Immunoreactive recombinant Alt a 1 expressed in Pichia pastoris

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RATIONALE
Alternaria sensitivity has been shown to be one of the major risk factors associated with asthma (1). 80% of alternaria-sensitized patients have specific-IgE to Alt a 1 (2). The structure of recombinant Alt a 1 expressed in E.coli has recently been determined by X-ray crystallography (Fig. 1, Ref. 3). However, this E.coli-expressed rAlt a 1 is no longer commercially available. Our goal was to produce rAlt a 1 in Pichia pastoris, a eukaryotic system more closely related to Alternaria alternata.

METHODS
Recombinant Alt a 1 was expressed in Pichia pastoris and purified by size exclusion chromatography (SEC). Pichia-expressed rAlt a 1 (RP-AA1) was compared to E.coli-expressed rAlt a 1 (RE-AA1) by SDS-PAGE, Far-UV CD spectroscopy, monoclonal antibody (mAb) ELISA and chimeric-IgE antibody ELISA. IgE binding of RP-AA1 was also compared to Streptavidin-CAP values for RE-AA1.

RESULTS
Purification of recombinant Alt a 1: Recombinant Alt a 1 was expressed in Pichia pastoris and purified over a SEC. The yield for RP-AA1 was 193 mg/L of culture. Silver-stained SDS-PAGE of the yeast culture supernatant showed a doublet at 35 kDa and a dimer at 30 kDa (Fig. 2, Lane 1) as seen in a previous study (4). After the SEC step, the dimer was the predominant band (Lane 2). RE-AA1, on the other hand, shows both the monomer and dimer equally (Lane 3). The calculated molecular weight of RP-AA1 was 26 kDa based on the SEC molecular weight calibration curve, suggesting that Alt a 1 presents as a dimer in solution (Figs. 1-3).

CD Spectral Analysis:
Differences in the CD spectra may be attributed to the variation in the molecular forms - RP-AA1 presents as a dimer while RE-AA1 shows both the monomer and the dimer (Fig. 2, 4).

CONCLUSIONS
Pichia-expressed recombinant Alt a 1 presents as an allergen dimer in solution, as previously described (2-4). It is almost identical to E.coli-expressed Alt a 1 in mAb and IgE binding assays. Alt a 1 produced in a eukaryotic system is a useful reagent for diagnostic assays to determine sensitization to Alternaria, and has other applications in T-cell studies, histamine release assays and mouse models of asthma.

REFERENCES
1) Buch & Portnoy. The role and abatement of fungal allergens in allergic diseases. JACI 2001; 107:S430-40

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