial reactions.

Statistics

The results from the first day were compared with the results obtained on the second day using Student's *t*-test for paired analysis. The calculations were performed on a microcomputer (Apple MacIntosh, Cupertino, CA, U.S.A.) with the aid of a statistical software package (Statview 512+, Brainpower Inc, Calebasas, CA, U.S.A.).

Results

Of the total of twenty-six patients, fifteen were tested with timothy grass pollens and eleven were tested with birch pollen allergens. A total of nine patients demonstrated a late-phase cutaneous reaction with a mean area of 1065 mm². The results from the initial and second prick tests for all twenty-six patients are shown in Fig. 1. There was no significant change in the size of the induced weal-and-flare reactions at rechallenge, as compared with the initial challenge in either the histamine-induced or the allergen-induced reactions. The results from the subgroup of patients who had previously displayed nasal allergen-induced hyperresponsiveness are shown in Fig. 2. There was again no significant change. As bronchial allergen hyperresponsiveness is linked to the presence of a late phase, a separate evaluation was made of those patients who had a dermal late-phase reaction following the initial allergen challenge, and the results are shown in Fig. 3. There was no tendency towards any increase in the allergen-induced reaction on the second day, compared with the first day in this subgroup. Probability (*P*) values obtained in the comparisons between the reactions obtained on day 1 and those obtained on day 2 are given in Table 1.

Discussion

In the present study we failed to demonstrate any allergen-induced hyperreactivity following dermal immediate allergic weal-and-flare reactions. This was also true when only looking at patients who had demonstrated late-phase reactions in the same location, the skin, or patients who had demonstrated allergen-induced hyperreactivity in the airways. As previously shown in the airways [17] we also failed to demonstrate any increased sensitivity after histamine challenge.

In the present study we chose to use the recently introduced Phazet skin-prick test device, which contains the allergens and histamine as freeze-dried preparations on the tip of the skin-prick test needle. This device has previously been shown to give good

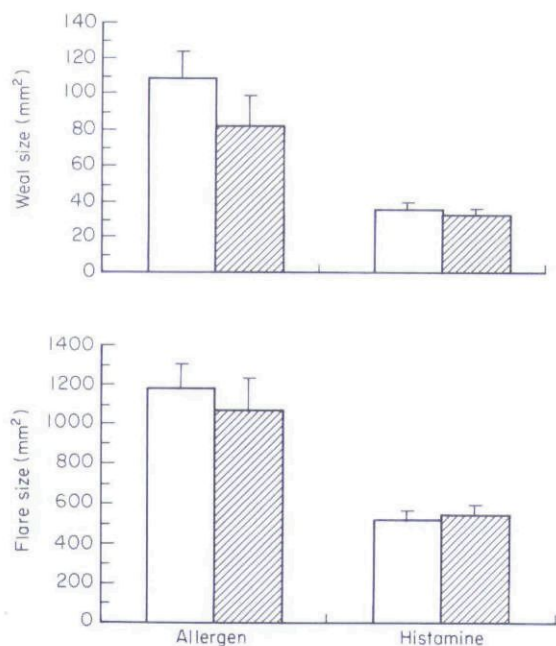


Fig. 1. Area of weal (upper panel) and flare (lower panel) reactions obtained at the initial skin-prick test and the re-test 24 hr later. Results obtained from twenty-six unselected hay fever patients after allergen and histamine tests. The changes observed are non-significant. (□) Initial challenge, (▨) rechallenge after 24 hr.

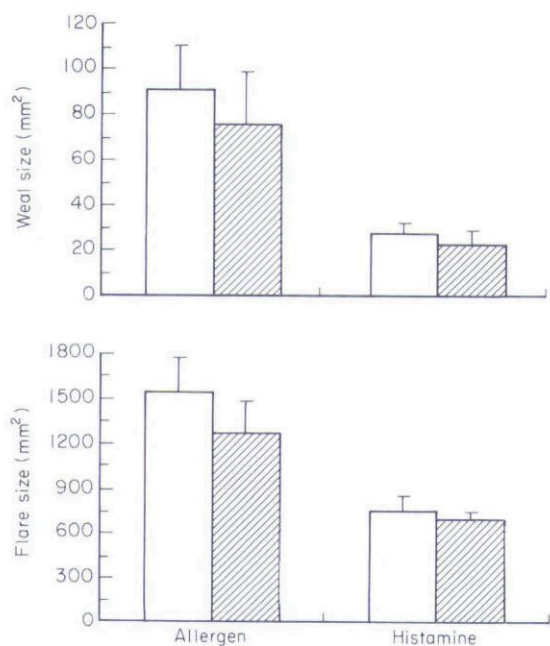


Fig. 2. Area of weal (upper panel) and flare (lower panel) reactions obtained at the initial skin-prick test and the re-test 24 hr later. Results obtained from nine hay fever patients with demonstrated nasal allergen-induced hyperresponsiveness. Allergy and histamine tests are shown. The changes observed are non-significant. (□) Initial challenge, (▨) rechallenge after 24 hr.

