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ELISA-DETERMINED SEROLOGICAL REACTIVITY AGAINST PURIFIED TRICHOPHYTIN IN DERMATOPHYTOSIS

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Abstract. Serological activity in 62 patients with dermatophytosis was investigated using purified trichophytin preparation as antigen (produced by the ethylene glycol method). Antibodies of IgG and IgM classes were determined by means ef enzyme linked immunosorbent assay (ELISA). In comparison with 79 controls consisting of groups of healthy blood donors and dermatological outpatients, the dermatophyte-infected patients showed a significantly higher mean IgG response to trichophytin antigen preparation. On the other hand several individual cases were low responders. In a group of 10 control children, no IgG activity against trichophytin could be demonstrated. IgM class antibodies showed no significnat differences between the groups investigated. These findings indicate that differences in the amounts of antibodies of IgG class against trichophytin as measured by the ELISA method reflect differences in the degree of mycotic sensitization between groups of individuals having experienced varying degrees of exposure to dermatophytes. Nevertheless, the determination of IgG antibodies in dermatophytosis seems to be of limited clinical value.

Key words: Dermatomycosis; ELISA; Trichophytin

Cell-mediated immunity (CMI) in dermatophytosis has been studied by several investigators and the importance of CMI in host resistance to fungal infections has been underlined (3, 4, 5). Interest in the serology of dermatophytosis is almost as old as the interest in CMI (9). Many studies have demonstrated circulating antibodies to dermatophyte antigens in dermatophytosis (2). However, the importance of these antibodies and their clinical significance is still unclear. The specificity of the antibodies detected has been questioned as often crude and non-defined antigens have been used (12). In a previous report, CMI was investigated in patients with dermatophytosis to antigenic preparations of dermatophytes, using so-called purified trichophytin produced according to the ethylene glycol method (6). In the present study the same antigen preparation has been employed in a sensitive system for detection of antibodies, namely the enzyme-linked immunosorbent assay (ELISA). The purpose of this study was to investigate the occurrence of IgG and IgM antibodies to trichophytin in patients with dermatophytosis, compared with non-infected individuals and to determine whether or not differences existed between patients with various clinical dermatophyte infections.

MATERIALS AND METHODS

Patients. Consecutive patients attending the Department of Dermatology, Södersjukhuset, Stockholm, who gave informed consent to a blood test, were investigated. Patients with clinical signs of immunological disorders or undergoing immunosuppressive medication were excluded.

This group comprised 62 patients with dermatophysis of whom 50 had fungal infection verified by culture and 12 had clinical dermatophytosis, i. e., positive direct microscopy but negative culture. Patients with a duration of infection of more than one year were considered chronic cases.

Controls. Sera collected from the following categories of donors: 1) 43 outpatients with various dermatological conditions. Anamnesis revealed no fungal infection. 2) 36 healthy blood donors. 3) 10 children with clinical signs of virosis.

Antigens

Strains of *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* isolated from active human skin lesions were cultured on defined liquid media. Antigens were extracted from the resulting mycelia by the ethylene glycol method as described elsewhere (7). The protein contents of antigen preparations were measured according to Lowry et al. (11) using bovine serum albumin as the standard. The protein values were for *T. rubrum* 11%, *T. mentagrophytes* 15% and *E. floccosum* 21%, dry weight.

ELISA

Antibodies to the dermatophyte antigens were determined by an indirect ELISA technique (15). The wells of irradiated polystyrene microplates (type M 29AR, Greiner,

Table 1. *Mean relative ELISA values in patients with dermatophytosis compared with control subjects using T. mentagrophytes antigen*

Standard deviation in parentheses

Subject group	No. of individuals	IgG	IgM
Dermatophytosis pats.	62	1 775 (1 335)	996 (599)
Chronic	25	1 684 (953)	781 (439)
Non-chronic	37	1 836 (1 551)	1 170 (655)
Control group	79	1 118 (852)	945 (675)
Outpatients	43	1 244 (898)	1 064 (750)
Blood donors	36	977 (778)	827 (561)
Children	10	●	169

Statistically significant difference for IgG between patients and controls (ANOVA $p<0.001$, multiple contrasts performed on the 5% level).

