

Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*

Part II. Identification of the major IgE-binding antigens by crossed radioimmuno-electrophoresis

Steven Krilis, Ph.D.,* Brian A. Baldo, Ph.D., and Antony Basten, Ph.D.

Sydney, North Ryde, and St. Leonards, Australia

Crossed immunoelectrophoresis demonstrated 51 antigens in a water-soluble extract of the house dust mite Dermatophagoides pteronyssinus. Autoradiography demonstrated that 11 of the antigens bound IgE antibodies in the sera of house dust mite-allergic patients. IgE antibodies in 23 different sera reacted with from one to eight antigens. On the basis of Lowenstein's (1978) definition of a "major allergen," two of the antigens, numbers one and 36 would be described as "major allergens." Apart from antigen number 36 that has already been demonstrated to be an important allergen in patients allergic to D. pteronyssinus, the clinical importance of the other 10 IgE-binding antigens has yet to be assessed. Reasons against use of the term "major allergen" and its replacement with the title "clinically important allergen" are advanced. (J ALLERGY CLIN IMMUNOL 74:142-6, 1984.)

Despite the prime importance of the house dust mites *D. pteronyssinus* and *Dermatophagoides farinae* as widespread sources of potent inhalant allergens in both temperate and tropical localities¹⁻⁵ and the claim for the isolation of the major allergen from *D. pteronyssinus*,⁶ no comprehensive and systematic study has been carried out to investigate the number and clinical importance of *Dermatophagoides* allergens.

After our isolation of an important allergen⁷ and demonstration of the existence of a number of antigenically distinct allergens in water-soluble extracts of *D. pteronyssinus*,⁸ we undertook a study to identify the *D. pteronyssinus* antigens most frequently bound by IgE antibodies in the sera of house dust-mite aller-

Abbreviation used

CRIE: Crossed radioimmuno-electrophoresis

gic patients. This study, with the use of the technique of CRIE,⁹ identified 11 IgE-binding mite antigens. The advantages and disadvantages of the technique and its value and relevance for identifying allergens is critically reviewed.

MATERIAL AND METHODS

House dust-mite extracts, human sera, rabbit antisera, and RAST were described in the previous paper.⁸

Crossed immunoelectrophoresis and CRIE

These were carried out according to the methods of Weeke¹⁰ and Weeke and Lowenstein⁹ and described in detail elsewhere.⁷

RESULTS

Crossed immunoelectrophoresis of *D. pteronyssinus* extract

Examination of water-soluble extracts of three different batches of "media-free" mites (Commonwealth Serum Laboratories, Parkville, Australia)

From the Clinical Immunology Research Center, University of Sydney; Commonwealth Scientific and Industrial Research Organization Wheat Research Unit, North Ryde; and Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, N.S.W., Australia.

Received for publication June 21, 1983.

Accepted for publication Feb. 5, 1984.

Reprint requests: Steven Krilis, Ph.D., Clinical Sciences Building, St. George Hospital, Belgrave St., Kogarah, N.S.W. 2217 Australia.

*Dr. Krilis is a recipient of a Research Fellowship from the National Health and Medical Research Council of Australia.

