

Letter to the Editor

Predominant localization of the major *Alternaria* allergen Alt a 1 in the cell wall of airborne spores*To the Editor:*

The fungus *Alternaria*, a common plant pathogen, occurs worldwide and releases allergen-bearing spores especially in late summer and early autumn.¹ Increased levels of *Alternaria* spores in the atmosphere have been linked to severe manifestations of respiratory allergy (ie, severe rhinitis and asthma), frequent occurrence of hospital delivery, and even asthma-related death.²

Alt a 1 is the most important *Alternaria* allergen. It reacts with serum IgE from more than 95% of *Alternaria*-sensitized patients, contains most of the *Alternaria*-specific IgE epitopes, and shows high allergenic activity. Several other *Alternaria* allergens have been characterized, but they are recognized by fewer patients and their allergenic activity appears to be much lower.³ In this study, we investigated the ultrastructural localization of Alt a 1 and Alt a 8, another well-described *Alternaria* allergen which is less frequently recognized by *Alternaria*-sensitized patients than Alt a 1 (ie, 41%)³ by immunogold electron microscopy. In order to obtain highly specific antibody probes recognizing the protein backbone of the allergens, peptides of Alt a 1 (aa 19-52) and Alt a 8 (aa 146-173) with predicted high surface accessibility were synthesized and used for immunization of rabbits as described.⁴ The *Alternaria* strain CBS 103.33 (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) was cultivated on a Sabouraud glucose agar plate (Becton Dickinson, Heidelberg, Germany) for 7 days at room temperature. Pieces measuring 1 mm² were excised from the dry mould and fixed overnight at 4°C in phosphate buffer (0.1 mol/L, pH 7.4) containing 4% (w/v) paraformaldehyde and 0.5% (v/v) glutaraldehyde. Samples were dehydrated through an ethanol series and afterwards infiltrated and polymerized in a LR White resin (Sigma, St Louis, Mo). Ultrathin sections were cut from embedded fungal tissue and mounted on gold grids. The sections were preincubated in 5% (w/v) BSA in 100 mM phosphate buffer (pH 7.4) and incubated with protein G purified (Pierce, Rockford, Ill) anti-Alt a 1 and anti-Alt a 8 antibodies. Sections were then exposed

to goat anti-rabbit IgG antibodies coupled to 10-nm gold particles diluted in phosphate buffer (0.1 mol/L, pH 7.4).

Interestingly, Alt a 1 was exclusively found in the cell wall, and there in the melanin layer of older spores (Fig 1, A) whereas no significant labeling was found within the cytoplasm, in the hyphae or in younger spores (data not shown). In contrast, Alt a 8 could be localized only in the cytoplasm of hyphae (Fig 1, B and C) but not in spores (data not shown). In the cytoplasm of hyphae it accumulated in electron-dense material within vacuole-like compartments.

The distinct and exclusive localization of Alt a 1 in the cell wall observed by us is in contrast to data obtained in 2 earlier studies describing a diffuse labeling of the cytoplasm and cell wall.^{5,6} This diffuse localization observed in the earlier studies may have been caused by different culture conditions. In fact, we used air-grown mold mimicking natural growth conditions, whereas in the other studies molds were grown under artificial liquid culture conditions. In this context, it has been shown recently that different culture conditions can influence the formation of a hydrophobin layer that may affect immune recognition of fungi.⁷ It is also possible that growth in liquid media may have caused elution and dislocation of Alt a 1 in liquid cultures, a phenomenon that has been found to cause diffusion of allergens also from pollen grains upon hydration.⁸

The localization of Alt a 1 in the spore wall observed in this study is in agreement with data suggesting that Alt a 1 might be involved in spore germination.⁹ Spores are essential for the survival and dispersal of fungi and some of them—including those of *Alternaria*—are resistant against adverse climatic conditions. In fact, Alt a 1 also represents an extremely stable protein whose conformation and IgE epitopes even resist heat treatments of 95°C (Twaroch et al, unpublished data, 2011).

The localization of the less frequently recognized Alt a 8 allergen in vacuole-like compartments of the hyphae is in accordance with its natural function as a mannitol dehydrogenase. The sugar alcohol mannitol is besides trehalose an important storage carbohydrate in fungi that constitutes approximately 11% of *Alternaria alternata* dry weight.

It is tempting to speculate that the localization of Alt a 1 in the airborne spore fraction, and in particular in the cell wall of *Alternaria* spores, may contribute to symptoms in the upper airways and eventually to sensitization and boosting of IgE responses

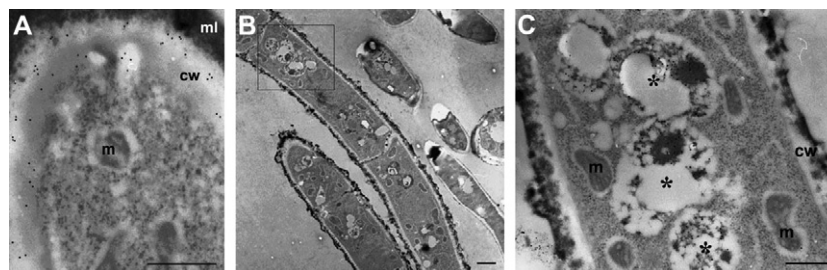


FIG 1. Localization of Alt a 1 and Alt a 8 by immunogold electron microscopy. **A**, Specific labeling of Alt a 1 in the melanin layer of *Alternaria* spores. **B** and **C**, Ultrathin section of hyphae of *Alternaria alternata* after staining with anti-Alt a 8 antibodies. **C**, Enlargement of the inset in **B**. Electron-dense material in vacuolar-like compartments is marked by asterisks. cw, Cell wall; m, mitochondria; ml, melanin layer. Scale bars = 0.5 μ m.

via the nasal mucosa and thus explain the high clinical relevance of this allergen.

Teresa E. Twaroch, MSc^a

Elsa Arcalís, PhD^b

Katja Sterflinger, PhD^c

Eva Stöger, PhD^b

Ines Swoboda, PhD^{a*}

Rudolf Valenta, MD^a

From ^athe Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria; ^bthe Department for Biotechnology, Institute for Applied Genetics and Cell Biology, University of Natural Resources and Applied Life Sciences, Vienna, Austria; ^cthe Department for Biotechnology, Institute for Applied Microbiology, University of Natural Resources and Applied Life Sciences, Vienna, Austria. E-mail: rudolf.valenta@meduniwien.ac.at.

*I. Swoboda is currently at the Section of Molecular Biotechnology, University of Applied Science, Campus Vienna Biocenter, Vienna, Austria.

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