Immunological Aspects of Dermatophyte Infections in Hereditary Palmo-Plantar Keratoderma

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Family and personal histories of atopy, total IgE level in serum, AB0 blood groups, trichophytin reactions, IgE RAST, IgG RAST and precipitating antibodies were investigated in patients with hereditary palmo-plantar keratoderma and dermatophytosis. 44% of the patients with dermatophytosis had a personal and/or family history of atopy and 67% a total IgE >100 kU/ml. No significant differences in the rate of dermatophytosis were found between atopics and non-atopics, nor were there differences between patients with a total IgE above and those with a level below 100 kU/ml. The determination of AB0 blood groups showed that T. mentagrophytes occurred significantly more often in patients with blood group A. All delayed trichophytin reactions were negative. A positive immediate trichophytin reaction was found in only 1 patient, who also had a raised level of IgE antibodies against dermatophytes. The level of specific IgG antibodies was increased in patients infected with T. rubrum and E. floccosum, which two species were found to have at least one antigen in common. Homologous precipitating antibodies occurred in 54% of the patients with hereditary palmo-plantar keratoderma, which is a considerably higher value than that reported in dermatophyte infected patients without this inherited disorder. *Key words: Blood groups; IgE; IgG; Precipitating antibodies.* (Received September 22, 1983.)

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Susceptibility to infections with dermatophytic fungi varies from person to person. Evidence from previous reports suggests that patients afflicted with endocrinological disorders (1, 2) or malignant diseases (3) are more susceptible, and it has recently been shown that patients suffering from hereditary palmoplantar keratoderma (HPPK) of the Unna Thost variety have an increased frequency of such infections (4, 5).

A personal and a family history of atopy has been found to be almost three times more common in chronically dermatophyte-infected patients compared to control subjects (6, 7). Likewise a high total serum IgE level could be correlated to chronic fungus infections (8). Immunologic cross reactivity between a glycoprotein isolated from *T. mentagrophytes* (TM) and human isoantigen A has been demonstrated in vitro in samples taken from patients who were continuously infected as has the presence of IgG antibodies against dermatophytes (9, 10, 11).

In the northernmost county of Sweden (Norrbotten) the frequency of dermatophyte infections in HPPK of the Unna Thost variety was shown to be 35.0% (12). The prevalence was later found to be 37.6% (13). Dermatophytosis in HPPK was classified as chronic if having a duration of several years. Dermatophytid reactions were not seen, but vesicular eruptions on normal skin close to the hyperkeratotic border were found during certain periods.

It was, therefore, considered of interest to study the relation between dermatophyte infections in HPPK and immunological aspects such as family and personal histories of atopy, trichophytin reactions, levels of IgE, IgG and occurrence of precipitating antibodies.

Table I. *Family and/or personal history of atopy and total IgE level in serum related to different dermatophyte species and to the total number of dermatophyte infections in patients with HPPK*

NS=not significant

	TR	%	TM	%	EF	%	Total	%	*p* value	Neg.	%
Personal and/or family history of atopy (n=32)	6	19	5	16	4	13	15	44	NS	17	56
Non atopics (n=53)	12	23	9	17	5	9	26	49		27	51
Serum IgE >100 kU/ml (n=12)	2	17	4	33	2	17	8	67	NS	4	33
Serum IgE >100 kU/ml (n=73)	13	18	9	12	6	8	28	38		45	62

MATERIAL AND METHODS

All patients included in the study were examined for fungi, using direct microscopy and conventional culture. Specimens were taken from palms and soles by means of a curette. The material for culture was inoculated on Sabouraud's glucose agar without cycloheximide and incubated for three weeks at 27°C. Cultures were read every week for three weeks and then discarded (14, 15).

History of atopy. 85 patients with HPPK were questioned about a family history of atopy, and it was further ascertained whether or not the patients themselves suffered from allergic rhinitis, bronchial asthma and/or atopic dermatitis.

IgE levels in serum. In the same 85 patients total IgE was determined using Phadebas radioimmunoassay method (16, 17).

Blood groups. 135 patients with HPPK were grouped according to the AB0 blood group system. The distribution of blood groups in the material was related to that found in the blood grouping of 13 319 persons at the Central Laboratory of the hospital during 1982. The chi-square test was used for statistical calculations.

