BASOPHILS IN ALLERGEN-INDUCED PATCH TEST SITES IN ATOPIC DERMATITIS

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Summary

Atopic dermatitis often occurs in patients who have high IgE levels and positive immediate skin tests to several common allergens. However, there is considerable doubt about the role played by allergens in this disease. Patch testing for 48 h at superficially abraded skin sites revealed that allergens could induce eczematous lesions in atopic dermatitis patients but only in those who also gave a positive immediate skin reaction to the same allergen. Lesions induced by the purified house dust mite antigen, antigen P1, contained mononuclear cells, basophils, eosinophils, and neutrophils. These patients also had raised specific serum IgE against antigen P1, and their leucocytes released histamine upon exposure to the same antigen. Thus an acute eczematous lesion can be induced by the application of inhalant allergens to the skin.

Introduction

Atopic dermatitis is a common debilitating skin condition occurring in all age-groups, though predominantly in the young. Most patients have high IgE levels and positive immediate skin tests to several inhalant allergens.1,2 They also often have allergic rhinitis, asthma, and/or food allergy.

DR MACDOUGALL AND OTHERS: REFERENCES

Skin affected by eczema is infiltrated with mononuclear cells, which suggests that the condition may be a form of delayed hypersensitivity (DH). However, allergens that cause immediate responses do not usually give rise to DH skin lesions. This failure of allergens to produce delayed or eczematous lesions has often been given as a reason for not regarding allergens as an important cause of eczema.3,4 However, the very small quantities of antigen used in skin testing atopic patients, or the local effects of vasoactive regarding allergens as an important cause of eczema.3,4 cell-mediated immune responses to candida and tuberculin.6

Patients with atopic dermatitis often improve after admission to hospital, even though treatment is not changed.7 Many of these patients are sensitive to allergens derived from the house dust mite Dermatophagoides pteronyssinus and are exposed to high levels of these allergens at home,8,9 but not when they are in the relatively dust-free environment of a hospital. We therefore decided to re-investigate the effects of exposure of the skin to dust mite and other allergens.

Materials and Methods

Seventeen patients (aged 7—61) with severe atopic dermatitis were skin tested (by prick technique) with a variety of common inhalant allergens (Bencard Ltd., U.K.). These same allergens and extracts of floor dust were dialysed against saline before being used for patch testing. Antigen P1 was purified from an extract of D. pteronyssinus culture by concentration over an 'Amicon PM10' filter, fractionation on 'Sephadex G-100', and 'pericon' block electrophoresis.10 Controls consisted of ten non-allergic volunteers with negative prick tests and six volunteers who had a history of rhinitis or asthma and positive prick tests but no history of atopic eczema.

Patients were only patch-tested when their dermatitis was in remission, and tests were done on skin that was macroscopically normal. A 2 cm x 2 cm area of skin was gently abraded by removing the upper layers of the epidermis but without causing any bleeding. 0.5 ml of aqueous allergen or normal saline was applied with a pad of sterile gauze, the area was occluded, and the dressing retained with hypoallergenic tape. Lesions were generally inspected at 48 h, when a biopsy specimen was also taken by the use of a 4 mm disposable biopsy punch (Stiefel Laboratories U.K. Ltd.). Care was taken to infiltrate local anaesthetic around the biopsy site but not into it. The biopsy specimens were immediately placed in Karnovsky's fixative.11 After 24 h they were washed in four changes of 0.1 mol/l sodium cacodylate pH 7.4 (Sigma) at 4°C and dehydrated in varying concentrations of alcohol. Specimens were embedded in hydroxyethyl methacrylate and 1-2 µm sections were stained with Giemsa.12 All sections were approximately 4 mm x 2 mm, and the whole area was examined, the few cells in the epidermis and on the surface not being counted.

Total serum IgE levels were measured by double antibody inhibition radioimmunoassay, and specific antibodies to antigen P1 of both IgE and IgG isotypes were assayed by the use of an antigen-binding radioimmunoassay technique.13,14 Tests for in-vitro leucocyte histamine release in response to antigen P1 were done for all subjects.15 Dust samples were collected from the homes of the patients tested and assayed for antigen P1 content.9

Results

Patch testing with allergens in seventeen atopic dermatitis patients produced a positive macroscopic response after 48 h in 38 out of 38 tests (table I). These lesions were evident at 24 h and persisted until 72 h. Most patients showed confluent papular erythema (+ +) while the stronger responses also showed oedema and exudation (+ ++). Scaling occurred in most lesions as they resolved over 4—10 days. Most patients were tested with more than one allergen, including extracts of house dust mite, grass pollen, guineapig dander, cat dander, and their own floor dust. Saline produced patchy erythema (+) on four occasions, and no reaction (0 or ±) in 19 patch tests on the same patients. These patients, who lived in England, had not been naturally exposed to ragweed pollen, and showed negative immediate skin tests with ragweed extract. In seven out of eight cases no striking response to patch tests with ragweed extract was demonstrable.

Ten patients were tested with the purified dust mite allergen, antigen P1. All ten showed positive patch responses to the 5 µg dose (in preliminary experiments we found that 0.05 µg would elicit a mild reaction), while nine out of ten non-allergic controls showed no reaction (table II). The ten patients had marked immediate hypersensitivity to antigen P1, as judged by the intradermal skin test and in-vitro leucocyte histamine release (table II); they also had high levels of serum IgE and IgG antibodies against antigen P1 (table III). Total serum IgE levels were very high in the patients with atopic dermatitis (geometric mean 5460 IU/ml), which was in keeping with the fact that most of these patients had had extensive skin involvement within the previous 6 months (table III). Six dust-mite-allergic individuals who did not have dermatitis were also patch-tested with antigen P1 and four of them showed positive responses (table II).

