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AN INVESTIGATION OF POLLEN EXTRACTS FROM DIFFERENT DECIDUOUS TREES IN PATIENTS WITH SPRINGTIME ALLERGY IN SWEDEN

By

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Allergy to pollen from deciduous trees is a common cause of allergic rhinitis and asthma during the spring in Sweden. Juhlin-Dannfelt discovered (2) that patients with these disorders often get allergic symptoms from eating nuts or raw fruits. Using intracutaneous tests (i. c. tests) and, in some cases, provocation tests and Prausnitz-Küstner tests, he could also detect the kind of tree pollen which usually caused the symptoms, i. e. mainly birch but also hazel, alder, ash, sailow-willow and oak.

Humoral reagins of the IgE class directed against different allergens can be detected with the radioallergosorbent test (RAST) (4). This test can also be used to study allergenic activities and to obtain information on relationships between various allergens. In the present investigation the RAST, i. c. tests and provocation tests have been used to study pollen extracts from different deciduous trees. The present study has been made in order to compare the extracts and illustrate whether they have any allergens in common.

MATERIALS AND METHODS

Ten patients (18-27 years of age, 4 ♀ 6 ♂) with a typical clinical history of allergy to pollens from deciduous trees were examined. All 10 had rhinitis and

4 also had asthma. All but one had noticed itching in the mouth while eating hazelnuts or raw fruits. Extracts from alder, ash, beech, birch, elm, hazel, oak and willow (willow-mix) were investigated. The extracts were manufactured by Dome Inc. and were standardized in protein nitrogen units (PNU).

The *i. c. tests* were made by injecting 0.1 ml of 10 PNU/ml on the back of the patients. If a positive reaction did not develop, testing was continued with solutions of 100 and 1,000 PNU/ml. The test was considered positive if the sum of two opposite diameters of the wheal measured more than 20 mm. A positive reaction to the 10 PNU solution was graded as +++, to the 100 PNU solution as ++, and to the 1,000 PNU solution as +.

The *provocation tests* were carried out as intra-nasal instillations of 0.2 ml of the extracts. Before each provocation, Coca's solution was instilled as a control. Testing was made with a solution of 100 PNU and subsequently continued with 1,000 and 10,000 PNU solutions, if a positive reaction was not obtained. All provocation tests were carried out in the autumn when the patients were free from symptoms. A positive reaction was characterized by sneezing, running nose and/or obstruction of nasal air passage, and often itching in the nose or throat. The evaluation of the provocation test may be difficult, and the results of some tests, in which the patients showed weak reactions to the strongest solution, were regarded as doubtful and graded as +? or —?. The grading of the provocation tests were made without any knowledge of the results of the RAST.

The amount of reagins in serum reacting with the different pollen extracts was semiquantitatively estimated by the *RAST method* (4) and expressed in sorbent units (3). The RAST was considered positive when the reaction was more than 1 sorbent unit and negative when less than 0.3 units.

In the *inhibition tests* 20 μ l of serum was incubated with 20 μ l of an allergen solution of 10,000 PNU/ml before analysis. Inhibition tests were also made with cellulose-coupled allergen; in these tests 20 μ l of serum was mixed with 0.5 mg allergosorbent in 0.5 ml, and the mixture was rotated for 24 hours at room temperature. After centrifugation, 0.4 ml of the supernatant was tested according to the RAST method.

RESULTS

The result of *i. c. tests*, provocation tests and RAST for the 10 patients tested with the 8 extracts are given in Table 1. The relationship between the amount of reagins directed against the different extracts and the result of *i. c.* and provocation tests with the extracts is summarized in Fig. 1. It appears that there is a rather good correlation between the different test methods. The results of these tests can conveniently be divided in three groups according to the allergen used.