Trichophytin reactions. A commercial antigen with purified trichophytin prepared on an extract

Table II. *Blood groups of the AB0 system in 135 patients related to different dermotophyte species and to the total number of dermatophytes in patients with HPPK*

	TR		TM		EF		Total		Neg.	
	n	%	n	%	n	%	n	%	n	%
Blood group A (n=72)	11	15	14*	19*	5	7	30	42	42	58
Blood group 0 (n=50)	7	14	4	8	7	14	18	36	32	64
Blood group B (n=5)	0	0	0	0	0	0	0	0	5	100
Blood group AB (n=8)	2	25	0	0	1	13	3	38	5	62
Total	20	15	18	13	13	10	51	38	84	62

* Increased in comparison with the other blood groups, $p<0.05$.

from TM was used (Trichophyton®, Miles and Dome). A prick test was performed with a dilution containing 5000 nitrogen units per ml, preserved in 0.4% phenol with the addition of 50% glycerin. As controls histamin 1 mg/ml and 0.9% sodium chloride were used. Immediate reactions were read after 20 min, delayed reactions after 72 hours. An immediate or delayed reaction consisting of erythema and induration of 5 mm or more was considered positive. 37 patients with HPPK were included in this material, 25 healthy persons without dermatophyte infections and 11 patients with dermatophytosis but without HPPK served as controls.

Specific IgE RAST (Pharmacia, Uppsala, Sweden). In 20 HPPK patients with dermatophyte infections specific IgE antibodies against TR, TM and EF were determined using the Phadebas RAST method (17). Dermatophyte antigen extracts 1/30 w/v of the species TR (CBS 363.62), TM (CBS 426.70) and EF (CBS 233.69) were prepared by extraction of homogenized mycelia from the individual species in 0.05 M sodium phosphate buffer, pH 7.4. The extraction was performed during 16 hours at 4°C. The discs for the RAST assay were prepared by covalent coupling of the dermatophyte extract to the activated paper discs. The dermatophyte raw materials were purchased from Allergon AB, Ängelholm, Sweden. Sera from patients who had total IgE >100 kU/ml were investigated for IgE antibodies against a reduced RAST panel consisting of ten common extrinsic allergens that frequently elicit positive reactions in patients with allergic rhinitis or bronchial asthma. The allergens tested were *Phleum pratense, Betula verrucosa, Artemisia vulgaris*, egg white, milk, cat epithelium, horse dandruff, dog dandruff, *Dermatophagoides farinae* and *Cladosporium herbarum*.

Specific IgG RAST (Pharmacia, Uppsala, Sweden). In the same 20 patients specific IgG antibodies were determined by RAST assay, using the Phadebas IgG RAST reagents together with specially prepared dermatophyte allergen discs (18). The same extractions of dermatophytes as were used for the determination of specific IgE antibodies were also used for this test. 10 healthy persons constituted the control group. Dermatophyte antibodies against TR, TM and EF were related to the homologous species and to the total number of dermatophytes, irrespective of their species. For statistical calculations Student's *t*-test was applied.

Crossed immunoelectrophoresis (CIE) (Rigshospitalet, Copenhagen, Denmark). The presence of precipitating dermatophyte antibodies was investigated by use of CIE with an intermediate gel (19, 20). 24 HPPK patients with dermatophyte infections were included in this part of the study, and the dermatophytes were distributed as follows: TR 14, TM 6 and EF 4. Blood samples were taken from the patients as soon as the diagnosis of dermatophytosis was established by direct microscopic examination of skin scrapings from palms and soles. Results from CIE were correlated to the definite diagnosis made after study of the cultures, and compared with results from previous investigations of blood samples from patients with acute and chronic dermatophytosis, but without HPPK, performed at the same laboratory and using the same method.

Table III. *The levels of the IgG RAST for TR, TM and EF in sera from 20 patients with HPPK infected with one of those dermatophyte species*

The figures indicate mean levels expressed in per cent of total counts per minute and standard deviations. The *p* values refer to comparison with the control vlue for each species specific IgG

	IgG RAST					
Dermatophytes	TR	*p*	TM	*p*	EF	*p*
T. rubrum (n=8)	19.5±5.6		30.8±8.7	<0.02	25.0±9.4	<0.05
T. mentagrophytes (n=5)	19.7±1.9		24.6±5.3		19.8±5.8	
E. floccosum (n=7)	21.9±4.8		27.3±7.4	<0.05	23.3±5.9	<0.05
Total number of dermatophytes (n=20)	20.4±4.5		28.0±7.6	<0.01	23.1±7.4	<0.05
Controls (n=10)	22.6±8.0		20.9±6.7		17.4±6.5	

