

Skin window eosinophil response in house dust allergy

**Joseph D. Bullock, M.D., Joann G. Bodenbender, M.T.,
William C. Deamer, M.D., and Oscar L. Frick, M.D., Ph.D.**
San Francisco, Calif., and Columbus, Ohio

Fifty-eight patients were studied by the skin window technique of Rebuck and Crowley. Ten control subjects had no skin window eosinophilia to house dust or mite extracts. Seven of 8 house dust-allergic patients, who received no immunotherapy, had skin window eosinophil responses to house dust extract, and all 8 had skin window eosinophilia to mite extract ($p < 0.001$). Seven of 8 house dust-allergic patients, who were receiving immunotherapy but were doing poorly clinically, had skin window eosinophil responses to house dust extract, and all 8 had skin window eosinophilia to mite extract ($p < 0.001$). Only 7 of 32 house dust-allergic patients, who were receiving immunotherapy and were doing well clinically, had skin window eosinophil responses to house dust extract ($p > 0.1$), but 29 had skin window eosinophilia to mite extract ($p < 0.001$). It is suggested that immunotherapy with improvement of clinical allergic symptoms may cause a decrease in release of mediators, perhaps including "eosinophil chemotactic factor."

For over 50 years house dust allergy has been recognized as a significant cause of bronchial asthma and perennial allergic rhinitis.^{1, 2} The search for a single house dust antigen has yielded many possibilities since house dust contains small quantities of many known allergens: Cotton linters, kapok, animal danders, molds, bacteria, algae, insects, and human dander have all been suspect. Recently, attention has focused on the house dust mite, *Dermatophagoides*.³⁻⁵ This tiny arachnid is found in large numbers in house dust samples from many parts of the world.⁶ They are particularly abundant in house dust collected from furniture which contains human keratin, upon which the mites subsist.⁷ Inhalation of minute amounts of mite exoskeleton or excrement produces allergic symptoms.⁸ Skin test results with purified mite extract correlate well with clinical house dust allergy.⁴ Purified mite extract is approximately 100 to 1,000 times as potent as house dust (weight by volume).⁴ In vitro and in vivo neutralization tests have shown a close but not complete identity of house dust and mite allergens.⁸

House dust extracts are often quite irritating, and positive skin reactions may represent a specific antigen-induced wheal and flare reaction or a nonspecific

From the Department of Pediatrics, University of California, and Department of Pediatrics, Ohio State University.

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Reprint requests to: J. D. Bullock, 150 East Broad St., Columbus, Ohio 43215.

wheel and flare reaction. The present study was designed to further evaluate mite and house dust allergens in children with bronchial asthma and/or perennial allergic rhinitis with the use of the skin window technique of Rebuck and Crowley.⁹

MATERIALS AND METHODS

Patients studied

Group A. Ten patients were studied in this control group. Six of these were atopic children who were not allergic (clinically or by skin tests) to house dust or to mite extract. Also studied were 4 nonatopic patients (negative past and present history of allergy, negative family history of allergy, low nasal and peripheral eosinophil counts, and negative skin tests to other allergens). All four had a 2+ or greater scratch test (over 20 mm. erythema) to house dust but not to mite extract.

Group B. Fifty-two children with perennial allergic rhinitis and/or bronchial asthma who were clinically allergic to house dust were chosen from the University of California Pediatric Allergy Clinic. All had a 2+ or greater scratch test to one or more commercial house dust extracts, and 48 also had a 2+ or greater scratch test to extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides culinae* (house dust mites). These 48 children were chosen for study in Group B.

A retrospective evaluation of allergic symptoms and medications required was made when it became apparent that there might be a relationship between skin window eosinophilia and clinical status. Group B was, therefore, subdivided into three subgroups (B_1 , B_2 , and B_3). A patient with a favorable clinical course following house dust immunotherapy (B_3) had asthma symptoms (wheezing, severe coughing on exertion, and night coughing) less often than 2 days a week and required bronchodilators less frequently than 2 days a week. Thirty-two patients fulfilled these criteria. A patient who fared poorly following house dust immunotherapy (B_2) fulfilled two or more of the following criteria: (1) required alternate-day corticosteroids (2 patients); (2) required disodium cromoglycate (2 patients); (3) required daily bronchodilators (6 patients); (4) had daily asthma symptoms (7 patients). Eight patients fulfilled these criteria.

These two groups were otherwise identical. Both received maximal tolerated doses of house dust extracts. There was no difference in the commercial sources of extract or the duration of therapy.

