

## TPIS – an IgE-binding wheat protein

P. Rozynek\*, I. Sander, U. Appenzeller, R. Cramer, X. Baur, B. Clarke, T. Brünig, M. Raulf-Heimsoth

**Key words:** allergen; expression library; wheat.

● A MAJOR CAUSE of IgE-mediated allergy in bakers is the inhalation of wheat flour. After 2D electrophoresis more than 100 of spots of a water/salt-soluble fraction of wheat proteins are recognized by serum IgE antibodies from wheat-allergic patients (1).

Using DNA technology, wheat allergens can be cloned, biotechnologically produced as pure recombinant proteins, and characterized.

An aliquot ( $1 \times 10^8$  pfu) of a phagemid preparation, derived from *in vivo* mass excision (2) of a wheat endosperm cDNA library (3) (CSIRO, Canberra, Australia) was digested with the restriction enzymes *EcoRI* and *XhoI*. The cDNA inserts in the size region between 500 and 3000 base pairs (bp) were ligated into *EcoRI/XhoI* restricted pJuFo2 vector (4).

Electrocompetent *E. coli* XL1-blue cells were electroporated with the ligation mixture, according to the manufacturers instructions (Bio-Rad, Munich, Germany). Thereafter, the biopanning procedure was applied to enrich IgE-binding phagemids as described (4) using a pooled serum from eight allergic bakers. The wheat-specific IgE antibody concentration of the serum pool was 54.7 kU/l, determined with the Pharmacia CAP method.

Five rounds of affinity selections resulted in a library with  $6 \times 10^6$  cfu potentially coding for wheat allergens.

In our previous study (1) triose-phosphate isomerase (TPIS) was found to be detected by five out of 10 sera. TPIS could be amplified from the pJuFo cDNA expression library with the following specific primer pair allowing site-directed cloning into *EcoRI/HindIII*-cut pMal c2 expression vector (New England Biolabs, Frankfurt, Germany):

TPIS\_5': 5'-CCGAATTCATGG-GCCGCAAGTTCTTCGTC-3'  
TPIS\_3': 5'-GAAAGCTTTTAG-GCGGACTTCACGGCGGC-3'

PCR conditions were 96°C, 5 min; (94°C, 1 min; 54°C, 1 min; 72°C, 2 min) 32 cycles; 72°C, 10 min. The resulting construct generates a fusion protein with a maltose binding protein (MBP) coded by pMal c2 suitable for affinity purification with an amylose resin (New England Biolabs).

This is the first detection of TPIS in wheat (EMBL accession number: AJ278243). Sequence comparison of the deduced amino-acid sequence of the wheat TPIS gene, against other cereal triose-phosphate isomerases, demonstrated a high level of sequence identity (88.1–96.4%). Comparison with data of Posch et al. (5) showed that seven of eight amino acids of a latex allergen, which was identified as a TPIS, are identical to the wheat TPIS.

Sera obtained from 60 allergic bakers with a wheat-specific IgE antibody range of 3.65–24.9 kU/l, including four of the sera used for biopanning, were tested by EAST (Allergopharma, Reinbek, Germany) with self-prepared allergen disks with rTPIS-MBP according to the method described by Ceska et al. (6). MBP served as negative control. Only one of the sera tested reacted with rTPIS-MBP (2.3 kU/l), without reaction to MBP (<0.35 kU/l). This serum belonged to the serum pool used for biopanning. Preincubation of this serum with native wheat-flour extract (80 µg) inhibited binding to rTPIS-MBP completely, indicating identity of allergenic epitopes in native and recombinant TPIS.

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### \*BFGA

Institut Ruhr-Universität Bochum  
Bürkle-de-la-Camp-Platz 1  
D-44789 Bochum  
Germany  
Tel: 49 234 3074 604  
Fax: 49 234 3074 610#  
E-mail: rozynek@bgfa.ruhr-uni-bochum.de

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## Cytokines and Th2 cells in AEP of smoking

Y. Tanino\*, E. Yamaguchi, K. Takaoka, Y. Fukui, S. Konno, N. Hizawa, M. Nishimura

**Key words:** acute eosinophilic pneumonia; challenge test; eotaxin; IL-5; smoking; Th2 cells.

● CIGARETTE SMOKING has recently been shown to cause acute eosinophilic pneumonia (AEP) (1), although the precise pathogenic mechanism remains largely unknown. We describe temporal changes in the levels of several cytokines in a patient who responded positively to a smoking challenge and we evaluate the roles of cytokines in this process.

A healthy 22-year-old Japanese man started smoking 20 cigarettes per day on October 5, 1999. Two weeks later, he developed a fever and a productive cough.