

Cross-reactivity between T cells and bacteria

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Key words: bacteria; molecular mimicry; type I allergy.

• Up to 20% of the population of the industrialized world suffer from allergies. Hypersensitivity to airborne allergens is the most important cause of hay fever, leading to itchy eyes and scratchy throats during the flowering period. Several studies on groups I, V, and IX grass-pollen proteins revealed an extensive interspecies cross-reactivity at the B- and T-cell level (1, 2). Therefore, the analysis of the primary structure of these proteins led to the definition of particular allergenic motifs (3).

Here we report on a sequence within the secreted α -proteins of pathogenic *Neisseria* spp., which share a high degree of homology to a major allergenic T-cell epitope. In addition, we found several other environmental and human proteins which contain similar motifs (Fig. 1). Initial T-cell assays with peripheral blood mononuclear cells (PBMC) of several atopic and healthy donors demonstrated a variable but significant stimulation capacity of the meningococcal α B2b protein and the derived peptides (data not shown). To investigate whether potential cross-reactivity between the neisserial sequence and the allergenic epitope exists, we performed single T-cell cloning. PBMC from a blood donor which exhibited strong proliferation after stimulation with the grass-pollen epitope Bet v IX- and the α B2b protein-derived peptides were chosen for cloning (mean SI [stimulation index] after 10 days of 12.5 and 5.5, respectively). The actual allergic state of this atopic donor [HLA-DR4/DRw53, DRB1 (0401), DRB4 (0107)] could be defined by positive RAST values against pollen extracts

of *Phleum pratense* (69.2 kU/l) and *Betula verrucosa* (0.81 kU/l), and total IgE titer (300 kU/l). An immunoblot against pollen extracts of *Poa pratense* and the meningococcal α B2b protein also exhibited a specific IgE titer in the serum (data not shown). For cloning, PBMC were stimulated in 96-well, round-bottomed plates (Nunc, Roskilde, Denmark) with Bet v IX peptide concentrations of 10 μ g/ml for 6 days in RPMI-1640 medium supplemented with 1% Pen/Strep solution (Sigma) and 5% inactivated autologous serum. The T-cell lines were maintained in culture until day 21 with several medium changes in the presence of interleukin-2 (IL-2, 25 IU/ml, Vitromex, Germany). Lines which were considered positive by means of a ^{3} H-TdR (Amersham, UK) incorporation assay after 10 days were cloned in the presence of irradiated (5000 rad) allogenic PBMC (1×10^5 cells/well), phytohemagglutinin (PHA-P, 1 μ g/ml, Sigma), and IL-2 (25 IU/ml).

Two stable Bet v IX-specific T-cell clones were obtained. Their specificity was assessed in duplicates by incubating 1×10^4 cells/well for 4 days with 1×10^5 irradiated autologous PBMC and with either a Bet v IX- (N-PAAELQVIDKADA-C), α B2b- (N-AAELLAKQRAEAE-C), Uro3- (N-PAGELLARVRAERL-C), or parvalbumin-

(N-FAADLRALTDAETK-C) derived peptide (10 μ g/ml), or medium alone. After 3 days, the cells were pulsed with 1 μ Ci ^{3} H-TdR, and radioactivity was determined after 18 h by liquid scintillation counting. Cytokine production was measured 24 h later by specific ELISA. FACS analysis was done with anti-CD3-FITC and anti-CD4/CD8-PE antibodies. The clones represented TH0/TH2 types (CD3/4 positive, CD8 negative) secreting considerable to high amounts of IL-4 (clone HG1: 935 pg/ml IL-4 and 5238 pg/ml IFN- γ ; clone HG2: 2613 pg/ml IL-4 and <30 pg/ml IFN- γ). Their specificity (HG1: mean SI of 55.5; HG2: mean SI of 27.3) was confirmed by two independent proliferation assays against the initial Bet v IX peptide. Interestingly, they also cross-reacted with the α B2b- (mean SI of 74.0 and 6.9, respectively) and Uro3- (mean SI of 18.5 and 6.0, respectively) derived peptides. A homologous parvalbumin-derived peptide from salmon could not activate both clones (mean SI of <1.5).

To our knowledge, this is the first report that TH0/2-type allergenic T-cell clones cross-react with sequences from bacterial proteins, which are normally thought to elicit a TH1 response. These results raise the possibility that microbial antigens play a role in skewing the TH1/TH2 balance toward TH2-based

