

Predominance of the major allergen (*Alt a* I) in *Alternaria* sensitized patients

J. KLEINE-TEBBE, M. WORM, S. JEEP, F. MATTHIESEN*,
H. LÖWENSTEIN* and G. KUNKEL

Department of Clinical Immunology and Asthma Outpatient Department, Universitätsklinikum Rudolf Virchow, Freie Universität Berlin, Germany and *ALK Research, Horsholm, Denmark

Summary

Thirty-nine patients sensitized to *Alternaria* were evaluated using titrated skin-prick test (SPT), histamine release studies (HR), inhibition of RAST and immunoblotting studies. To determine the relevance of the major allergen, *Alt a* I, specific rabbit antibodies against *Alt a* I and *Alt a* B were used. The antibodies were preincubated at different concentrations: (i) with the *Alternaria* allergen dose required for maximum response in the HR assay (10 BU/ml) and (ii) with the *Alternaria* antigen coupled to RAST paper discs (1000 BU/disc). Dose dependent inhibition of histamine release ($n=30$, $\bar{x}=80\% \pm 4\%$, $IC_{30}=0.69 \mu\text{g/ml}$) and of RAST ($n=7$, $IC_{30}=4.4 \mu\text{g/ml}$) was found in all patients sensitized to *Alternaria* as indicated by allergen induced HR. The greater the response to *Alternaria* in HR, the higher the antibody concentrations necessary for inhibition ($P < 0.05$). Immunoblot experiments ($n=25$) using SDS-PAGE showed in all cases IgE- and IgG binding at approximately 28 kD, which is the size reported for the major allergen, *Alt a* I. In two cases, slight IgE binding at 45 and 66 kD was also found, while in two other patients, only IgE binding at 66 kD was seen. Our findings emphasize the major importance of *Alt a* I in patients sensitized to *Alternaria*.

Clinical and Experimental Allergy, Vol. 23, pp. 211–218. Submitted 27 March 1991; revised 17 August 1992; accepted 7 September 1992.

Introduction

In recent years, the role of *Alternaria* as an important aeroallergen in Europe and North America became evident since 20–30% of individuals with IgE mediated inhalant allergies were found to be positive by skin prick or intradermal tests [1,2] using *Alternaria* allergen extracts.

Earlier investigations to characterize the *Alternaria* allergens failed primarily due to inappropriate methods. Also, the wide range of different allergens in *Alternaria* extracts limited clear cut results [1,3–5]. After isolation of a protein fraction with a MW of 25–50 kD, *Alt a* I was described [6]. Later, important single allergens were investigated using crossed immunoelectrophoresis [7].

The relevance of *Alt a* I as a major allergen was established in a collaborative study using several *in vitro* methods [8]. Depending on the origin of the raw material, spores versus mycelia, the methods used, either culturing and harvesting the fungal antigens, or the preparation and storage of the allergenic extracts, differences in the content of allergenic compounds were found using several biological and immunological methods [9–11]. Results of collaborative studies [12,13] enabled the selection of a candidate for the international standard for *Alternaria* extracts, a useful tool for better control of extract potency and quality. However, the selected antigen was not useful due to its poor stability.

Despite this work, little knowledge exists about the frequency of IgE-mediated sensitization to the major allergenic compound of *Alternaria*. Since monospecific antibodies against *Alt a* I and *Alt a* B have been shown to bind to these allergens [14,15], we felt that they might be useful tools for studying their allergenic relevance in cases of clinical sensitization to *Alternaria*.

Correspondence: Professor Dr G. Kunkel, Freie Universität Berlin, Universitätsklinikum Rudolf Virchow, Abteilung für klinische Immunologie und Asthma-Poliklinik, Augustenburger Platz 1, 1000 Berlin 65, Germany.

SDS-PAGE/Westernblot

The appearance of several proteins, most with a molecular weight between 10 and 70 kD, has been described in other studies using SDS-PAGE for the separation of *Alternaria* extracts [28,29]. IgE binding capacities of *Alternaria alternata* extracts might be reduced by dissociating conditions during SDS-PAGE [30]; however, a great variety of IgE-binding components in mould allergens were detectable after immunoblotting probably due to the refolding of allergens during the blotting procedure where SDS was partially removed [30].

Several bands were visualized using anti-*Alt a I*. The strongest band was the 28 kD band also recognized by the IgE antibodies of the patient's sera. Most of the other bands recognized apparently represent the proposed *Alt a I* subunit (MW 14 kD) and high MW aggregates of the 28 kD MW band [17]. Two bands at 25 kD and 36 kD cannot be explained by this reasoning, and might be due to impurities in anti-*Alt a I*. Another possible explanation is non-specific binding of the rabbit IgG-antibodies to carbohydrates, which have been found to be an important fraction of *Alternaria* extracts [31,32], and which would not stain as protein. However, as demonstrated by different immunoelectrophoretic techniques, anti-*Alt a I* appears to be highly specific for the 28 kD MW band, which has been shown to occur also as a band at 14 kD MW under reducing conditions [17].

