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Intracutaneous Tests with Pityrosporon Extract in Atopic Dermatitis

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In patients with atopic dermatitis and in a control group prick and intracutaneous tests were performed with Pityrosporon extracts. Moreover, specific IgE against Pityrosporon was determined in these patients. Significant differences existed between the results obtained with these methods in both groups of patients. Therefore, both skin tests with Pityrosporon extract and IgE determinations may contribute to a diagnosis of AD; moreover, it is likely that Pityrosporon may be of significance in the pathogenesis of AD.

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Yeasts of the genus Pityrosporon (*Malassezia furfur*) are known to constitute a major proportion of the saprophytic microflora of human skin and are associated with a number of disease conditions including pityriasis versicolor, folliculitis, seborrhoeic dermatitis, dandruff, acne vulgaris and systemic infections of compromised patients. Clemmensen and Hjorth (1) and Waersted and Hjorth (2) found evidence by means of prick tests and by treatment with ketoconazole that *Pityrosporon orbiculare* is of importance in several cases of atopic dermatitis, especially those patients with head and neck dermatitis.

Because no other atopic allergen is known to us giving an immediate type reaction only or almost only in patients with atopic dermatitis (AD), we intended to do a similar investigation. This was extended, however, to intracutaneous tests—which we believe sometimes to be more reliable especially in AD patients because of difficulties in the evaluation of the skin tests—and to specific IgE determinations by means of RAST for the same reason.

MATERIALS AND METHODS

Patients and controls

109 patients were skin tested (prick and/or intracutaneous tests); of these patients, 57 had AD according to the criteria of Hanifin and Rajka (3), 52 patients served as controls. The group of controls consisted of: (*a*) 41 atopic patients with asthma and/or rhinitis; 25 of these had one or more positive skin tests to atopic allergens such as housedust. *Dermatophagoïdes pteronyssinus*, pollen or animal danders. (*b*) 7 non-atopic patients with allergic contact dermatitis. (*c*) 4 non-atopic patients with seborrhoeic dermatitis or pityriasis versicolor. Specific and total IgE were determined in 34 patients with AD (diagnosis made according to the criteria of Hanifin and Rajka (3)) and in 10 atopic controls suffering from asthma and/or rhinitis.

Skin tests

Intracutaneous tests were performed with 0.05 ml of two different extracts of *Pityrosporon orbiculare:* extract A prepared by ALK (Copenhagen, Denmark) and extract B prepared in our own laboratory. Prick tests were performed with extract A only. All tests were read after 20 min, 6 hours and 24 hours. Moreover, all patients were tested with control solution, histamin HCl 1:100000, house dust 0.5% (HAL), *Dermatophagoïdes pteronyssinus* 10 U/ml (HAL), grass pollen 100 U/ml (HAL), tree pollen 100 U/ml (HAL), cat- and dog dander 0.01% (HAL). The results of the skin tests were graded 1+ through 3+; a 1+ reaction a wheal of no more than 5 mm diameter with erythema, a 2+ reaction a wheal of 5–8 mm with erythema and a 3+ reaction a wheal of 8–12 mm with pseudopods and erythema.

Pityrosporon extracts

A) Extract prepared by ALK (Copenhagen, Denmark); this extract was used in a concentration of 5 mg/ml in the prick test and 0.05 mg/ml in the intracutaneous test. B) Extract prepared in our own laboratory; the inoculating pure organism *Malassezia furfur=Pityrosporon ovale* was obtained through the Centraal Bureau voor Schimmelcultures (Baarn) from Gist-Brocades Inc. (Delft, The Netherlands).

A liquid medium was prepared containing NeoPeptone (Difco) 10 g, extract of baker's yeast (10 g) and D-glucose (20 g) per 1000 ml of distilled water. The solution was heat-sterilized and subsequently 50 ml of sterile olive oil was dispersed into the medium. The organism was cultured in this broth for 3 days at 37°C. The growth was removed by filtration and the culture filtrate was cleared of residual oil by centrifugation and prolonged dialysis. The culture filtrate antigens were finally obtained by lyophilization. This extract was used in a concentration of 0.5 mg/ml in the intracutaneous test.

