

Effect of local allergen priming on early, late, delayed-phase, and epicutaneous skin reactions

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Allergic disease is reflected in a chronic inflammatory response to an allergen. It is thought that local allergen priming underlies this chronicity. To assess the effect of allergen priming on the amplitude and histologic effect of the allergic reaction, four sequential, intracutaneous skin tests were done with 48-h intervals in 13 patients allergic to the house-dust mite *Dermatophagoides pteronyssinus* (Dpt). Reactions were measured at 15 min, and at 6, 24, and 48 h. Subsequently, epicutaneous tests were done on Dpt-primed spots ($n=5$). At 6, 24, and 48 h, reactions increased after priming ($P<0.006$), with unaltered early reactions. Epicutaneous reactions to Dpt on primed spots were larger than in epicutaneous controls on similarly primed skin. Local intradermal priming results in greater inflammatory responses at both intra- and epicutaneous challenge. This mechanism may explain the chronicity of allergic reactions at epithelial surfaces.

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The chronic nature of allergic asthma, rhinitis, and atopic dermatitis is well established. This chronicity suggests that a continuing allergic reaction may have some permissive effect for further allergen-driven inflammation. This is illustrated in both pollen-allergic rhinitis and allergic asthma where the threshold of specific reactions of the nose and lower airways is lowered in the pollen season (1). Frequent intranasal challenges also lead to increased sensitivity to allergens (2, 3).

Intradermal skin reactions are widely used as a model for allergen-induced inflammation of the upper and lower airways. Usually, the skin is challenged once before evaluating the inflammatory response. Such an approach evidently does not take into account the chronic nature of the reactions in the airway mucosa. The chronicity in allergen exposure may influence both the clinical magnitude and the histologic quality of the reaction. Repetitive challenge of the skin has earlier been found to induce diminishing immediate reactions, with unaltered histamine responses (4, 5). Little is known, however, about the late-phase and delayed-

phase reactions at rechallenge, the duration of the reaction, and the effect of allergen priming on epicutaneously induced allergic reactions.

We deal with these points in this study. Thirteen subjects allergic to house-dust mite were sequentially challenged four times with intradermal application of house-dust-mite allergen. Reactions were measured at time intervals up to 96 h after challenge. After sequential intradermal challenge, epicutaneous reactions with house-dust mite were assessed.

Material and methods

Subjects

The study was approved by the hospital ethics committee. The subjects ($n=13$) were student volunteers who suffered from bronchial asthma. All showed a positive skin test reaction (diameter ≥ 5 mm) to house-dust-mite allergen (ALK, Copenhagen, Denmark). None of the patients suffered from atopic dermatitis.

Skin tests

Intracutaneous tests. We induced skin reactions with house-dust-mite allergen (ALK, Copenhagen, Denmark) by injecting 30 BU/ml intracutaneously in the back of all 13 patients at four different locations (1–4; Table 1). We repeated the testing three more times with Dpt (*Dermatophagoides pteronyssinus*) at the Dpt spots. Intervals between the tests were 48 h.

We read the reactions at 15 min and 6, 24, and 48 h after challenge and scored the wheal diameters as a measure of the early responses. Indurations were determined for the later phases of the reactions according to a procedure suggested by Sokal (6), and the maximal diameter of each reaction was expressed in millimeters. In the case of similar challenge patterns, the average diameters were used for subsequent analysis.

Epicutaneous tests. In five of the patients, Dpt was given epicutaneously 48 h after the four intracutaneous tests at location 1 (Dpt-Dpt-Dpt-Dpt) and at a nonprechallenged control spot (location 5). Plasters with control solution were put on a Dpt-prechallenged spot (Dpt-Dpt-Dpt-Dpt; spot 2) and at a control spot (6). All four epicutaneous reactions were measured 48 h later.

Immunohistologic analysis

Monoclonal antibodies. For immunophenotyping of distinct cell types, a series of mouse monoclonal antibodies was used, including OKT1, OKT11, OKT8 (aCD8), OKIa (aHLA-DR) (Ortho Pharmaceutical Corp.), and Leu3a (aCD4) (Becton Dickinson). An indirect, two-step immunoperoxidase method was used on frozen tissue sections (4–6 µm).

Statistics

Nonparametric tests were used for statistical analysis: the Mann-Whitney test for comparisons in reaction diameters and Spearman's rank correlation test for correlation studies. Only *P* values less than 0.05 were considered significant.