In agreement with our findings, the *Alt a I* protein fraction first isolated was found to have a molecular weight of 25–50 kD [6]. Another purified preparation of *Alt a I* was characterized by gel filtration to have a MW of 60 kD [7]. In contrast, Vijay *et al.* evaluated one fraction with high allergenic activity and a MW of 20 kD [33], while Bengtson *et al.* [28] identified two protein bands (approximately 16 and 18 kD) as major allergens. Allergen extracts of different origin and variable contents could explain the different results [5,10,11]. Recent data obtained using SDS-PAGE for the investigation of *Alt a I* fractions of different allergen extracts [14,15,17] confirm our findings (*Alt a I*, MW of 28 kD) or give similar results: *Alt a I*, MW of 29 kD [34] and 31 kD [32]. While in one study, 71% of the patients had specific IgE antibodies to this protein as measured by CRIE [14,17], we found an IgE-binding band recognized by anti-*Alt a I* in all patients' sera (100%). Furthermore, for 84% of sera this was the only IgE binding site.

Two proteins (MW 44 and 66 kD) with much less IgE antibody binding capacity than *Alt a I* might be identical to AgB and AgC that have been described as minor allergens of *Alternaria* (MW of 40 and 50 kD) [8]. However, we found only rare evidence for sensitization towards these secondary allergens, which might be the

result of our allergen extract containing only a small amount of minor allergens. Further clinical evaluations are necessary to define the relevance and role of minor allergens in patients with sensitization to *Alternaria*.

Acknowledgment

We gratefully acknowledge Dr Anne Kagey-Sobotka (Johns Hopkins Asthma and Allergy Center, Baltimore, U.S.A.) for editorial assistance.

References

- 1 Aas K, Leegard J, Aukrust L, Grimmer O. Immediate type hypersensitivity to common molds. *Allergy* 1980; 35:443–51.
- 2 Yee EG, Bahna SL. Skin testing and RAST to molds in patients with respiratory allergy. *J Allergy Clin Immunol* 1986; 77:200 (abstract).
- 3 Agarwal MK, Jones RT, Yunginger JW. Shared allergic and antigenic determinants in *Alternaria* and stemphylium extracts. *J Allergy Clin Immunol* 1982; 70:437–44.
- 4 Hoffmann DR, Kozak PP, Gillman SA, Cummins LH, Gallup J. Isolation of spore specific allergens from *Alternaria*. *Ann Allergy* 1981; 46:310–16.
- 5 Yunginger JW, Jones RT, Gleich GJ. Studies on *Alternaria* allergens II. Measurement of the relative potency of commercial *Alternaria* extracts by the direct RAST and by RAST inhibition. *J Allergy Clin Immunol* 1987; 58:405–13.
- 6 Yunginger JW, Jones RT, Nesheim ME, Geller M. Studies on *Alternaria* extracts III. Isolation of a major allergic fraction (*Alt I*). *J Allergy Clin Immunol* 1980; 66:138–47.
- 7 Nyholm L, Löwenstein H. Identification and characterization of allergens from *Alternaria alternata*. *Allergy* 1982; 37, Suppl.1:40 (abstract).
- 8 Nyholm L, Löwenstein H, Yunginger JW. Immunochemical partial identity between two independently identified and isolated major allergens from *Alternaria alternata* (*Alt I* and *Ag I*). *J Allergy Clin Immunol* 1983; 71:461–7.
- 9 Vijay HM, Young NM, Jackson GED, White GP, Bernstein IL. Studies on *Alternaria* allergens. V. Comparative biochemical and immunological studies of three isolates of *Alternaria tenuis* cultured on synthetic media. *Int Archs Allergy Appl Immunol* 1985; 78:37–42.
- 10 Vijay HM, Young NM, Bernstein IL. Studies on *Alternaria* allergens. VI. Stability of the allergen component of *Alternaria tenuis* extracts under a variety of storage conditions. *Int Archs Allergy Appl Immunol* 1987; 83:325–8.
- 11 Steringer I, Aukrust L, Einarsson R. Variability of antigenicity and allergenicity in different strains of *Alternaria alternata*. *Int Archs Allergy Appl Immunol* 1987; 84: 190–7.
- 12 Helm RM, Squillace DL, Aukrust L, Borch S, Baer H, Löwenstein H, Zmamirowski R, Nitchuk W, Yunginger JW. Production of an International Reference Standard *Alternaria* extract. *Int Archs Appl Immunol* 1987; 82:178–89.
- 13 Helm RM, Squillace DL, Yunginger JW and Members of the International Collaborative Trial. Production of a