Specific IgE

For direct RAST-testing, the culture filtrate antigens as described above were coupled to cellulose paper discs with cyanogen bromide by a modification of the method of Ceska, Eriksson and Varga (4). The RAST was further carried out with radioactive anti IgE, purchased from Pharmacia AB (Uppsala, Sweden). The results were expressed in percent

Table I. *Intracutaneous tests with Pityrosporon extract read after 20 min*

	Total no. of patients		No. of positive reactions	
Atopic dermatitis	40		35 (87.5%)	
Controls: rhinitis and asthma (positive reactions to inhalants)	25	41	5 (20%)	5 (12%)
Controls: rhinitis and asthma (negative reactions to inhalants)	16		0 (0%)	
Total	81		40 (49%)	

binding of added radioactivity to the allergen-coated discs. The maximum binding in this system was 45%.

Statistical calculations

For statistical analysis, all significance tests are based on a normal distribution (5).

RESULTS AND DISCUSSION

Skin test results in the patients with atopic diseases are presented in the Tables I and II. From these tables it is apparent that there is a statistically significant difference between the reactions after 20 min in patients suffering from AD and in patients with other atopic diseases ($p < 0.001$). In our group of 40 patients with atopic dermatitis tested intracutaneously, four patients reacted neither to Pityrosporon, nor to any inhalant allergens. If we exclude these four patients, as many as 34 of the remaining group of 36 patients (94%) reacted positively to the intracutaneous test

Table II. *Prick tests with Pityrosporon extract read after 20 min*

	Total no. of patients		No. of positive reactions	
Atopic dermatitis	44		26 (59%)	
Controls: rhinitis and asthma (positive reactions to inhalants)	25	34	0 (0%)	0 (0%)
Controls: rhinitis and asthma (negative reactions to inhalants)	9		0 (0%)	
Total	78		26 (33%)	

Table III. *IgE to pityrosporon*

	No. of patients	Elevated Pityrosporon IgE i.e. ≥ 2% binding
Atopic dermatitis	34	22 (65%)
Controls: rhinitis and asthma (positive reactions to inhalants)	10	0 (0%)

with Pityrosporon. No reactions were observed after 6 and 24 hours.

A group of 26 patients with AD was tested by both prick and intracutaneous tests with Pityrosporon extract A. As expected, the results showed that both methods in general produced qualitatively identical but quantitatively different results.

The results obtained with extracts A and B in the intracutaneous tests were almost always identical and have therefore not been presented separately.

Taking these findings together, one may conclude that with intracutaneous tests almost every patient with AD reacts to Pityrosporon, although some of the atopic controls also reacted. With prick tests all atopic controls are negative, but on the other hand a number of patients with AD are also negative.

Intracutaneous tests performed in 7 non-atopic patients with allergic contact dermatitis and in 4 non-atopic patients with seborrhoeic dermatitis or pityriasis versicolor produced negative results.

We could not find cross reactions in the skin test between Pityrosporon and possibly related allergens, for example *Candida albicans*.

In contrast with the findings of Waerstedt and Hjorth (2) we could not find any relation between the localization of the dermatitis and the result of the skin

Table IV. *Relation between total IgE and specific IgE to Pityrosporon in patients with atopic dermatitis*

Total IgE (kU/L)	Specific IgE (percentage of binding)			
	<2%	2–5%	5–10%	>10%
<1 000	7			
1 000–5 000	5	3	5	1
5 000–10 000		4	2	
>10 000		1	3	3
Total number of patients	12	8	10	4

test. In Table III the results are shown of the specific IgE determinations. As with the skin tests statistically significant difference ($p<0.001$) was observed between the AD patients and the atopic controls. In our patients with AD the highest values of specific IgE to Pityrosporon (up to 33% binding) were seen mostly in patients with high total IgE values. The relation between total and specific IgE is shown in Table IV.

High IgE values were generally seen in patients with generalized skin lesions. Again, there was no relation to skin lesions localized especially in the head-neck region, however. In our opinion all these data strongly point to a probable relationship between Pityrosporon and AD. To clarify further this point, more investigations are needed (e.g. isolation and enumeration of these yeasts from the skin in AD, patch-tests with Pityrosporon extract in patients with AD, specific therapy against Pityrosporon in these patients). Such investigations are now in progress in our Department.

Finally, skin tests with Pityrosporon extract and specific IgE determinations against this allergen may obviously contribute to the diagnosis of AD.

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