Histologically the positive patches induced with antigen P1 showed a moderate to severe dermal inflammatory cell infiltrate most prominent around blood vessels. This contrasted with the scarcity of inflammatory cells in the responses to saline in nine out of ten of these patients and in the responses to the allergen in nine out of ten of the non-allergic controls. In biopsy specimens of positive lesions taken at 48 h the epidermis contained occasional inflammatory cells. At 72 h epidermal changes, including focal spongiosis and microvesiculation, were more evident. Differential cell counts done blind showed highly significant increases in the number of basophils in positive lesions (table IV)—e.g., the dermatitic patients showed a mean of 4½ basophils in response to antigen P1, while non-allergic controls had 1½ basophils. No differences in absolute mast cell counts were noted in the different groups. Eosinophils were a predominant infiltrating cell in the positive biopsies; they
TABLE II—RESULTS OF PATCH TESTS, INTRADERMAL SKIN TESTS, AND TESTS FOR LEUCOCYTE HISTAMINE RELEASE

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Patch test at 48 h</th>
<th>Intradermal skin test* (15 min) antigen P1 (µg/ml)</th>
<th>IgP1 leucocyte histamine release† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topic dermatotic (n=10)</td>
<td>++</td>
<td>10⁻³</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>10⁻⁴</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>10⁻⁵</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>10⁻⁶</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>10⁻⁷</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>10⁻⁸</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>10⁻⁹</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>10⁻¹⁰</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10⁻¹¹</td>
<td>45</td>
</tr>
<tr>
<td>Non-atopic controls (n=10)</td>
<td>−</td>
<td>N.D.</td>
<td>&lt;10⁻⁹</td>
</tr>
</tbody>
</table>

*Done with 0-02 ml of serial 10-fold dilutions of antigen P1. A wheal of >6 x 6 mm was regarded as the endpoint. Skin tests negative at 10 µg/ml are recorded as >101.
†Nine out of ten patients showed histamine release (>10% above background) with dilutions of antigen P1 from 1 µg/ml to 10⁻⁶ µg/ml. Values shown are % release above background using 10 µg/ml.
§One subject gave a (+) reaction and one gave a (±) reaction.

TABLE III—TOTAL SERUM IgE AND SPECIFIC ANTIBODIES TO ANTIGEN P1

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total IgE* (units/ml)</th>
<th>IgE anti P1 binding activity†</th>
<th>IgG anti P1 binding activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatotic (n=10)</td>
<td>5460</td>
<td>206</td>
<td>811</td>
</tr>
<tr>
<td>Atopic (n=6)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Non-atopic (n=10)</td>
<td>9</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(13–50)</td>
<td>(0)</td>
<td>(3–9)</td>
</tr>
</tbody>
</table>

*1 unit IgE was assumed to be equivalent to 2·4 ng.
†Results obtained from a control curve and expressed in arbitrary units of binding activity (1 BA unit ≈ Ing). Results are expressed as geometric mean values. Figures in parentheses represent one standard deviation range above and below the geometric mean. An unpaired t test was used to compare the results of both the patient group and the atopic non-dermatotic group with the non-atopic control group. All results were converted to log values before statistical analysis.

Discussion

Our study has shown that a modification of the usual method of presenting immediate hypersensitivity allergens can consistently induce an eczematous delayed skin response. The dose of allergen used was larger than that necessary to produce an urticarial wheal in the same patients, but it is similar to that used to elicit delayed hypersensitivity responses. Furthermore, we found that dust from the beds of eight of our patients contained high levels of antigen P1 (50 ± 10⁻⁵, [SE] µg/g). Abrasion and prolonged exposure, both features of the technique used, simulate naturally occurring conditions, since scratching is an important feature of the disease and exposure to house dust allergens is likely to be prolonged and repeated.

The basophil infiltrate reported here is similar to that which occurs in induced contact dermatitis and in the late cutaneous response. Furthermore, electron microscope evidence of basophil degranulation, as has been reported in contact dermatitis, was present in some of the patch responses. The presence of basophils is interesting because these patients' leucocytes released histamine in response to the same antigen, and it is probable that the infiltrating basophils were sensitised to antigen P1. The presence of basophils may also imply that sensitised T cells are involved in the skin response, since human T cells can release a basophil chemotactic factor. Unlike induced contact dermatitis, the patch responses always showed a marked eosinophil infiltration. Recruitment of eosinophils into allergen-induced patch sites probably depends on the release of eosinophil chemotactic factor(s) from either mast cells or basophils. Neither basophils nor eosinophils have been reported to be increased in naturally occurring lesions of atopic dermatitis; however, it has been suggested that eosinophils may participate in the development of the lesions. The differences in histological findings between...
Basophil

Under the electron microscope basophil leucocytes (magnif. × 12 285; bar = 1 µm) had multilobed nuclei, large variable granules (diam. 860 nm ±226), and knob-like surface protrusions. The granules (below) (magnif. x 48 940; bar = 0·2 µm) were particulate and had a variable number of myeloid membranous figures.

atopic dermatitis and the patch test lesions may reflect three features of these patches. Firstly, they are of a known duration (48 h); secondly, they probably represent a fairly intensive local stimulation; and thirdly, they have not with cutaneous basophil hypersensitivity (CBH) in guineapigs— they both contain basophils and eosinophils, they have a similar time course, and they are elicited by similar doses of antigen. The basophils infiltrating guineapig lesions are known to be sensitive to the eliciting antigen and can release mediators locally. It seems likely that as in guineapig CBH the response to allergens in our patients involves both T cells and anaphylactic antibodies, and that both lesions represent an overlap between immediate and delayed hypersensitivity.

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REFERENCES