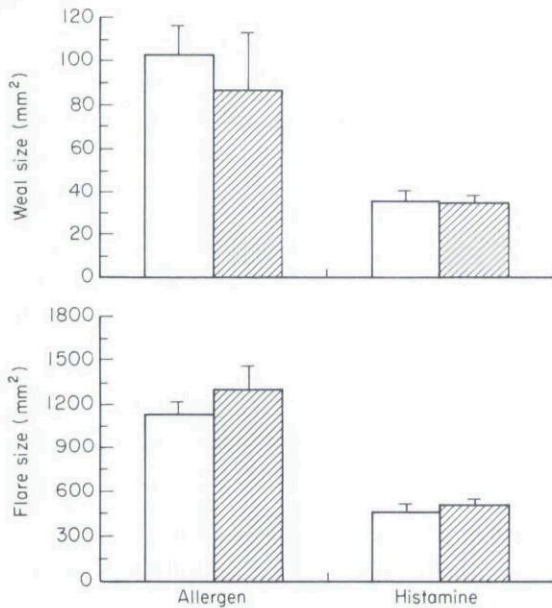


Fig. 3. Area of weal (upper panel) and flare (lower panel) reactions obtained at the initial skin-prick test and the re-test 24 hr later. Results obtained from nine hay fever patients with a cutaneous late phase following the initial allergen test. Allergen and histamine tests are shown. The changes observed are non-significant. (□) Initial challenge, (▨) rechallenge after 24 hr.

reproducibility [18, 19] and has been used by us for earlier pharmacological studies [20, 21]. The allergen dose given is equivalent to skin-prick tests with a 100 000 BU/ml solution, a high allergen dose that has been chosen to minimize any false negative results in the routine clinical prick test situation. The same argument would also be appropriate in the present study as it would be arguable that a somewhat vigorous initial immediate reaction might predispose the patient to a late-phase reaction and

Table 1. Statistical significance levels obtained in the comparisons between weal and flare reactions on day 1 with those obtained day 2

Patient group	Challenge agent	Reaction	<i>P</i> value*
Unselected group (<i>n</i> = 28)	Allergen	Weal	0.17
		Flare	0.38
	Histamine	Weal	0.26
		Flare	0.36
Hyperreactive of nose (<i>n</i> = 9)	Allergen	Weal	0.30
		Flare	0.34
	Histamine	Weal	0.15
		Flare	0.42
Dermal late phase (<i>n</i> = 9)	Allergen	Weal	0.53
		Flare	0.24
	Histamine	Weal	0.59
		Flare	0.36

* Student's paired *t*-test: two-tailed.

hyperresponsiveness to the reintroduction of allergen as shown in bronchial allergen challenge experiments [1]. The timing of the rechallenge experiment in relation to the initial one might be crucial. In the airways it appears that 24–48 hr is the time at which the allergen-induced hyperresponsiveness is at its maximum. Shorter times, such as 4 hr, are not enough, while the hyperresponsiveness appears to wane after 5–6 days [22]. The time selected in the present study, 24 hr, is also a practical time interval since the rechallenge can be performed on the same time of day, thus avoiding any diurnal variation in responsiveness. Furthermore, although a refractory period is a possibility as has been suggested for the skin late phase [23] this has not, to our knowledge, been reported for any immediate skin tests using the present time interval.

There may be several possible explanations for the lack of allergen-induced hyperreactivity following the skin reactions, compared with reactions of the upper and lower airways. One possible explanation is related to methodological differences in the way the allergens are introduced to the challenged organ. In airway challenges the allergens are applied to the mucosal surface, usually in the form of a solution. In the asymptomatic allergic patients the allergens must then traverse the epithelial lining as an initial step in order to react with the sensitized mast cells, the majority of which are presumably located below the basement membrane. In the skin-prick tests the allergens are deposited in part below the epidermis, with the aid of the skin-prick needle. Consequently, there is no epithelial lining to be traversed in order to initiate the reaction. If these features alone are taken into account, a crucial point in the development of allergen-induced hyperreactivity appears to be the status of the airway epithelium in terms of its barrier function. Thus, our findings appear to confirm previous reports by Hogg [24] and Eiwood *et al.* [25] that the epithelial lining of the airways performs a crucial regulatory function in the development of airway hyperreactivity.

Another possible explanation of the differences in the reactions of the skin and airway mucosa might be that these two reactions differ in the pathophysiological events taking place after the initial contact between the sensitized mediator cell and the appropriate allergen has been established. This must be considered a definite possibility in light of recent findings that mast cells of the skin differ in several aspects from mast cells in airway mucosa. Thus, it appears that mast cells in airway mucosa possess a migrating capacity [26] and differ in their phenotypic expression of proteoglycans and proteases from skin mast cells [27, 28]. Recent evidence also suggests that they also differ from skin mast cells in terms of pharmacological responsiveness [29]. At present it is not known whether they also differ in the mediators released, which are responsible for any induced hyperresponsiveness. As a result it can only be speculated that the release of PAF, which has been suggested to be such a late phase 'hyperresponsiveness mediator' [30], differs in skin, compared with the airways.

The present findings further spotlights the importance of studying the organs that are implicated in continuous allergic diseases. Although basic inflammatory reactions might be similar in terms of pathophysiology they might also differ in some fundamental ingredients in the development of the actual allergic disease.

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