Skin test diagnosis of grass pollen allergy with a recombinant hybrid molecule

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Background: A recombinant hybrid molecule (HM) consisting of 4 major allergens from timothy grass (Phl p 1, 2, 5, and 6) was expressed in *Escherichia coli*, purified, and characterized regarding its immunologic properties.

Objective: We sought to determine whether the recombinant HM can be used for the diagnosis of grass pollen allergy by means of skin testing.

Methods: Skin prick testing was performed in 32 patients with grass pollen allergy and in 9 control individuals by using increasing concentrations (4, 12, 36, and 108 μg/mL) of the HM and using commercial grass pollen extract. Specific IgE reactivities against the HM, grass pollen extract, and a panel of purified grass pollen allergens (recombinant Phl p 1, 2, 5, 6, 7, 12, and 13 and natural Phl p 4) were measured by means of ELISA, and timothy grass pollen–specific IgE levels were determined by using ImmunoCAP.

Results: Grass pollen allergy was diagnosed in all patients by means of skin testing with the HM. No false-positive skin test responses were obtained in the control individuals. There was an excellent correlation between IgE levels obtained with the HM and natural grass pollen extract measured by means of ELISA ($r = 0.98$, $P < .0001$) and by means of ImmunoCAP ($r = 0.98$, $P < .0001$).

Conclusions: The recombinant HM permitted accurate and specific *in vivo* diagnosis of grass pollen allergy in all tested patients. It can be considered a well-defined tool for the diagnosis and perhaps for immunotherapy of grass pollen allergy.

Clinical implications: A recombinant HM can replace traditional allergen extracts for skin test–based diagnosis of grass pollen allergy. (J Allergy Clin Immunol 2007;120:315-21.)

Key words: *Allergy, grass pollen, recombinant hybrid allergen, skin testing, diagnosis*

Grass pollens are one of the most important airborne allergen sources worldwide and can elicit severe forms of allergic manifestations, such as asthma.[1-4] Pollens from various grass species contain highly cross-reactive allergens that, depending on initial biochemical studies and on the frequencies of patients' IgE reactivities, have been assigned to certain groups. Thirteen allergens are present in grass pollens, and the main frequencies of reactivity are attributed to group 1 (>90%), group 2 (>60%), group 5 (<90%), and group 6 (76%).[5] Certain allergen groups are not present in every grass species. For example, it has been shown that Bermuda grass pollen does not contain detectable amounts of group 2, group 5, or group 6 allergens.[6] On the other hand, timothy and rye grass pollen contain the majority of IgE epitopes present in most grass species.[6] For this reason, timothy grass pollen allergens have been characterized in great detail at the molecular level.[7] Recombinant timothy grass pollen allergens mimicking the immunologic properties of natural extract–derived allergens have been produced.[8] It has been shown that individual recombinant timothy grass pollen allergens can be used for reliable *in vitro* and *in vivo* diagnosis of grass pollen allergy and can replace traditional extracts.[9-12] A clinically successful immunotherapy trial performed with the major recombinant timothy grass pollen allergens Phl p 1, 2, 5, and 6 has recently been reported.[13] The crude grass pollen extracts available represent complex and variable mixtures of allergenic and nonallergenic components in which certain allergens might be underrepresented or vary strongly as regards their amounts.[14] Hence it might be desirable to replace traditional grass pollen extracts for diagnosis and therapy with recombinant allergens. To facilitate the diagnostic and therapeutic applications of recombinant allergens for grass pollen allergy, we engineered a recombinant hybrid molecule (HM) containing the majority of grass pollen–specific epitopes.[15,16] We produced a recombinant HM consisting of the 4 major timothy grass pollen allergens Phl p 1, 2, 5, and 6 in *Escherichia coli* and characterized the purified molecule regarding its immunologic properties.[16] *In vitro* IgE measurements indicated that the HM can be used for the diagnostic identification of patients with genuine grass pollen allergy. This result has been achieved because the HM was engineered to contain major grass pollen–specific marker allergens only, which, when used in combination,

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Abbreviation used
HM: Hybrid molecule

allowed accurate diagnosis of grass pollen allergy in different populations. It did not contain cross-reactive panallergens (ie, Phl p 7 and Phl p 12), which are present in several allergen sources unrelated to grass pollen.[16]

Here we report the results of a clinical study in which we investigated whether the recombinant HM can be used for the *in vivo* diagnosis of grass pollen allergy by means of skin testing.