Eight patients had never received house dust immunotherapy (B_1). Four had responded well to house dust environmental control alone, and 4 were new to the Pediatric Allergy Clinic.

Allergenic extracts

The following extracts in the indicated concentrations (w/v) were used for scratch testing and on the skin window abrasions: control of Coca's solution in 50 per cent glycerine; house dust (Berkeley Biological), 1:40; house dust (Hollister-Stier), 1:10; house dust (Endo), 1:30; *Dermatophagoides pteronyssinus* (Bencard), 1:500; *Dermatophagoides culinae* (farinae) (Bencard), 1:500.

Skin window technique

The skin window technique used was similar to that of Rebuck and Crowley⁹ with a few modifications: The anterior surface of the forearm was scrubbed with iodine and alcohol and dried with a sterile gauze pad. Several 5 × 5 millimeter areas of skin were abraded to the prickle cell layer with a to-and-fro movement under gentle pressure with a No. 15 Bard-Parker blade. Each child was tested with control solution, house dust extract, and mite extract. In patients receiving house dust immunotherapy, the skin window studies were performed with the commercial house dust extract used in immunotherapy. Patients not

receiving immunotherapy were tested with the house dust extract producing the largest skin test reaction. *D. pteronyssinus* extract produced a larger skin test reaction than did *D. culinae* in all allergic patients and was thus used in the skin window testing. One drop of solution was dropped on the abraded area, which was covered with a 30×30 millimeter glass slide and a piece of cardboard. Micropore tape and an elastic bandage taped at both ends secured the windows. After 24 hours the slides were removed and allowed to air dry for 24 hours. The slides were mailed from San Francisco to Columbus, Ohio, where they were stained with Giemsa stain and read "blind" by a laboratory technician who knew nothing about the patients and who has had 6 years of experience in reading skin window slides. There were some clumps of cells on all slides, but areas of the slide were examined in which the cells were spread out evenly enough to be identified easily, and a 300 cell differential white blood cell count was performed on each slide. Peripheral white blood cell differential counts were performed prior to testing.

The allergen-stimulated skin window was compared to the control window. A threefold increase in percentage of eosinophils in the allergen window over the control window was considered significant. If the control window contained fewer than 1.5 per cent eosinophils, an increase of 3 percentage points was required for a positive result (i.e., 1.0 per cent to 4.0 per cent).

Reproducibility of the skin window technique was demonstrated as follows: Six children received skin windows in which exudation was stimulated by house dust extract, mite extract, and glycerinated Coca's solution. Five months later skin windows were repeated using the same extracts. The percentage of skin window eosinophils did not vary more than 4 per cent in any case.

Scratch testing

All children were scratch tested with the Chalazion knife. The control and five allergenic extracts were applied to the scratches. Scratch tests were read at 15 and 30 minutes and graded as follows: 0 = erythema less than 10 mm.; 1+ = erythema 10 to 20 mm. plus wheal of 2 mm.; 2+ = erythema over 20 mm. plus wheal of 2 mm.; 3+ = erythema over 20 mm. plus enlargement of wheal; 4+ = erythema over 20 mm. plus pseudopodia. The data were subjected to the chi square test with Yates correction factor.

RESULTS

The results are summarized in Table I. None of the 10 control patients (Group A) had skin window eosinophilia to Coca solution control, house dust extracts, or mite extracts. None of these windows contained more than 0.5 per cent eosinophils.

The 48 patients in Group B were judged to be atopic individuals and had a clinical history of house dust allergy. Each had a positive scratch test to one or more house dust extracts as well as to both mite extracts. The Coca solution control skin window contained fewer than one per cent eosinophils in all 48 house dust-allergic patients. Eight of these children (Group B₁) had never received house dust immunotherapy. Seven of the 8 (88 per cent) had positive skin window eosinophilia to house dust extract ($p < 0.001$), and all 8 (100 per cent) had skin window eosinophilia to mite extract ($p < 0.001$). The mean percentage of eosinophils on the 8 house dust skin window slides was 16 per cent (range 2 to 30 per cent). The mean percentage of eosinophils on the 8 mite skin window slides was 49 per cent (range 22 to 68 per cent).