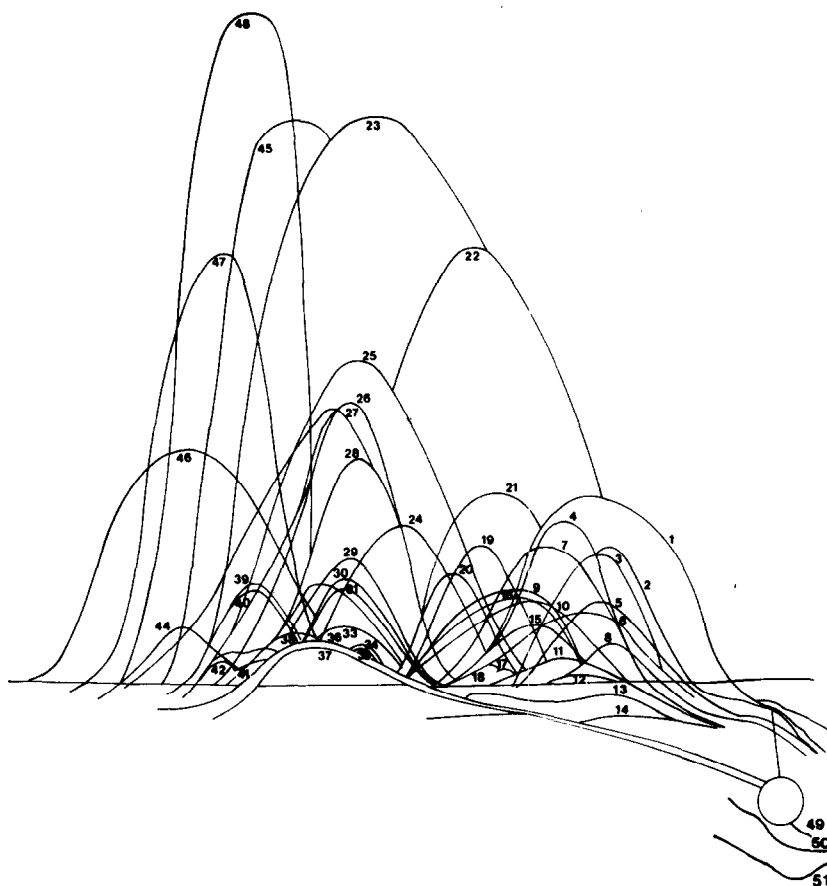


FIG. 1. Drawing of individual precipitin lines obtained after crossed immunoelectrophoretic analysis of a water-soluble extract of *D. pteronyssinus* with a hyperimmune rabbit antiserum (see Baldo et al⁷).

revealed 48 different anode-migrating and three cathode-migrating arcs. The reference pattern of 51 arcs detected is illustrated in Fig. 1, a diagrammatic representation of previously published gels and our more recent findings with antigens that move to the cathode⁷ (Krilis et al.⁸). Little qualitative difference was observed between the different batches of mite extract, but quantitative differences between some arcs, for example numbers 1, 22, 36, 46, and 48 were apparent.

Identification of IgE-binding antigens

Sera from different house dust-mite allergic patients were used in CRIE experiments in an attempt to identify the different mite antigens that bind human IgE and to establish the frequency of binding of the antigens. No positive reactions could be detected with many of the sera even though they had been obtained from patients clearly diagnosed as clinically allergic to *D. pteronyssinus*. In general, only those sera demonstrating at least a 3+ RAST reaction (Pharmacia Phadebas RAST Radioimmunoassay Directions for

Use pamphlet) produced positive autoradiographs with ¹²⁵I-labeled anti-human IgE. Twenty three sera, however, reacted with from one to eight arcs. The patterns of antigens detected with some of the sera are demonstrated in Fig. 2.

Nonspecific adsorption of IgE antibodies or ¹²⁵I-labeled anti-IgE was not observed. Sera from patients who were not allergic to mites but who nevertheless had high levels of IgE (e.g., to pollens), did not react with any of the precipitin arcs.

Frequency of reactivity of antigens with serum-IgE antibodies

Eleven of the 51 arcs were detected by autoradiography after reaction with patient's sera and ¹²⁵I-labeled anti-human IgE. The frequency of binding of different antigens is demonstrated in Fig. 3. Two antigens, numbers 1 and 36, reacted with more than 80% of the 23 sera, whereas antigens 22 and 48 reacted with more than 50% and 40% of the sera, respectively. Antigens 21, 23, and 25 bound IgE from 22% to 26% of the allergic sera. The remaining four