Protein	Sequence	Organism
Bet v IX	P A A E L Q V I D K A D A	<i>B. verrucosa</i>
Lol p I	S A G E L E L Q F R R V K	<i>L. perenne</i>
Phl p I	P A G E L Q I I D K I D A	<i>P. pratense</i>
Poa p IX	P A G E L Q V I D K V D A	<i>Poa pratense</i>
α B2b-Protein	K A A E L L A K Q R A E A	<i>N. meningitidis</i>
Pan1	P A G E L P V I D A V T T	<i>N. gonorrhoeae</i>
Uro-3	P A G E L L A R V R A E R	<i>P. aeruginosa</i>
IF-2	D V A E L N V I V K A D V	<i>H. influenzae</i>
Protein G	A A A D A L A K A K A D A	<i>Streptococci</i>
Katalase	V I A S L A V L A K A D A	<i>P. vitalae</i>
Parvalbumin	F A A D L R A L T D A E T	<i>G. callaris</i>
Hemoglobin	S A G Q L E A A F K V L S	<i>L. pectinata</i>
YQEY	T I A E V G A S S K A D M	<i>B. subtilis</i>
THD1	I F G E F I V P E K A E T	<i>L. esculata</i>
Profilin	K A Y E L A L Y L R R S D	<i>H. sapiens</i>
Parathyrosin	A A A E L S A K D L K E K	<i>H. sapiens</i>
RAD1	K A S E L R I Q L R A R	<i>H. sapiens</i>
	K A Y E L A L Y L R R S D	

Figure 1. Comparison of major cross-reacting T-cell epitope of plant pollen proteins Bet v IX (*Betula verrucosa*), Poa p IX (*Poa pratense*), Lol p I (*Lolium perenne*), and Phl p I (*Phleum pratense*) with homologous sequences retrieved from SwissProt and Pir databases (releases 2/1999). Boxed letters indicate conserved amino-acid residues according to motif suggested by Mohapatra et al. (3).

immunity since colonization of the upper-respiratory tract (e.g., with *Neisseria meningitidis*, *Haemophilus influenzae*, or *Streptococcus* spp.) occurs early in life, resulting in 1/4 individuals being temporarily affected. It is known that irritating stimuli, such as LPS, can convert a tolerizing immune response into an allergic IgE-based response (4). Intriguingly, we recently found that neisserial IgA1 protease that is secreted in conjunction with the cross-reacting α -proteins is a potent inducer of proinflammatory cytokines (Lorenzen et al., submitted). Therefore, colonization with *N. meningitidis* and related pathogens might trigger allergic responses against otherwise harmless proteins. Furthermore, structural similarity with host proteins (Fig. 1) may stimulate the allergic memory over the winter season.

The nasopharyngeal carriage of microbial pathogens in children with a history of atopic diseases (5) and the increased development of allergic symptoms in meningococcal stock-vaccinated patients (6) support the significance of our observations. However, the clinical relevance of our findings remains to be established.

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Hypersensitivity syndrome from isoniazid

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Key words: drug reactions; hypersensitivity syndrome; isoniazid; positive patch test.

● We present a 34-year-old woman with a personal background of smoking and simple chronic bronchitis with bronchial hyperreactivity. Diagnosed with pulmonary tuberculosis, she began triple tuberculostatic treatment with isoniazid, rifampicin, and pyrazinamide. After 2 months, she visited the hospital with a severely pruritic urticariform cutaneous eruption; treatment was started with chlorpheniramine, and pyrazinamide was discontinued because the treatment had been completed. One week later, she was admitted to the emergency department because of a worsening of the eruption, with edema in the face and neck, purpuric lesions in the lower extremities, cutaneous-mucous icterus, lymphadenopathy, asthenia, and a fever of 38°C.

A case of severe cutaneous reactions secondary to isoniazid.

The emergency analysis was as follows. Leukocytes numbered 11.5×10^9 ; eosinophilia was 8%, 62% segmented, 15% lymphocytes (not activated), and 14% monocytes; and hepatic function was altered with aspartate aminotransferases of 1459 U/l and Quick time of 32%. The viral serologies and ANAs were negative; renal function, ionograma, proteinograma, α -fetoprotein, and urine sediment were normal; and the levels of lactate dehydrogenase were high. The abdominal ecograph showed homogeneous hepatomegaly, and the thorax radiology a decrease of the one tuberculous infiltrate of the right lower lobe. The patient was admitted to the unit and started treatment with methylprednisolone at 0.5 mg/kg body weight and endovenous dexchlorpheniramine. Cycloserine, ethambutol, and streptomycin replaced the initial antituberculostatic treatment. After 15 days, the hepatic function and the eosinophilia were normalized. The cutaneous eruption persisted with lower intensity for several months.

The patient was referred to our service for evaluation of hypersensitivity to tuberculostatics 1 year later, when she was asymptomatic. The test of lymphocytic transformation to rifampicin, pyrazinamide, and isoniazid was negative. Epicutaneous tests were also carried out with 1% rifampicin in vaseline, 10% pyrazinamide in alcohol (ethanol 70%), and 1% isoniazid in saline, showing a strongly positive reaction to isoniazid after 48 and 96 h. Ten epicutaneous tests with isoniazid administered to healthy controls were negative.

The incidence of cutaneous reactions to drugs in the general population is unknown and varies according to the drugs involved. The hypersensitivity syndrome refers to a serious specific reaction secondary to drugs that affects the skin (1). Clinically, patients present cutaneous rash and fever (87%), and often hepatitis (51%), arthralgia, lymphadenopathy (75%), and/or blood alterations (fundamentally eosinophilia of 30% and atypical lymphocytes). The