Nürtingen, West Germany) were coated with test antigen at a concentration of 1–10 μg dry weight per ml in 0.05 M sodium carbonate buffer, pH 9.6, at 4°C for at least a week. Before use the plates were washed three times with phosphatebuffered saline (PBS) containing Tween 20 (0.05% w/v). The serum samples were diluted 1 : 1000 in PBS–Tween containing 1% bovine serum albumin and 0.2 ml was added to each well. After 90 min incubation at 37°C the plates were washed three times with PBS–Tween and 0.2 ml of alkaline phosphatase-conjugated anti IgG or anti-IgM was added to the wells. The conjugates had been prepared according to Engvall & Perlmann (1) and Wasserman et al. (14) respectively. The conjugates were used at dilutions of 1 : 1000 and 1 : 4000 in PBS–Tween–albumin for IgG and IgM respectively.

The plates were incubated overnight at room temperature and after washing three times with PBS–Tween 0.2 ml *p*-nitrophenylphosphate (1 mg/ml in 1 M diethanolamine–HCl buffer, pH 9.8, containing 0.5 mM $MgCl_2$) was added. The reaction was stopped after 1 h by the addition of 50 μl 3 M NaOH. The absorbance at 405 nm was measured with a Titertek Multiskan plate reader (Flow Laboratories, Irvine, Scotland). The ELISA values given in the tables refer to the absorbance value × 1000 after correction for the spontaneous substrate hydrolysis blank.

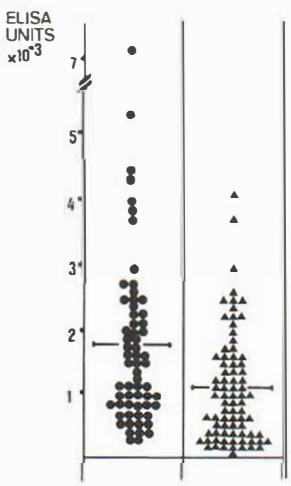

Fig. 1. Relative IgG ELISA values to trichophytin from *T. mentagrophytes* in patients with dermatophytosis and control individuals (dermatological outpatients and blood donors). ●, patients; ▲, controls.

Statistical analyses

Logarithm-transformed values of the four main groups of IgG and IgM respectively were found to fulfil the requirements for performing statistical test by single-factor analyses of variance (ANOVA). The individual values of the experiments shown in Table III were also logarithm-transformed and analysed with two-factor analysis of variance. When ANOVA-tests were statistically significant multiple comparison procedure was carried out according to the method of Scheffes' multiple contrasts (16). All multiple comparisons were performed on the 5% level of significance.

RESULTS

The amounts of IgG and IgM antibodies to purified trichophytin from *T. mentagrophytes* as measured by the ELISA method are shown in Fig. 1 and Table 1. The results are expressed as relative units. Fig. 1 shows the ELISA values of IgG antibodies in the dermatophyte-infected patient group compared with control individuals, either dermatological outpatients or healthy blood donors. The highest mean IgG values were found in the patient group, followed by the control outpatients, while the blood donors showed the lowest mean values (Table 1). The differences between the IgG values of the dermatophyte-infected patients and the control group was statistically significant (Table 1). However, several dermatophyte-infected patients showed low individual IgG values (Fig. 1).

The differences between mean IgM values of pa-

Table II. *Mean relative ELISA values in patients with dermatophytosis divided into groups according to localization of infection and dermatophyte isolated using T. mentagrophytes antigen*

Standard deviation in parentheses

	n	IgG	IgM
A. Localization			
Tinea cruris	26	1 379 (955)	1 105 (733)
Tinea pedis	10	2 200 (1 150)	951 (427)
Tinea corporis	7	2 530 (2 366)	1 336 (612)
Nails	10	2 421 (1 475)	851 (759)
B. Infecting dermatophyte			
T. rubrum	25	2 272 (1 656)	949 (623)
T. mentagrophytes	8	1 447 (926)	1 200 (599)
E. floccosum	16	1 396 (1 013)	1 121 (796)

tients and controls were small and not statistically significant (Table I).

Ten children, 3–5 years of age, were investigated as another control group. Very low amounts of IgM antibodies and no IgG antibodies were found (Table I).