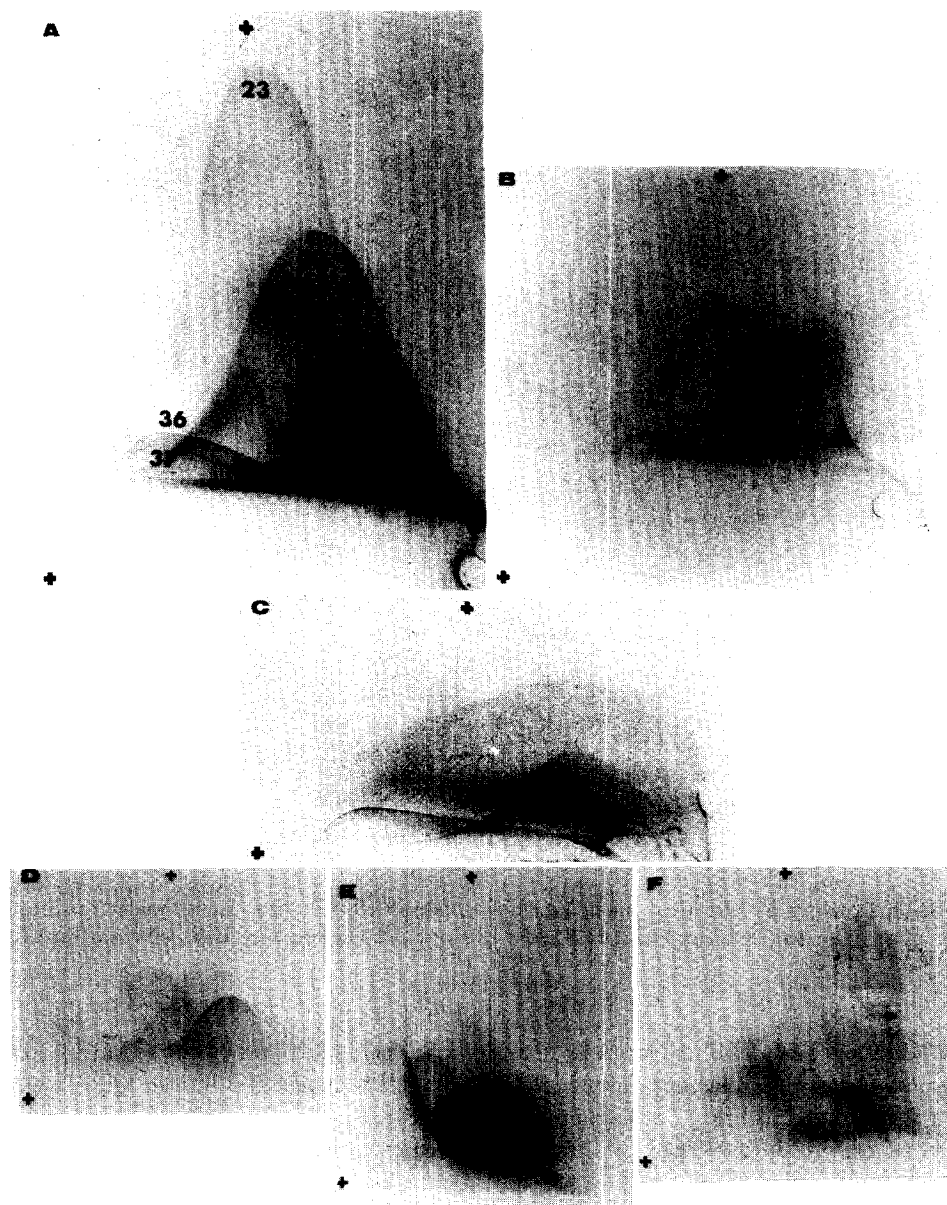


FIG. 2. Autoradiographs obtained with sera from different house dust-mite (*D. pteronyssinus*) allergic subjects (A through F) after crossed immunoelectrophoresis of a water-soluble extract of *D. pteronyssinus*. The numbered precipitin lines demonstrated in A correspond to the antigen numbers demonstrated in Fig. 1.

antigens detected on the autoradiographs, numbers 37, 41, 42, and 50 were positive with only two or three sera (9% to 13%).

DISCUSSION

CRIE has been used to identify allergens in a variety of allergen sources including timothy pollen, animal hair and epithelia, flour, codfish, fungi and house dust (see Lowenstein¹¹ for review and references). In some of these studies, significant correlations between CRIE and RAST were obtained.¹²⁻¹⁵

In our crossed immunoelectrophoresis study no IgE-binding antigens were detected with some of the sera despite the fact that there was no doubt about the allergic status of the patients and that serum dilutions and exposure time for autoradiography were suitable. In general, only sera that reacted strongly with mite allergens in the RAST to give at least a 3+ reaction (classified according to Pharmacia RAST reference discs) proved positive. Sera that were negative from patients who were proved to be mite-allergic with clear case histories implicating house dust mites and