Results

Macroscopic appearance of the skin reactions at sequential testing

Intracutaneous tests. Fig. 1 shows the diameters of the reactions at 15 min and 6, 24, and 48 h after testing. The reactions at 15 min (early) did not change after rechallenge. The reactions at 6 h (late) were significantly larger at the third ([test 1]

Table 1. Pattern of repeated intra- and epicutaneous challenges with house-dust mite (Dpt) and grass pollen

Position	Test 1	Test 2	Test 3	Test 4	Test 5
1	Dpt IC	Dpt IC	Dpt IC	Dpt IC	Dpt epi
2	Dpt IC	Dpt IC	Dpt IC	Dpt IC	Contr epi
3	Dpt IC	Dpt IC	Dpt IC	Dpt IC	
4	Dpt IC	Dpt IC	Dpt IC	Dpt IC	
5					Dpt epi
6					Contr epi
7				Dpt IC	
8				Dpt IC	

Position: Skin position at patient's back, at which repeated challenges were done.
Dpt IC: intracutaneous (IC) skin test with Dpt (30 BU/ml).
Dpt epi: epicutaneous application of Dpt (for period of 48 h beginning at 48 h after fourth IC test).
Contr epi: epicutaneous application of control solution.

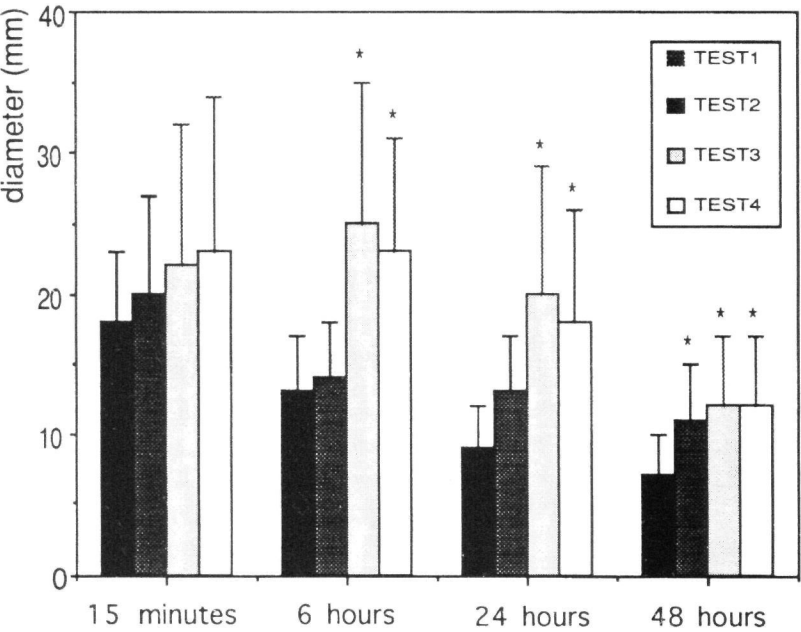


Fig. 1. Diameters of skin reactions after repeated challenge with house-dust mite (Dpt). Reaction diameters (in millimeters) after repeated challenge with 48-h intervals (positions 1–4). *Increase in relation to diameters at test 1 (*P* < 0.006).

13–25 mm; *P* = 0.003) and fourth (23 mm, *P* = 0.002) challenges. The reactions read at 24 h were larger at each rechallenge, approximately doubling in size (test 1: 9 mm, test 2: 13 mm, *P* = 0.006; test 3: 20 mm, *P* = 0.002; test 4: 18 mm, *P* = 0.003). The same pattern was seen for the reaction at 48 h (test 1: 7 mm, test 2: 11 mm, *P* = 0.003; test 3: 12 mm, *P* = 0.002; test 4: 12 mm, *P* = 0.002).

Epicutaneous tests. Application of house-dust mite on skin plasters on unprimed skin failed to elicit any visible or palpable reaction. When the skin was primed intracutaneously with house-dust mite (four times), application of these plasters resulted in larger reactions after a 48-h exposure (on average, 80% of the reaction before application of the plaster) in all five patients tested, compared to

control plasters (52% of the reaction before application of the plaster) on similarly primed skin (Fig. 2).

Histology

In unchallenged skin, small numbers of mononuclear cells are usually present, mainly grouped around organelles such as vessels, hair follicles, and sweat glands. After challenge, the numbers of cells increased considerably. CD4-positive lymphocytes comprised most of these cells, followed by eosinophils. Cell numbers stayed high for up to 96 h after the tests.

Discussion

Repeated intracutaneous challenge with allergen resulted in an increase in late, delayed-phase, and epicutaneous reactions.

Sequential challenge of the skin was done earlier at different challenge intervals. Shaikh et al. (7) did intracutaneous challenges with intervals of 1, 2, and 3 weeks, using both anti-IgE and several allergens. The early reaction was unaltered at rechallenge; however, the late-phase reactions appeared to be smaller. The number of allergen-challenged patients (3–5) was relatively small. Andersson & Pipkorn (4) repeated intracutaneous skin tests with pollen once after an interval of 12 h. Early reactions at rechallenge were smaller with unaltered histamine reactions, but the effect on late-phase reactions was not mentioned. These data underline the importance of the challenge interval in determining the priming effect. We chose a 48-h interval because of the observation that at that point of time numerous activated cells were still accumulated at the reaction site but the macroscopic reaction was declining. As the presence of activated cells such as lymphocytes, eosinophils, mast cells, and possibly Langerhans' cells may account for the observed priming effect on the reaction, an interval of weeks is likely to be too long. Andersson & Pipkorn's observation on the decline of early reactions at 12-h intervals may be explained by depletion of mast-cell histamine content. Furthermore, we observed significant increases only at the third and fourth challenges. It was noted that the most striking degree of increase occurred after the third challenge. This effect appeared to level off at the fourth challenge, suggesting that at repetitive exposure reactions grow up to an individual maximum. Of course, this is compatible with the clinical observation that with continuous exposure reactivity does not increase indefinitely. The effect of intracutaneous priming on epicutaneous reactions has not been described before.

