

Inevitably, referees may not agree, and the editors will need more advice. We must make an eventual decision as to acceptance, revision, or rejection; and I plan to be involved in all such final decisions.

This continues to put the burden of detailed review of articles on the almost 500 reviewers on whom the editors call. The referees cannot be

thanked enough for the hours they spend without pay in making the JOURNAL the leading publication in Allergy and Immunology. The new editorial team solicits their continued cooperation and hopes to see further growth and development of the JOURNAL.

Philip S. Norman, MD
Editor

A major antigen of *alternaria alternata* with potential for safe and effective immunotherapy

Most allergen extracts that are used in immunotherapy are crude mixtures and contain large amounts of nonallergenic proteins. Variability in allergenic potency among different batches of extracts is a recognized clinical pitfall.¹ Injection of allergen extracts also poses the risk of a generalized systemic reaction induced by a relative overdose of the allergenic material to which that patient is sensitive. Thus the physician is often faced with the paradox of only being able to administer small amounts of extract material to highly sensitive patients who actually require high doses of extract for effective immunotherapy. Another confounding variable is the fact that IgE antibody synthesis may be observed even in nonatopic persons who receive injection of naturally occurring allergens.² Hence, it is therefore possible that crude allergenic extracts could induce IgE synthesis in response to irrelevant antigens and thereby broaden rather than reduce the allergenic load in the patient. The ideal immunotherapeutic preparation would be one that had very potent immunogenicity without allergenicity.

Variability of mold extracts may present an even

Abbreviations used

IEF:	Isoelectric focusing
Ig:	Immunoglobulin
RAST:	Radioallergosorbent test
pI:	Isoelectric point

greater technical problem because investigators must consider interstrain differences, culture conditions, and optimum methods for extracting complex mixtures of mycelial mats and spores. Considering these obstacles, it is not surprising that the scientific search for major mold allergens has often been inconsistent. Portnoy et al. (pages 930-8) have addressed some of these issues by assessing the effects of time and extraction buffers on extractable proteins and allergens from four different strains of *Alternaria alternata*. Resolution of proteins in this study was greatly improved by two-dimensional electrophoresis. As expected, the extraction of specific allergens was dependent on strain, extraction time (1 hour vs 24 hours) and to a lesser extent, extraction buffer. In general, larger molecular weight allergens (42, 50, 70, 140 kd) were extracted rapidly within 1 hour and their appearance was not affected by protease inhibitors. Allergens below 40 kd in weight exhibited no clear-cut differences with respect to extraction time. Portnoy et al. concluded that extraction conditions must be optimized for obtaining particular allergenic glycoproteins from *A. alternata*. Of additional interest, however, was their identification of a 30/31 kd molecule after

From Bureau of Drug Research, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada.

Accepted for publication Dec. 22, 1992.

Reprint requests: Hari M. Vijay, PhD, FAAAAI, Health Protection Branch, Sir F. G. Banting Building, Ottawa, Ontario, Canada K1A 0L2.

J ALLERGY CLIN IMMUNOL 1993;91:826-8

Copyright © 1993 Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/8/45149

1 hour of extraction in three of the four sampled *A. alternata* strains. This major allergenic fraction derived from *A. alternata* had previously been observed and reported by four independent laboratories.

Studies on *A. alternata* mycelial and broth extracts³⁻⁵ have identified a major protein component reactive with human atopic serum that can be purified by gel filtration on Sephadex G-100 (Pharmacia, Ltd., Montreal, Quebec, Canada) followed by ion-exchange chromatography. Comparisons with standard proteins on Sephadex G-100 indicated that its molecular weight was 30 kd. After reduction, it produced a doublet pattern on sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with molecular weights of 14.5 and 16 kd. The doublet pattern was confirmed by western blotting with human atopic sera. Nonreduced samples showed a band at 29 kd, which also reacted strongly with specific IgE antibodies. This antigenic protein, which was described earlier as "hypoallergen,"³ is now termed "Alt a-29." Nondenaturing isoelectric focusing (IEF) of the protein showed three bands in the 4.15 to 4.55 isoelectric point (pI) region. Immunoblots of the IEF showed that all of the bands were reactive with human atopic serum.

Alt a-29 can induce IgG antibodies in rabbits but does not stimulate IgE antibodies in either rats or mice. This protein antigen is a potent inhibitor of the specific binding of IgE antibodies obtained for *Alternaria*-allergic patients in the RAST. In addition, this antigen has been found to be nonmutagenic by the Ames test and free of mycotoxins. This protein therefore appears to possess the appropriate constellation of biologic characteristics required for an "ideal" therapeutic agent as described above. It remains to be established whether this protein is allergenic in humans.

In the past year, three other reports of *A. alternata* components that are evidently descriptions of the same protein have appeared. Matthiesen et al.⁶ described a protein under nonreduced conditions in SDS-PAGE, which produced a 28 kd IgE binding band. They called this protein Alt a1. After reduction, Alt a1 showed a 14 kd band that indicated a disulfide-bonded dimeric structure. Both reduced and nonreduced forms bound monospecific rabbit antibodies and a murine monoclonal antibody against Alt a1. These authors showed that Alt a1 inhibited the specific IgE antibody in inhibition experiments such as SDS-PAGE immunoblotting. Protein sequencing of the 14.5 and 16 kd components of Alt a-29 produced identical N-terminal sequences, and comparison with the Alt a1 N-terminal sequence of Matthiesen et al.⁶ with Alt a-29 indicated

that these proteins have 85% homology over the first 20 amino acids.