TABLE I

Patient no		Alder	Ash	Beech	Birch	Elm	Hazel	Oak	Willow mix
1	I.c. test	+++	+	+	+++	+	+	++	—
	Prov. test	+	—	—	+	—	—	+	—
	RAST	28	neg.	0.7	250	neg.	neg.	0.3	neg.
2	I.c. test	++	—	+	+++	—	—	+	+
	Prov. test	+	—?	—	+	—?	—	—?	—
	RAST	7.6	0.5	neg.	26	neg.	neg.	neg.	0.4
3	I.c. test	++	++	++	+++	+	+++	+	+
	Prov. test	+	—	—?	+	—	—	+	—
	RAST	38	0.7	neg.	215	neg.	neg.	2.2	0.9
4	I.c. test	+++	+	+	+++	+	+	++	—
	Prov. test	+	—	—?	+	—	—	+	—
	RAST	150	neg.	7.8	460	neg.	neg.	5.0	neg.
5	I.c. test	+++	++	++	+++	+	+	++	+
	Prov. test	+	—	—	+	—	—	+	—
	RAST	7.8	neg.	neg.	110	neg.	neg.	0.3	0.3
6	I.c. test	+++	+	+	+++	+	—	+	+
	Prov. test	+	—	—	+	—	—	+	—
	RAST	64	7.2	neg.	160	2.6	neg.	2.8	1.2
7	I.c. test	+++	+	+	+++	+	+	+++	+
	Prov. test	+	—	—	+	—	—	+	—
	RAST	37	neg.	neg.	210	neg.	neg.	0.9	0.5
8	I.c. test	+++	+	++	+++	+	+	++	—
	Prov. test	+	—	—?	+	—	—	+	—
	RAST	205	neg.	1.3	210	neg.	neg.	16	neg.
9	I.c. test	++	+	++	+++	+	+	+	+
	Prov. test	+	—	—?	+	—	—?	—	—
	RAST	9.4	neg.	1.8	36	neg.	neg.	0.8	neg.
10	I.c. test	++	—	+	+++	+	—	++	+
	Prov. test	+	—	—	+	—	—	+	—
	RAST	12	neg.	neg.	27	neg.	neg.	1.2	neg.

The results of intracutaneous tests, provocation tests and RAST with 8 tree pollen extracts in 10 patients with clinical allergy to pollens from deciduous trees.

for alder allergen. Patient no 6 who also had a high RAST titer and +++ i. c. test to alder, had only a weak positive provocation test reaction with this allergen. He exhibited no symptoms in april.

For ash, beech, elm, hazel, and willow-mix no patient reacted positively to provocation tests, i. c. reactions were weaker, some even negative and the RAST titers were weak or positive in only 13 of the 50 tests. With the oak extract 6 patients gave a positive, 3 a doubtful, and one a negative provocation test; 6 i. c. tests were positive with 100 PNU/ml; the RAST reactions were generally weak. For the patients whose allergic symptoms continued for some time into June—when chiefly oak is in flower—no correlation between clinical history and the test results with the oak extract could be seen.

The inhibition tests gave the same results using both methods. The reactions to the different pollen extracts were completely inhibited when the same allergen was used for inhibition and test. Birch extracts also inhibited all positive reactions to the other tree pollens. In three instances, however, the inhibition was only partial, namely the alder reactions of patients nos. 6 and 8, and the oak reactions of patient no 8. Inhibition of the birch reactions with the extracts that had given a positive RAST showed that alder extract partially inhibited the birch reactions for patients nos. 3, 5, 6, 7, 8, 9 and 10, and that oak extract gave a partial inhibition for patient no 8. No other extract gave any inhibition of the birch reaction.

DISCUSSION

In these patients, the strongest allergen was birch, followed by alder and oak. This corresponds remarkably well with the analyse of air pollen frequency, which show that birch pollen is always the dominating element representing between 50 and 90 per cent of the total tree pollens. The frequency of alder pollen in different investigations is given as 5 to 30 per

cent, while the frequencies from deciduous trees other than birch and alder in central Sweden is less than 10 per cent (1).

The frequency variations depend upon the district in which the investigation has been made. It may be concluded that all 10 patients get clinical symptoms from birch pollen, and that 4 of them are also sensitive to alder pollen. The possible rôle of oak pollen is uncertain, while the other 5 pollens investigated probably have no clinical significance. The results of the inhibition tests, where the birch pollen extracts were able partially or totally to inhibit the reactions to all other pollen extracts, shows that the birch extract contains several allergens or allergenic determinants, of which at least one is present in the other extracts. As the RAST reactions to birch were partially inhibited by extracts from alder (patients nos. 3, 5, 6, 7, 8, 9, and 10) and oak (patient no 8), we may assume that some of the reagins which reacted with birch extract have also affinity for alder and oak extracts.

In patient no 6 the birch extract only partially inhibited the reactions to alder and oak, and in patient no 8 the birch extract only partially inhibited the reactions to alder. This indicates that these two patients have reagins more particularly directed against allergens in alder and oak extracts respectively. Thus, although the birch pollen extract may be the dominating allergen, there are specific allergens in the alder and oak pollen extracts.

SUMMARY

10 patients with a clinical history of springtime allergy in Sweden have been investigated by means of intracutaneous test, provocation tests, and radioallergosorbent tests (RAST) for reagins in serum against pollen extracts from eight different springtime flowering trees. Inhibition tests using the RAST has shown that there are allergenic determinants in common between the different extracts, but also that some extracts have specific allergens.

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