

# Prohevein-like defense protein of tobacco is a cross-reactive allergen for latex-allergic patients

Anna-Riitta Hänninen, BSc,<sup>a</sup> Nisse Kalkkinen, PhD,<sup>b</sup> Jari H. Mikkola, MSc,<sup>c</sup> Jari Helin, PhD,<sup>b</sup> Kristiina Turjanmaa, MD,<sup>d</sup> Timo Reunala, MD,<sup>d</sup> and Timo Palosuo, MD<sup>a</sup>  
Helsinki and Tampere, Finland

**Key words:** Tobacco, latex allergy, prohevein, IgE

Plants produce defense-related proteins (DRPs) during stress response caused, for example, by microbial pathogens, chemical substances, mechanical damage, UV light, and ozone.<sup>1</sup> Many DRPs are cross-reactive allergens.<sup>2</sup> For example, several DRPs resembling prohevein, a major allergen of natural rubber latex (NRL), can be bound by IgE from sera of patients allergic to NRL.<sup>2,3</sup> Tobacco is a widely used model plant in studies of stress responses. Here we studied whether tobacco products contain proteins that cross-react with prohevein. Recently, allergen cross-reactivity was observed between tobacco and various other species of the Solanaceae family, which comprises many edible plants, such as tomato, potato, and eggplant.<sup>4</sup>

## METHODS

The methods used have been described previously in detail.<sup>2</sup> Briefly, a prohevein-like protein was purified from tobacco leaf extract by chitin affinity and reversed-phase chromatography. Immunologic properties of this potential tobacco allergen were analyzed by immunoblotting and ELISA. The protein was identified by amino acid sequencing of tryptic internal peptides and the molecular mass was determined by mass spectrometry. Further characterization was obtained by mass mapping. In IgE ELISA sera from 63 NRL-allergic patients (50 female and 13 male; mean age 30 years; range 1 to 64 years; 55 had IgE against prohevein) were used. Serum specimens for immunoblot experiments were from 12 of these patients (11 female and 1 male; mean age 41 years, range 22 to 64 years). As controls we used sera from 97 individuals (70 female and 27 male; mean age 34 years, range 2 to 75 years). Seventy-two of these subjects were atopic, showing skin prick test (SPT) responses to at least three common environmental allergens such as birch pollen. SPT with the tobacco protein (concentration 100 µg/mL in PBS, pH 7.2) was performed on four NRL-allergic

### Abbreviations used

CBP20<sub>LP</sub>: CBP20-like protein  
DRP: Defense-related protein  
NRL: Natural rubber latex  
SPT: Skin prick testing  
Win: Wound-induced

patients and on four control patients. The study was approved by the Ethical Committee of Tampere University Hospital.

## RESULTS

In immunoblotting a 21-kd protein of tobacco, purified by chitin affinity and reversed-phase chromatography, was bound by IgE from pooled sera of NRL-allergic patients. The binding was dose dependently inhibited by prohevein (inset in Fig 1, lanes *c* to *f*) and the protein was also recognized by IgG from hevein-immunized rabbit serum (data not shown). In mass spectrometric analysis the purified 21-kd protein had an average mass of 19.2 kd. Sequenced internal peptides of the protein (the obtained sequences were GHLIVNYEF, IVDQCSN, and YGWTAFC) revealed 100% identity to a previously described 20-kd chitin-binding protein (CBP20) of tobacco having a 73% sequence identity to prohevein.<sup>5</sup> Fragments of CBP20 were also identified from the 21-kd tobacco allergen by mass mapping. Together these methods identified more than 40% of the sequence of CBP20 without any contradictory signals. Hereafter, the 21-kd tobacco allergen is called a CBP20-like protein (CBP20<sub>LP</sub>).