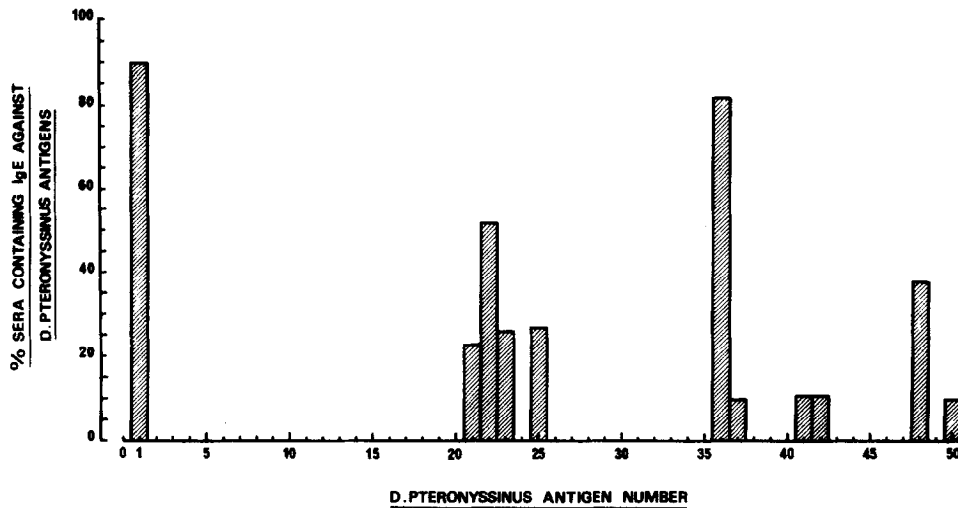


FIG. 3. Histogram demonstrating the frequency of antigen binding of mite-specific IgE antibodies in individual sera from a panel of patients allergic to *D. pteronyssinus*. The antigen numbers correspond to the numbers assigned to the precipitin lines demonstrated in Fig. 1.

positive skin prick test reactions to *D. pteronyssinus* extracts generally had RAST scores of 1+ or 2+. We have made a similar observation in our studies of dicot pollen allergy where only strongly reacting RAST-positive sera have proved positive in crossed immunoelectrophoresis experiments with plantain pollen extract.¹⁶ These observations, together with the difficulties we had in relating CRIE findings to other methods of allergen detection such as skin prick tests, RAST, and histamine release⁸ lead us to conclude that CRIE should not be used alone to identify allergens. The procedure should be combined with biologic test methods such as the skin prick test or histamine-release assay. CRIE and the RAST, unlike the latter two methods, detect IgE binding and provide no data on the biologic properties of the antigens. In addition, the electrophoretic method identifies the major IgE-binding antigens, but it is the clinical relevance of the antigens that is important. Hence the term "major allergen" defined by Lowenstein¹¹ as "antigens that bind IgE from at least 50% of the sera of patients tested and demonstrate strong binding to at least 50% of these" should, we believe, be abandoned and replaced by the designation "major IgE-binding antigens." When the importance of an antigen has been unequivocally established by the use of methods that reveal biologic properties known to be possessed by allergens, the description "clinically important allergen" would, we suggest, be a more appropriate description of the antigen than the presently used title of "major allergen."

On the basis of Lowenstein's¹¹ definition of a "major allergen," only antigen numbers 1 and 36 in

our study would be designated "major allergens." However, apart from antigen 36 that has already been isolated and demonstrated to be clinically important (Baldo et al.⁷) we cannot yet say which of the other 10 IgE-binding antigens identified here are clinically important. Our findings and those of other workers¹⁷ with use of CRIE confirm that a number of antigenically distinct allergens exist.

The technique of CRIE, although successfully applied to the study of a number of allergen sources, does have some important disadvantages. These include the need to produce an antiserum for each allergen source studied, failure to detect allergens that are not precipitated by the antiserum (because complementary antibodies are not present or because of the low molecular weight of the allergen), and the problem of coprecipitation in precipitates situated beneath the specific IgE-binding precipitates.^{11, 18} In our study, control experiments with anti-IgE alone and sera from patients with high IgE levels but who were not allergic to house dust mites, clearly demonstrated that the radiostaining we observed was specific.