METHODS

Subjects

Thirty-two patients with grass pollen allergy were included in the study on the basis of positive skin test results to a mixture of natural grass pollen extracts, including timothy, orchard grass, and rye grass (100 IR/mL; Stallergènes Laboratories, Antony, France) and of a clinical history of grass pollen allergy (rhinitis, conjunctivitis, and/or asthma during May and June). This group included 18 men and 14 women (mean age, 31.9 years). For control purposes, we also included 9 more individuals: 5 nonallergic subjects (mean age, 26.6 years) and 4 subjects allergic to aeroallergens other than grass pollen allergens (mean age, 30.4 years). Demographic (age and sex) and clinical (symptoms and sensitization to other respiratory allergens according to our usual panel[17]) data were recorded for each patient and are displayed in Table I. After informed consent was provided, skin tests were performed, and blood was collected for serum sampling. Ten of the 32 patients agreed to provide a complementary blood sample for histamine release tests. Only patients who had not received specific immunotherapy in the last 5 years were allowed to participate in the study. None of the patients was taking antihistaminic medication at the time of the study. Ethical approval for skin testing with recombinant allergens in human subjects was obtained from the ''Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale'' of Strasbourg.

TABLE I. Demographic and clinical characterization of study subjects

	Demographic and clinical data			
Patient no.	**Age (y)**	**Sex**	**Allergies**	**Symptoms**
1	38	M	g, t	r, c, as
2	18	M	g	r, c, as
3	37	M	g	r, c, as
4	23	F	g, w	r, as
5	25	M	g, m	r, c, as
6	25	F	g, a	r, c
7	25	F	g, t, m	r, c
8	26	F	g, t, w	r, c
9	24	M	g, t	r
10	52	F	g, t	r, c
11	31	M	g, t, m	r, c
12	30	F	g, a	r, c, as
13	44	F	g, m, a	r, c, as
14	25	M	g, a	r, c, as
15	32	M	g, t	r
16	21	F	g, m	r
17	47	M	g, w, a	r, c
18	50	M	g, w, m	r, c
19	28	F	g, t, w, a, m	r, c
20	22	M	g, t	r, c
21	20	F	g, w	r, c
22	52	F	g, t, m	r, c, as
23	31	M	g, t, w	r, c
24	38	M	g, t	r, c
25	21	F	g, t, w	r, c
26	23	M	g, t	r, c
27	24	M	g, w, a	r, c
28	55	M	g, m	r, c
29	35	M	g, t, a	r, as
30	48	F	g	r, c
31	23	F	g, t, w, a, m	r, c, as
32	30	M	g, t	r, c
Demographic data for subjects allergic to allergen sources other than grass pollen				
	31	M	m	r, a
	33	F	a, m	r, c
	24	M	t, m	r, c
	40	F	t, co	r
Demographic data for nonallergic subjects				
	28	F	0	
	24	M	0	
	25	F	0	
	25	M	0	
	31	M	0	

M, Male; *g*, grass pollen; *t*, tree pollen; *r*, rhinitis; *c*, conjunctivitis; *as*, asthma; *F*, female; *w*, weed pollen; *m*, mites; *a*, animals; *co*, cockroach.

Allergen extracts and purified allergens

The natural grass pollen extract used for comparison was a mixture of 3 different grass species: *Phleum pratense* (timothy grass), *Dactylis glomerata* (orchard grass), and *Lolium perenne* (rye grass; Stallergènes Laboratories). It was used at a concentration of 100 IR/mL, as recommended by the manufacturer. The contents of group 1, 2, and 5 allergens were measured by using ELISA assays.[14,18] Recombinant Phl p 1, 2, 5, 6, 7, 12, and 13, as well as natural Phl p 4, used for the determinations of patients' IgE reactivity profiles were purified as previously described.[19-23] The recombinant HM consisting of Phl p 1, 2, 5, and 6 was expressed in *E coli* and purified as previously described.[15,16]

Skin testing

Four 3-fold increasing concentrations (4, 12, 36, and 108 μg/mL) of the HM were applied. The lyophilized HM was reconstituted and diluted with 0.9% sodium chloride solution on the day of the test, as previously described.[24]

The natural grass pollen extract (Stallergènes Laboratories) and the recombinant HM preparations at 4, 12, 36, and 108 μg/mL were applied in duplicates on the volar side of the 2 forearms. The 4 increasing concentrations of the HM were applied from the wrist to the elbow in reverse order. The test sites were placed 3 cm apart to avoid false-positive results. The skin was then pricked with Prick-Lancets (Stallergènes Laboratories). A negative control test was performed with saline solution, and a positive control test was done with histamine (10 mg/mL). The tests were performed by the same investigator. The wheal and the flare contours were outlined 15 minutes later with a skin-marking pen. The areas of the wheals and the flares were determined by means of digital planimetry (Adobe Photoshop; Adobe, San Jose, Calif) and analyzed by using National Institutes of Health Image software. For each allergen preparation, the mean value of the skin test reaction was calculated.