In ELISA, 73% (46/63) of sera from NRL-allergic patients showed significant IgE binding to the purified CBP20<sub>LP</sub> (Fig 1). All patients with positive results had IgE against prohevein. One control serum showed exceptionally strong IgE binding to the CBP20<sub>LP</sub> and 3 of the 97 control sera were marginally positive. One of the four patients allergic to NRL showed a positive SPT reaction to the CBP20<sub>LP</sub>. Diameters of the wheals (in millimeters) caused by the CBP20<sub>LP</sub>/histamine for the four patients were 4 of 6, 2 of 4, 0 of 4, and 0 of 4. In ELISA against the CBP20<sub>LP</sub> recordings for these patients sera were 1.050, 0.339, 0.763, and 0.176 OD units, respectively (Fig 1).

## DISCUSSION

These results show that tobacco contains a 21-kd prohevein-like allergen that is targeted by IgE from the majority of the NRL-allergic patient sera. From the data

From the <sup>a</sup>National Public Health Institute, Helsinki; the <sup>b</sup>Institute of Biotechnology, University of Helsinki; the <sup>c</sup>Institute of Occupational Health, Helsinki; and the <sup>d</sup>University and University Hospitals of Tampere, Helsinki and Tampere, Finland.

Supported by the Academy of Finland and the Yrjö Jahnsson Foundation. Received for publication Feb 3, 2000; revised June 21, 2000; accepted for publication June 22, 2000.

Reprint requests: Timo Palosuo, MD, Laboratory of Immunobiology, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland.

J Allergy Clin Immunol 2000;106:778-9.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/1/109617

doi:10.1067/mai.2000.109617

revealed from sequencing and mass mapping it can be reasoned that the purified 21-kd protein is with a very high probability CBP20 or a close homolog. To our knowledge, this is the first IgE-binding allergen characterized from tobacco. In SPT, one of the four NRL-allergic patients tested, having the highest IgE ELISA recording against the CBP20<sub>LP</sub>, showed a clear-cut positive reaction to it (Fig 1). However, further studies are needed to assess the clinical significance of this allergen and related proteins.

The CBP20 is more than 80% identical to the other known prohevein-like proteins in the Solanaceae family: the wound-induced (Win) proteins of potato and tomato.<sup>5</sup> These proteins are classified into the pathogenesis-related protein group 4. There are two domains in CBP20 (as in prohevein and wound-induced proteins): the N-terminal hevein-like domain and the C-terminal Win-like domain. Our results suggest that both domains contain epitopes cross-reacting with prohevein because the CBP20<sub>LP</sub> was recognized by rabbit antihevein antiserum (data not shown) and in the group of sera positive to the CBP20<sub>LP</sub> there were sera specific to prohevein but not to hevein (Fig 1). The Win domain of CBP20 is more than 70% identical to many group 4 pathogenesis-related proteins. The identity of the hevein-like domains of CBP20 and wound-induced proteins in potato and tomato is more than 90%, and its identity to many hevein-like domains of class I chitinases (such as the chitinase allergens from avocado, banana, and chestnut) is more than 70%.

The current study provides evidence that group 4 pathogenesis-related proteins from phylogenetically distant plants can cross-react to the IgE epitope level. Prohevein and hevein are likely to be primary sensitizers for NRL-allergic patients. However, other group 4 pathogenesis-related proteins may also act as independent allergens. The strong IgE binding to the CBP20<sub>LP</sub> from one control serum without IgE to prohevein is an example of this possibility (Fig 1, control group).

Allergenic properties of group 4 pathogenesis-related proteins are of special interest because the expression of these proteins is related to stress responses of plants and their amounts increase after certain agricultural treatments.<sup>1,2</sup> There has also been interest in using defense genes of tobacco as transgenes to increase the resistance of edible plants to various pathogens,<sup>6</sup> but such intents obviously call for cautiousness.<sup>1,2</sup> There has also been interest in these proteins as possible transgenes because of their antimicrobial properties.

We thank Helena Honkasalo, BSc, for skillful technical assistance.

## REFERENCES

1. Brederode FT, Linthorst HJ, Bol JF. Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding. *Plant Mol Biol* 1991;17:1117-25.
2. Hänninen A-R, Mikkola J, Kalkkinen N, Turjanmaa K, Ylitalo L, Reunala T, et al. Increased allergen production in turnip (*Brassica rapa*) by treatments activating defense mechanisms. *J Allergy Clin Immunol* 1999;104:194-201.