Eight children (Group B₂) had experienced poor clinical responses to conventional therapy, including environmental control and immunotherapy to house

TABLE I. Skin window eosinophil response

Subjects	Positive house dust skin window	Mean percentage eosinophils in house dust skin window	Positive mite skin window	Mean percentage eosinophils in mite skin window
Group A (control subjects)	0/10 (0%)	0	0/10 (0%)	0
Group B ₁ (no immunotherapy)	7/8 (88%) $p = < 0.001^*$	16	8/8 (100%) $p = < 0.001^*$	49
Group B ₂ (poor response to immunotherapy)	7/8 (88%) $p = < 0.001^*$	19	8/8 (100%) $p = < 0.001^*$	57
Group B ₃ (favorable course after immunotherapy)	7/32 (22%) $p = > 0.10^*$	3	29/32 (91%) $p = < 0.001^*$	25

*Level of confidence (p) is given for each group compared to Group A (control subjects).

dust. Seven of 8 (88 per cent) had positive skin window eosinophilia to house dust ($p = < 0.001$), and all 8 (100 per cent) had skin window eosinophilia to mite extract ($p = < 0.001$). The mean percentage of eosinophils on the 8 house dust skin window slides was 19 per cent (range one to 45 per cent). The mean percentage of eosinophils on the 8 mite skin window slides was 57 per cent (range 7 to 77 per cent).

Thirty-two children (Group B₃) had improved symptoms following conventional therapy, including immunotherapy for house dust. Twenty-nine of these 32 children (91 per cent) had positive skin window eosinophilia to mite extract ($p = < 0.001$). However, only 7 of the 32 (22 per cent) had positive skin window eosinophil responses to a house dust extract ($p = > 0.10$). The mean percentage of eosinophils on the 32 house dust skin window slides was 3 per cent (range 0 to 19 per cent). The mean percentage of eosinophils on the 32 mite skin window slides was 25 per cent (range 0 to 65 per cent).

Several children had over 10 per cent peripheral blood eosinophilia but had fewer than one per cent eosinophils in the Coca solution control skin window.

DISCUSSION

Mite extract gave strongly positive skin test reactions in 48 of 52 house dust-sensitive children (91 per cent), which is in agreement with the findings of other investigators.^{4, 5, 7} In previous studies we^{10, 11} showed that skin window eosinophil migration is specific and totally unrelated to peripheral blood eosinophil content. This was again confirmed in the present study, as several children had over 10 per cent peripheral eosinophilia but no eosinophils in the control skin window. No control patients (Group A) had skin window eosinophilia to house dust or mite extracts. Therefore, eosinophil migration into an antigen-stimulated window appears to be a specific migration phenomenon. Four of the control patients were apparently nonatopic but had positive scratch tests to house dust. Since the skin window was negative in all 4, it appears that this technique may be useful in differentiating a nonspecific wheal and flare reaction from an immune wheal and flare reaction.

As the study progressed and we began to review the data, we were impressed that children who were receiving immunotherapy for house dust and were relatively free of allergic symptoms had less skin window eosinophilia than did the untreated patients and patients who were receiving immunotherapy for house dust but having daily symptoms. Unfortunately, the medication and symptom score was not performed prospectively but was used in retrospect.

Children who had not received immunotherapy and children who had responded poorly to house dust immunotherapy had significantly greater eosinophil exudation into the house dust and mite skin window sites than did children who improved clinically following house dust immunotherapy ($p = < 0.001$). These data suggest that eosinophil chemotaxis may be affected by immunotherapy, as shown previously by Fowler and Lowell.¹² They found that skin window eosinophilia was significantly decreased in patients receiving grass or ragweed immunotherapy.

Kay and associates^{13, 14} have shown that guinea pig immune complexes stimulate the production of at least two "eosinophil chemotactic factors" (ECF) which selectively attract eosinophils. ECF-C is complement dependent, and ECF-A is complement independent. Possible mediators of the allergic reaction—histamine, bradykinin, slow reacting substance of anaphylaxis (SRS-A), serotonin, and prostaglandins E_1 , E_2 , and $F_{2\alpha}$ —by themselves do not attract eosinophils in the guinea pig or man¹⁴⁻¹⁷ although histamine does attract eosinophils in horses.¹⁸

One possible explanation for our findings lies in the fact that immunotherapy produces IgG (blocking) humoral antibodies.¹⁹ During the skin window procedure, oozing of serum into the site occurs. IgG (blocking) antibody may be released into the skin window site and may combine with antigen, making it unavailable for the IgE (skin-sensitizing) antibody which is cell fixed in the skin.^{20, 21} Therefore, an allergic reaction involving IgE may fail to occur, and eosinophils may not be attracted. This would not explain why the poor responders to house dust immunotherapy still had eosinophil exudation into the skin window site, since they presumably are making IgG (blocking) antibody also. Perhaps they are making blocking antibodies to antigens in house dust different from the allergen.

