Sabina Wünschmann, PhD September, 2012

Focus on…Alt a 1

Exposure to high levels of *Alternaria* spores in the US Midwest during the spring and summer months is a risk factor for asthma attacks and has been associated with respiratory arrest among children and young adults[1]. Sensitization and exposure to *Alternaria* species was also associated with asthma in the Inner City Asthma Studies and the most recent National Health and Nutrition Examination Survey[2-6]. Most research has focused on *A. alternata*.

Thirteen allergens of *Alternaria alternata* have been identified, however only Alt a 1 is considered a major allergen. Alt a 1 is a dimer of 29 kDa that dissociates into 14.5 and 16 kDa subunits under reducing conditions and is recognized by approximately 80% of *Alternaria*-allergic patients[7]. Alt a 1 has been cloned and the recombinant allergen has been used to measure IgE and IgG antibody responses in patients with *Alternaria* allergy. Recombinant Alt a 1 induces skin prick reactivity comparable with natural Alt a 1 or *A. alternata* extract and is sufficient for a reliable diagnosis of *A. alternata* sensitization [7-9]. Homologs of Alt a 1 have been identified as allergens primarily in other *Alternaria* species[10].

The three-dimensional structure of the *E. coli* expressed recombinant Alt a 1 has just recently been solved through collaborative NIH supported research studies between scientists at INDOOR Biotechnologies and crystallographers and structural biologists at the University of Virginia. Chruszcz et al published the crystal structure of Alt a 1, determined by means of x-ray crystallography in the JACI[11]. The study reveals that Alt a 1 has a unique β-barrel structure, comprised of 11 β-strands, which form a “butterfly-like” dimer that is linked by a single disulfide bond (Figure 1). Dimerization of Alt a 1 provides an explanation for the ability to use the same monoclonal antibody for capture and detection of the allergen in a sandwich ELISA.

INDOOR Biotechnologies recently expressed rAlt a 1 in *Pichia pastoris* (rAlt a 1-P). rAlt a 1-P was purified and compared to the *E. coli* expressed rAlt a 1 (rAlt a 1-E) by SDS-PAGE, mAb ELISA and IgE Ab ELISA. Under non-reducing conditions SDS-PAGE shows the rAlt a 1-P dimer at ~29kD (Fig. 2A, Lane 2) and the rAlt a 1-E dimer and monomer at ~29kD and ~15kD, respectively (Lane 3). Under reducing conditions only the rAlt a 1 monomer is visible (inset). Immunoreactivity of rAlt a 1 was measured using a mAb ELISA and by chimeric ELISA assay for IgE. No difference in mAb binding was seen between rAlt a 1 constructs (Fig.2B), and IgE binding of rAlt a 1-P showed an excellent correlation to that of rAlt a 1-E (Fig. 3).

Applications for recombinant Alt a 1 produced in *Pichia pastoris* (Product Code: RP-AA1) include T-cell studies, histamine release assays and mouse models of asthma and it will also be useful for allergen standardization and the development of improved allergy diagnostics.

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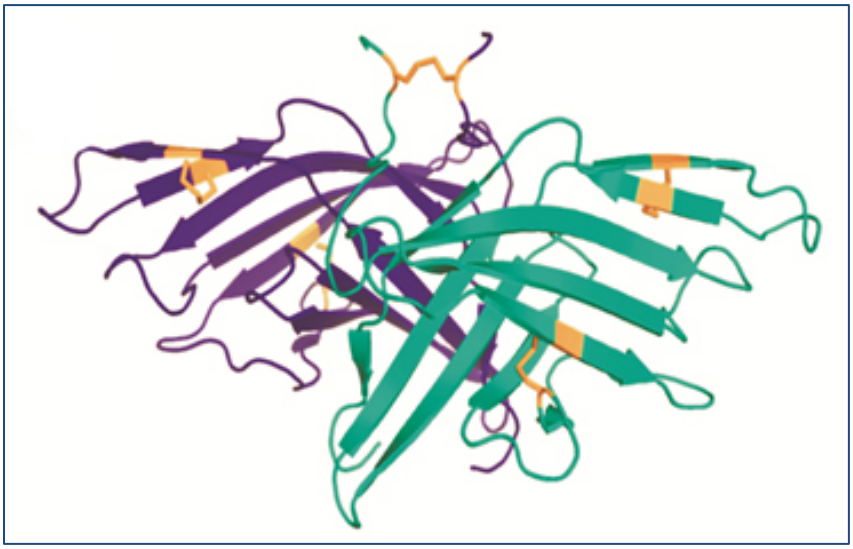

Fig.1: Alt a 1 dimer shown as a cartoon representation (Chruszcz et al, 2012)