Recently, Deards and Montague⁷ also reported a major allergen with a molecular weight of 29 kd (Alt a Bd 29K) from *A. alternata*. After reduction, this protein also produced a band at 15 kd in SDS-PAGE, which showed IgE binding activity. In IEF, the pI value of Alt a Bd 29K was 4.2. Paris et al.⁸ reported a 31 kd major allergen from *A. alternata* termed Alt a I₁₅₆₃, which in IEF showed a major band at pI 4.2 and two minor variants with pI 4.25 and 4.35. SDS-PAGE of this antigen after reduction was not performed, so the quaternary structure of this protein has yet to be determined.

From the above characteristics, it appears that Alt a-29, Alt a1, Alt a Bd 29K, and Alt a I₁₅₆₃ are probably the same protein. Variations in the physical characteristics of the antigens are within the errors of the methods or could be due to strain-related differences and/or growth conditions, as demonstrated by the current report of Portnoy et al. (pages 930-8).

Availability of a purified allergen derived from one of the most common allergenic fungi should have far-reaching clinical applications. As is currently the case with pollen, mite, and epidermal allergens,⁹ standardization of diagnostic and therapeutic mold extracts will be greatly facilitated by direct comparison with Alt a-29. A uniform standard may have even more utility in the case of mold extracts because of production variables caused by strain differences, culture conditions, and processing of mycelial mats or spores. General access to mold extracts with uniform potency should also improve the precision of in vivo and in vitro diagnostic tests and encourage expansion of controlled clinical studies for definitive efficacy assessment of mold immunotherapy.

H. M. Vijay, PhD

National Health and Welfare Canada

N. M. Young, PhD

National Research Council of Canada, Ottawa

I. H. A. Curran, PhD

National Health and Welfare Canada, Ottawa

D. F. Copeland, MD

Ottawa, Ontario, Canada

I. L. Bernstein, MD

University of Cincinnati, Cincinnati, Ohio

REFERENCES

1. Gleich GJ, Larson JB, Jones RT, Bear H. Measurement of the potency of allergy extracts by their inhibitory capacities in the radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* 1974; 53:158-69.

2. Greenert S, Bernstein IL, Michael JG. Immune responses of non-atopic individuals to prolonged immunization with ragweed extract. *Lancet* 1971;2:1121-3.
3. Vijay MH, Huang H, Lavergne G, Young NM, Bernstein IL. Studies on *Alternaria* allergens. II. Presence of two related antigens with contrasting allergenic properties in *Alternaria tenuis* extracts. *Int Arch Allergy Appl Immunol* 1981;54:410-6.
4. Vijay HM, Burton M, Young NM. Evidence that the hypoallergen of *Alternaria alternata* carries a dominant epitope of the major allergen. In: Sehon AH, Draft D, Kunkel G, eds. Epitopes of atopic allergens. Proceedings of Workshop, XIV Congress European Academy of Allergology and Clinical Immunology; Berlin; Sept. 1989. The UCB Institute of Allergy, 1989:14-7.
5. Curran IHA, Young NM, Burton M, Vijay HM. Purification and characterization of a low molecular weight antigen from *Alternaria alternata*. (*J ALLERGY CLIN IMMUNOL* 1992;89:283.
6. Matthiesen F, Olsen M, Lowenstein H. Purification and partial sequencing of the major allergen of *Alternaria alternata*. [Abstract]. *J ALLERGY CLIN IMMUNOL* 1992;89:241.
7. Deards MJ, Montague AE. Purification and characterization of a major allergen of *Alternaria alternata*. *Molecular Immunol* 1991;28:409-15.
8. Paris S, Debeaupuis JP, Prevost MC, Casotto M, Latgé JP. The 31 Kd major allergen, Alt a 1 1563 of *Alternaria alternata*. *J ALLERGY CLIN IMMUNOL* 1991;88:902-8.
9. Reed CE, Yunginger JW, Evans R. Quality assurance and standardization of allergy extracts in allergy practice. *J ALLERGY CLIN IMMUNOL* 1989;84:4-8.

AVAILABLE NOW! The FIVE-YEAR (1986-1990) CUMULATIVE INDEX TO THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY can be purchased from the Publisher for \$48.00. This comprehensive, 188-page reference guide is a current presentation of all topics included in the JOURNAL from January 1986 to December 1990 (volumes 77-86)—the past 10 volumes. It incorporates complete references to over 1500 original articles, abstracts, case reports, letters, editorials, and CME articles. It features 1662 subject headings, under which there are 5863 entries. Each subject entry lists the complete article title, author(s), volume, page, and year of publication. It also includes 5801 author entries, listing contributors, along with their respective titles, author-to-author referral, volume, page, and publication date.

To purchase, call or write: Mosby, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318, or telephone FREE 1-800-325-4177, Subscription Services, ext. 4351, or 314-453-4351. PREPAYMENT REQUIRED. Make checks payable to Mosby. (All payments must be in US funds drawn on a US bank.) Price: \$48.00 in the US, \$54.35 in Canada, and \$51.00 international (price includes mailing charges).