- proposed International Reference Standard *Alternaria* extract—II. Results of a Collaborative Trial. *J Allergy Clin Immunol* 1988; 81:651–63.
- 14 Matthiesen F, Larsen L, Løwenstein H. Purification and characterization of two important mold allergens, *Alt a I* and *Cl a h I* (abstract). XIVth Congr of the EAACI 1989. *Allergologie* 1989; 12:21.
 - 15 Matthiesen F, Løwenstein H. Quantitative determinations of allergenic components in *Cladosporium* and *Alternaria*. *NER Allergy Proc* 1988; 9:261 (Abstr. ICACI; Montreaux).
 - 16 Dreborg SKG, Einarsson R. The major allergen content of allergenic preparations reflects their biological activity. *Allergy* 1992; 47:418–23.
 - 17 Matthiesen F, Friberg L, Olsen M, Løwenstein H. Purification and characterisation of the major allergen of *Alternaria alternata*, *Alt A I*. *J Allergy Clin Immunol* 1993, in press.
 - 18 Harboe N, Ingild A. Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand J Immunol* 1973; 2(suppl.1):161–9.
 - 19 Sirangian RP. Automated histamine analysis for in vitro allergen testing—II. Correlation of skin tests results with in vitro whole blood histamine release in 82 patients. *J Allergy Clin Immunol* 1977; 59:214–22.
 - 20 Neville DM. Molecular weight determination of protein dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J Biol Chem* 1971; 246:328–34.
 - 21 Westphal W. Analysis of rye pollen (*Secale cereale*) allergens using patient IgE immunoprint, westernblot and monoclonal antibodies. *Int Arch Allergy Appl Immunol* 1988; 86:69–75.
 - 22 Ostergaard PA, Kaad PH, Schwartz B, Ingemann L. Bronchial provocation and histamine release test in asthmatic children with mold allergy. Annual meeting of the EAACI 1985, Stockholm (abstract 120).
 - 23 Reinders E, Chin HC, Yoo TH. Human leukocyte histamine release assays with whole ragweed and *Alternaria* antigens using technicon autoanalyser II. *Allergy* 1980; 35:391–8.
 - 24 MacGlashan DW, Peters SP, Warner J, Lichtenstein LM. Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to correspond to dimeric cross-links. *J Immunol* 1986; 136:2231–9.
 - 25 Nguyen K-L, Gillis S, MacGlashan DW. A comparative study of releasing and nonreleasing human basophils: nonreleasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. *J Allergy Clin Immunol* 1990; 85:1020–9.
 - 26 Miles RM, Parker JL, Jones RT, Dahlberg MJE, Yunginger JW. Studies on *Alternaria* allergens. IV. Biologic activity of a purified *Alternaria* fraction (*Alt-I*). *J Allergy Clin Immunol* 1983; 71:36–9.
 - 27 Steward MW. Antibodies: their structure and function. London: Chapman and Hall, 1983.
 - 28 Bengtsson A, Karlsson A, Rolfsen W, Einarsson R. Detection of allergens in mold and mite preparations by a nitrocellulose electroblotting technique. *Int Archs Allergy Appl Immunol* 1986; 80:383–90.
 - 29 Lombardero M, Duffort O, Cortes C, Carreira J. Immunchemische und biologische Charakterisierung eines Referenzextraktes aus *Alternaria alternata*. *Allergologie* 1988; 12:509–13.
 - 30 Karlsson-Borgå Å, Rolfsen W. Methodological considerations when using nitrocellulose immunoblotting from polyacrylamide gels to study the mold allergens *Aspergillus fumigatus* and *Alternaria alternata*. *J Immunol Meth* 1991; 136:91–102.
 - 31 Portnoy J, Pacheco F, Ballam Y, Barnes C. Separation of *Alternaria* into protein and carbohydrate fractions with phenyl sepharose. *J Allergy Clin Immunol* 1991; 87:789–93.
 - 32 Paris S, Debeaupuis JP, Prévost MC, Casotto M, Latgé JP. The 31 kD major allergen, *ALT a I*₁₅₆₃, of *Alternaria alternata*. *J Allergy Clin Immunol* 1991; 88:902–8.
 - 33 Vijay HM, Huang H, Laverne G, Young NM, Bernstein IL. Studies on *Alternaria* allergens II. Presence of two related antigens with contrasting allergenic properties in *Alternaria tenuis* extracts. *Int Archs Allergy Appl Immunol* 1981; 65:410–16.
 - 34 Deards MJ, Montague AE. Purification and characterisation of a major allergen of *Alternaria alternata*. *Mol Immunol* 1991; 28:409–15.