A solution to some of the difficulties with CRIE may be provided by the electrophoretic transfer or "Western blotting" technique.^{19, 20} The method may be applied to allergen identification by transferring protein zones from a separation gel to a nitrocellulose membrane and then treating the membrane first with serum from an allergic patient and then with ¹²⁵I-labeled anti-human IgE.^{8, 21, 22} The transfer method requires less time than CRIE, it uses smaller quantities of patient's serum, an antiserum is not required, and the method provides a simple and efficient means

for investigating cross-reacting antigens in taxonomically related species side by side on the same separation gel.

REFERENCES

1. Bronswijk JE Van, Sinha RN: Pyroglyphid mites (*Acari*) and house dust allergy. *J ALLERGY* 47:31, 1971
2. Buchanan DJ, Jones IG: Allergy to house dust mites in the tropics. *Br Med J* 3:364, 1972
3. Sarsfield JK: Role of house-dust mites in childhood asthma. *Arch Dis Child* 49:711, 1974
4. Wright GTL, Derrick EH: The role of allergens in the aetiology of asthma in Brisbane. *Med J Aust* 1:375, 1975
5. Kabasawa K, Ishii A, Murata H, Takaoka M: Clinical significance of the house dust mite *Dermatophagoides pteronyssinus* in asthmatic children in Japan. *Acta Allergol* 31:442, 1976
6. Chapman MD, Platts-Mills TAE: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*—antigen P1. *J Immunol* 125:587, 1980
7. Baldo BA, Krilis S, Basten A: Selective approaches to the isolation and standardization of allergens. In Inman FP, Mandy WJ, editors: Contemporary topics in molecular immunology. New York, 1981, Plenum Publishing Corp, vol 8, p 41
8. Krilis S, Baldo BA, Sutton R, Basten A: Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. I. Demonstration of multiple allergens by immunochemical and biological analyses. *J ALLERGY CLIN IMMUNOL* 74:132, 1984
9. Weeke B, Lowenstein H: Allergens identified in crossed radioimmuno-electrophoresis. *Scand J Immunol* 2(suppl 1):149, 1973
10. Weeke B: Crossed immunoelectrophoresis. *Scand J Immunol* 2(suppl 1):47, 1973
11. Lowenstein H: Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 25:1, 1978
12. Weeke B, Lowenstein H, Nielsen L: Allergens in timothy pollen identified by crossed radioimmuno-electrophoresis (CRIE). *Acta Allergol* 29:402, 1974
13. Lowenstein H, Markusen B, Weeke B: Identification of allergens in extract of horse hair and dandruff by means of crossed radioimmuno-electrophoresis. *Int Arch Allergy Appl Immunol* 51:38, 1976
14. Blands J, Lowenstein H, Weeke B: Characterization of extract of dog hair and dandruff from six different dog breeds by quantitative immunoelectrophoresis. Identification of allergens by crossed radioimmuno-electrophoresis (CRIE). *Acta Allergol* 32:147, 1977
15. Prahl P, Weeke B, Lowenstein H: Quantitative immunoelectrophoretic analysis of extract from cow hair and dander. Characterization of the antigens and identification of the allergens. *Allergy* 33:241, 1980
16. Baldo BA, Chensee QJ, Howden MEH, Sharp PJ: Allergens from plantain (*Plantago lanceolata*). Studies with pollen and plant extracts. *Int Arch Allergy Appl Immunol* 68:295, 1982
17. Lind P, Lowenstein H: Identification of allergens in *D. pteronyssinus* mite body extract by crossed radioimmuno-electrophoresis with two different rabbit antibody pools. *Scand J Immunol* 17:263, 1983
18. Aukrust L, Almeland TL, Aas K: The specificity of radiostaining in crossed radioimmuno-electrophoresis. *Scand J Immunol* 8:421, 1978
19. Towbin T, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. *Proc Natl Acad Sci USA* 76:4350, 1979
20. Burnette WN, "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 112:195, 1981
21. Baldo BA, Sutton R, Wrigley CW: Grass allergens, with particular reference to cereals. *Prog Allergy* 30:1, 1982
22. Sutton R, Wrigley CW, Baldo BA: Detection of IgE- and IgG-binding proteins after electrophoretic transfer from polyacrylamide gels. *J Immunol Methods* 52:183, 1982