An alternate explanation (Fig. 1) may be as follows: Human IgE (skin-sensitizing) antibody is cytophilic and binds to human basophils²⁰ and monkey mast cells,²¹ and presumably also to human mast cells. A specific antigen bridges the combining sites on two IgE molecules,²² apparently causing distortion of the cell membrane and stimulating mast cell and basophil degranulation with release of histamine and SRS-A.²³ Kay and associates²⁴ found that ragweed-sensitized human lung fragments (presumably their mast cells) free from complement released histamine, SRS-A, and eosinophil chemotactic factor (ECF-A) when challenged with ragweed extracts. Sadan and associates²⁵ showed that immunotherapy in ragweed-sensitive patients sometimes caused a decrease in basophil sensitivity (decreased histamine release) when basophils were challenged with ragweed. This decreased cellular sensitivity was related to clinical improvement of allergic symptoms. It is possible that immunotherapy causes a

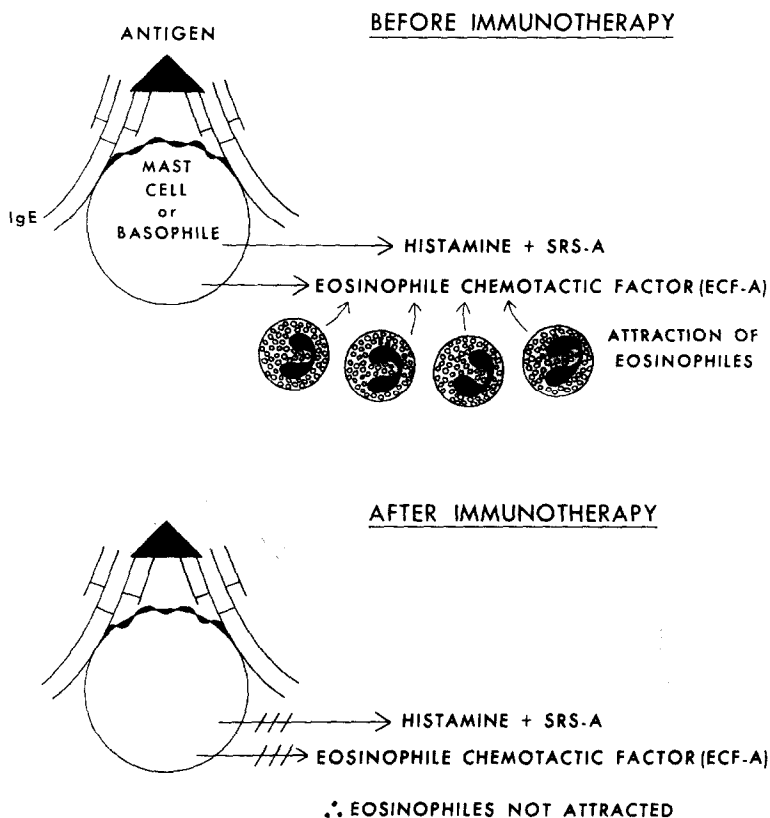


FIG. 1. Before immunotherapy: Antigen combines with mast cell or basophil-fixed specific IgE causing membrane changes and resulting in the release of histamine, slow reacting substance of anaphylaxis (**SRS-A**), and eosinophil chemotactic factor (**ECF-A**). Allergic symptoms occur and eosinophils are attracted to the area. **After immunotherapy:** Antigen again combines with cell fixed IgE, but histamine, **SRS-A**, and **ECF-A** are not released. Allergic symptoms fail to occur, and eosinophils are not attracted.

decreased release of histamine, SRS-A, and ECF-A from mast cells and basophils. The decreased release of histamine and SRS-A may bring about clinical improvement, and the decreased release of ECF-A would result in fewer eosinophils being attracted to the skin window site.

The latter hypothesis does not explain why the patients who were doing well on immunotherapy still had skin window eosinophilia to mite extract, although the percentage of eosinophils was considerably lower in these individuals (mean 25 per cent) than in the poor responders (mean 57 per cent). It is possible that the patients who improved on immunotherapy were allergic to a house dust component other than mite. It also is possible that if these patients were treated with mite extract, eosinophil chemotaxis might be further suppressed.

Eosinophil migration into areas of immune reactions has been observed for over 100 years, although the exact function of eosinophils still remains unknown. Litt²⁶ has shown that eosinophils phagocytize antigen-antibody complexes.

Archer²⁷ found that equine eosinophils release a substance which blocks histamine. Regardless of the function of the eosinophil, their migration into areas of immune reactions is an interesting observation which may be useful clinically in detecting immune reactions. The skin window technique may possibly be helpful in predicting the success of immunotherapy. Prospective studies are presently being undertaken to test this hypothesis.

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