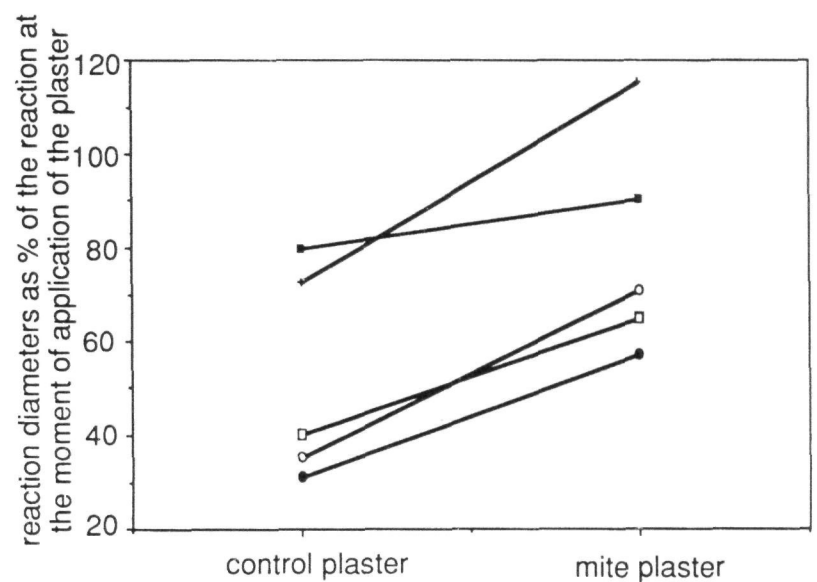


Fig. 2. Skin reaction diameters after epicutaneous application of Dpt after prechallenge. Epicutaneous tests with Dpt and control solution were done at 48 h after intracutaneous priming with Dpt (at position 1: Dpt; position 2: control). Plasters were removed after 48 h, and reaction was expressed as percentage of local priming reaction (just before application of plasters).

Local allergen-specific priming is likely to depend on the attraction and activation of cells at the reaction site. We found that CD4-positive lymphocytes and eosinophils were increased up to 96 h after challenge. The role of allergen-specific CD4-positive cells in the regulation of allergen-induced inflammation is becoming increasingly clearer. Allergen-specific T cells resemble the Th2 phenotype in mice, producing interleukin (IL)-4 and IL-5 as cytokines (8). Using *in situ* hybridization, Kay et al. showed that there was a predominance of Th2-type lymphocytes 24 h after intracutaneous challenge with house-dust mite (9). IL-5, GM-CSF, and IL-3 produced by these cells show potent activity in stimulating the growth of eosinophils, as well as attracting and activating them. Eosinophil-degranulation products contribute to the tissue damage caused by allergic inflammation. Mast cells, bearing allergen-specific IgE, may also be involved in allergen-specific priming. At continuous epicutaneous challenge of the skin during up to 8 days with house-dust-mite allergen, the number of mast cells showed a time-dependent increase (10). This may result from local production of growth-potentiating IL-3 by the attracted T cells (11). A similar influx of mast cells and basophils and an increased concentration of their mediators (histamine and prostaglandin) was observed at repeated nasal challenges (3).

On the basis of these mechanistic considerations, some of our observations in the skin might be extrapolated to the mucosal allergic reactions of the airways. These, too, are dominated by T cells and eosinophils. A study by Crimi et al. (12), who reported an allergen-specific priming effect on

late-phase bronchial reactions due to seasonal influences, supports this association. Potentiation of the late, but not the early, response was further observed by Rasmussen (13) in a study in which patients with allergic asthma underwent two bronchial allergen challenges with an interval of 2 weeks. Inhalation of small amounts of allergen (1–10 BU) was shown to increase nonspecific bronchial hyperreactivity in 11 of 13 patients tested (14). Comparisons with studies on the nose is difficult, as late-phase reactions of the nasal mucosa are seldom measured due to technical difficulties. The potentiating effect of the pollen season on the early reactions in patients with allergic rhinitis is well established. Moreover, chronic or recurrent nasal challenge has caused an increased sensitivity of the nasal mucosa to allergen or histamine (15). These data show that priming of late-phase reactions at sequential challenges is observed in both the skin and the lower airways.

The relevance of these observations is twofold. Firstly, mechanistically: as allergen reaches the airway mucosa continuously, any priming effect is likely to determine the severity of the allergic reaction and symptoms. Secondly, repetitious skin testing can be used as an extension of the conventional screening of allergy to provide additional information on the regulation of the allergic reaction. This may very well be useful to assess effects of immunomodulating therapy.

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