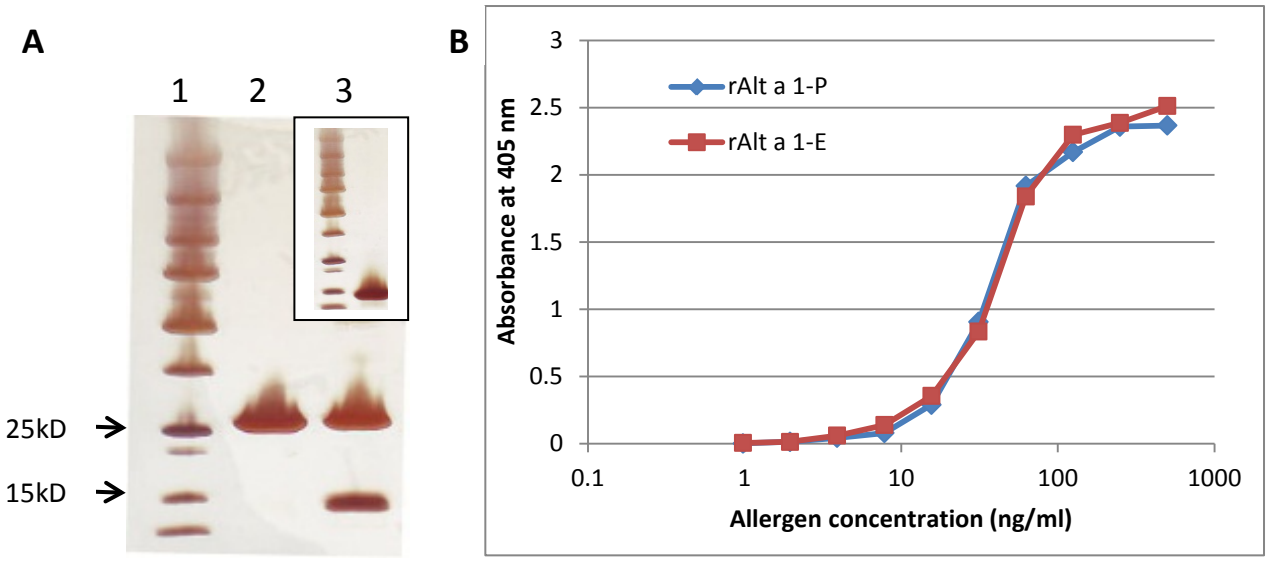

Fig.2. A: Non-reducing SDS PAGE of rAlt a 1 produced in *Pichia pastoris* (Lane 2) and in *E.coli* (Lane 3). SDS-PAGE of rAlt a 1 produced in *Pichia pastoris* under reducing conditions (inset).B: Alt a 1 ELISA.

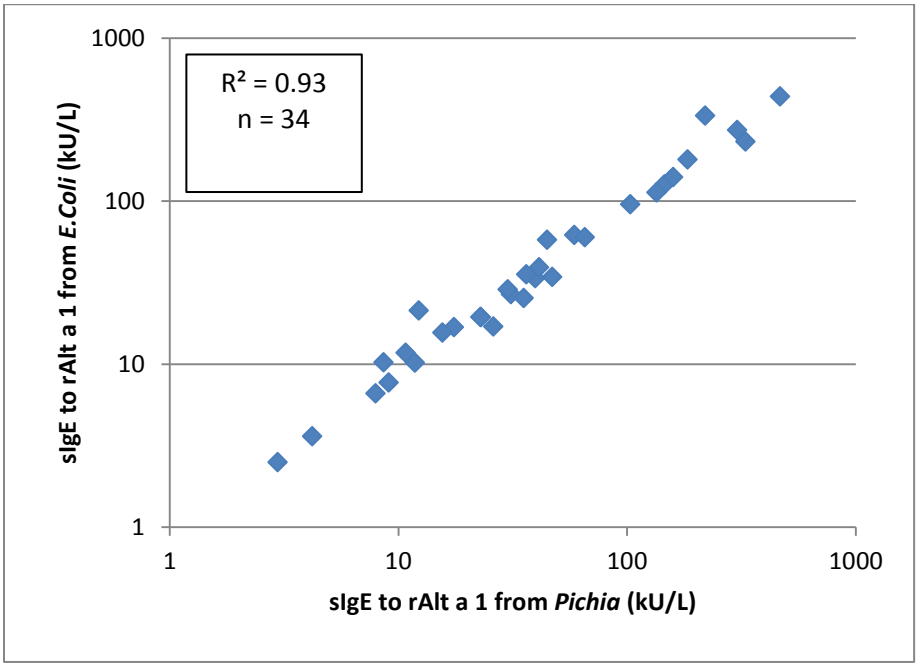

Fig.3: Correlation of IgE binding to rAlt a 1from *Pichia pastoris* and *E.coli.* IgE antibody binding was quantified using a chimeric IgE ELISA assay and sera from 34 Alternaria alternate allergic patients.

References

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