RESULTS

Among 85 patients with HPPK a family history of atopy was found in 22, of whom 10 (45%) were suffering from dermatophyte infections. 10 patients had a personal history of allergic rhinitis or bronchial asthma, none had atopic dermatitis, but it was possible to culture dermatophytes from the soles of 5. Patients with HPPK who had a personal and/or family history of atopy did not have a higher frequency of dermatophyte infections than that found in non-atopics (Table I). Total IgE level in serum, correlated with different dermatophyte species found in the material, is shown in Table I. It was found that patients with serum IgE level >100 kU/ml had a tendency to an increased frequency of dermatophyte infections when compared to patients with a serum IgE level <100 kU/ml. The difference was not, however, statistically significant.

The AB0 blood groups of 135 patients with HPPK were distributed as follows: A 53%, 0 37%, B 4%, AB 6%. The corresponding figures for the total number of blood groups determined at the Central Laboratory of the hospital during 1982 were: A 48%, 0 37%, B 10%, AB 5%. The relationship between the different blood groups in the 135 patients with HPPK and the different dermatophyte species is shown in Table II. It was also discovered that TM was found more often in patients with blood group A ($p<0.05$).

A positive immediate trichophytin reaction was found in only 1 patient (papule >5 mm). No case of delayed trichophytin reaction was seen. In the two control groups both immediate and delayed reactions were found to be negative. The patient, who had a positive trichophytin reaction also had specific IgE antibodies against TM and EF and a total IgE level of 623.5 kU/ml. Results from testing on the reduced RAST panel of common extrinsic allergens showed in all patients with an IgE level >100 kU/ml raised values of circulating specific IgE antibodies against most antigens of the panel.

Results of the determination of specific IgG antibodies against TR, TM and EF are shown in Table III. Precipitating circulating dermatophyte antibodies determined by crossed immunoelectrophoresis were found in 13/24 (54%) of the sera.

DISCUSSION

Family and personal histories of atopy showed that dermatophyte infections in HPPK did not appear more often in atopics than in non-atopics, and in this respect the findings documented in this paper did not concord with previous publications on chronic dermatophytosis in atopics (8, 21). The generally accepted higher frequency of dermatphyte infections in atopics was surpassed by the greater affinity to such infections in patients with HPPK and probably for this reason no statistically significant difference was found between atopics and non-atopics. The same result was valid in patients with serum IgE level >100 kU/ml compared with those with IgE level <100 kU/ml. This also corresponded poorly to earlier reports (16). Great affinity of dermatophytes to the hyperkeratosis of HPPK patients probably plays the same role in this part of the study.

Type A blood groups iso-antigen was found in a surprisingly high number of patients with TM infections. Subjects who possess tissue antigens which cross-react with the fungus seem to have an immunologic tolerance which may facilitate dermatophyte infection. This glycoprotein has been demonstrated not only in patients infected with TM but to a lesser degree also in infections caused by TR and EF (11). Commercial trichophytin gave only one positive immediate reaction and no delayed reactions. Since no reactions were found in the control groups either, the reliability of this trichophytin should be questioned. Earlier publications have also stated that activity and specificity of commercial trichophytin preparations vary (22).

As seen in Table III a significantly higher level of TM and EF antibodies could be shown to exist in patients with HPPK infected with TR and EF ($p<0.05$). The number of patients is small and conclusions should be drawn with caution. These results confirm, however, that TR, TM and EF have at least one antigen in common (11, 21). TM was a weak homologous and heterologous antibody inducer compared with TR and EF, and patients infected with TM did not produce higher levels of IgG antibodies against TR, TM and EF than the controls.

Precipitating antibodies determined by crossed immunoelectrophoresis have previously been found in about 10% of patients with acute and chronic dermatophytosis caused by different dermatophyte species (20). In the present material the corresponding figure was 54%. Dermatophytosis in patients with HPPK is usually confined to the palms and soles and does not propagate to other parts of the body. Restriction of dermatophyte infections to the hyperkeratosis and the appearance of vesicular eruptions along the hyperkeratotic border are probably manifestations of the immunologic defence mechanism, however, the material is too limited to permit general conclusions.

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