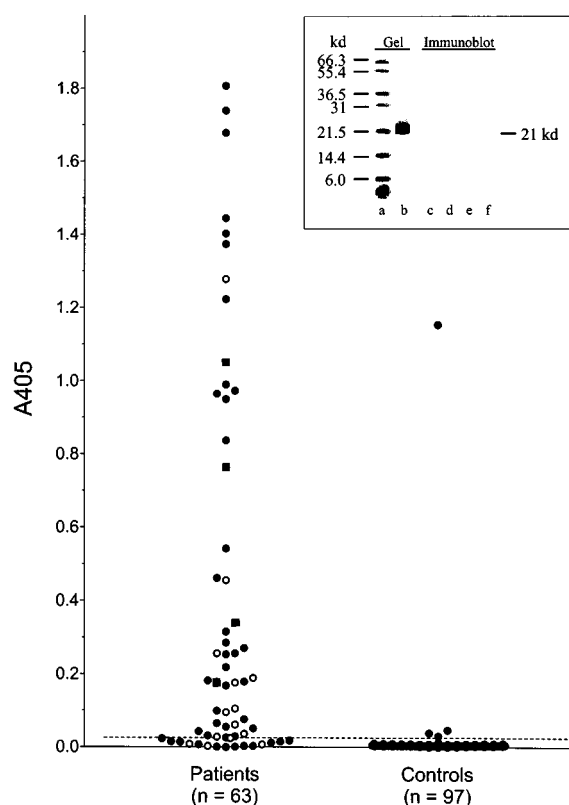


**FIG 1.** IgE binding to the purified 21-kd tobacco protein in immunoblotting and in ELISA. Sera from 46 of 63 (73%) of NRL-allergic patients show IgE binding to the CBP20<sub>LP</sub> in ELISA. Patients having IgE against prohevein but not against hevein (n = 14) are marked with open circles and patients selected to SPTs are marked with squares. Threshold value for positivity (mean absorbance unit value +3 SD of the controls) is indicated by the dotted line. One control serum showed strong IgE binding (OD value 1.15) to CBP20 and was omitted from the threshold value calculation and 3 control sera showed marginal reactivity to the CBP20<sub>LP</sub>. Inset: Lane a, Molecular weight markers; lane b, Coomassie blue staining of purified 21-kd tobacco protein in SDS-PAGE; lane c, IgE binding from the pooled sera of 12 NRL-allergic patients to purified 21-kd tobacco protein in immunoblotting; lanes d to f, inhibition of IgE binding by prohevein in concentrations of 1 µg, 1 ng, and 1 pg/mL. A dose-dependent inhibition is clearly seen. IgE binding was also inhibited by NRL in a concentration of 10 µg/mL but not by an unrelated allergen, ovalbumin (data not shown).

3. Mikkola J, Alenius H, Hänninen A-R, Kalkkinen N, Reunala T, Turjanmaa K, et al. Cross-reacting IgE-binding epitopes of chitin-binding protein domains in NRL and several plants [abstract]. *J Allergy Clin Immunol* 1998;101:S153.
4. Ortega N, Quiralte J, Blanco C, Castillo R, Alvarez MJ, Carrillo T. Tobacco allergy: demonstration of cross-reactivity with other members of Solanaceae family and mugwort pollen. *Ann Allergy Asthma Immunol* 1999;82:194-7.
5. Ponstein AS, Bres-Vloemans SA, Sela-Burlage MB, van den Elzen PJ, Melchers LS, Cornelissen BJ. A novel pathogen- and wound-inducible tobacco (*Nicotiana tabacum*) protein with antifungal activity. *Plant Physiol* 1994;104:109-18.
6. Shah DM. Genetic engineering for fungal and bacterial diseases. *Curr Opin Biotech* 1997;8:208-14.