In Tables I and II the results of IgG and IgM determinations are presented, with patients divided into groups according to different clinical parameters such as duration of infection, type of infection dermatophyte, and localization of infection. When comparing chronically infected patients with non-chronic patients the differences between mean ELISA values were small with respect to both IgG and IgM and not statistically significant. Patients with tinea cruris showed lower mean IgG values than those with tinea pedis, tinea corporis and nail infections (Table II A). The mean values of IgM antibodies showed only minor variations (Table II). *T. rubrum* infections gave higher IgG values than both *T. mentagrophytes* and *E. floccosum* infections, while IgM values did not differ between the dermatophyte infections (Table II B).

Table III shows the mean IgG titres of sera from randomly chosen patients with different culture-verified dermatophyte infections using purified antigens from *T. rubrum*, *T. mentagrophytes* and *E. floccosum*. The relative ELISA values obtained in this part of the study were generally lower, and, thus not directly comparable with values obtained in other experiments presented in this study. The only difference registered in this cross-sensitivity experiment was in patients infected with *T. rubrum*. These patients had a significantly lower IgG response to *T. rubrum* antigen than to *T. mentagrophytes* and *E. floccosum* antigen. However, the response to *T. rubrum* antigen, although not statistically significant, was also lowest in the other two patient groups, viz. patients infected with *T. mentaprophytes* and *E. floccosum*.

DISCUSSION

The most consistent serological finding in dermatophytosis has been the presence of antibodies in patients with deep skin lesions (13). In this study

Table III. *Mean relative IgG ELISA values for different dermatophyte antigens in patients with different dermatophytosis*

Standard deviation in parentheses

Dermatophyte infection	No. of patients	IgG ELISA antibodies to antigens from		
		T. rubrum	*T. mentagrophytes*	*E. floccosum*
T. rubrum	12	965 (758) (1)	1 427 (1 017) (2)	1 355 (1 103) (3)
T. mentagrophytes	6	543 (312)	753 (434)	565 (390)
E. floccosum	10	512 (257)	706 (434)	575 (358)

Statistical evaluation: significant difference between 1–2 and 1–3 (ANOVA $p<0.001$, multiple on the 5% level). All other differences not significant.

IgG and IgM antibodies to purified trichophytin were determined with a sensitive ELISA technique. Mean IgM values did not differ between patients and controls, while mean IgG values showed statistically significant differences between patients with clinical dermatophyte infection and control individuals. The higher mean IgG and IgM values in control outpatients vis-á-vis healthy blood donors may possibly indicate that affected skin of dermatological control patients more easily permits a mycotic sensitization or secondary dermatophyte infection. In the the young children (3–5 years of age) the results revealed low IgM and absence of IgG antibodies. This result may be ascribed to infrequent exposure to the dermatophytes in question at this age and renders it likely that ELISA antibodies of IgG class are indicative of sensitization to dermatophytes.

Dermatophyte antibodies do not seem to closely correlate to the clinical status of dermatophytosis. No differences in IgG or IgM values were noted between the two main groups of patients, viz. chronically infected and the non-chronic. There were certain differences in antibody values between patients with respect to other clinical parameters, e. g., patients with tinea cruris had lower mean IgG values than patients with infections at other sites and patients with *T. rubrum* infections showed higher mean IgG values than patients with *T. mentagrophytes* or *E. floccosum* infections. However, these groups were relatively small and the differences do not seem to allow of any reliable evaluation of clinical implications.

The results from the cross-reactivity study (Table III) reveal on the whole no substantial differences in the reactivity to these three antigens in patients infected with different fungi, thus suggesting that *T. rubrum*, *T. mentagrophytes and E. floccosum* possess common antigenic components. The reactions obtained with *T. rubrum* antigen were possibly weaker in all three patient groups, which might be due to some physical properties of the antigen preparation that influence its adsorption to the plastic wells and thus the sensitivity of the test. These findings are fairly similar to findings from experiments with the same antigens in sensitized guinea pigs in which substantial cross-reactivity of cell-mediated immune reactions was demonstrated (8). It can be mentioned in this context that the specificity of serological reactions in dermatophyte infections has been questioned and, there is reason to believe that antigen preparations from other dermatophytes and from Aspergillus species are capable of cross-reacting (2, 10). However, from a clinical standpoint this problem is of minor importance, since dermatophytosis and aspergillosis rarely occur concomitantly.

In conclusion, the determination of IgG antibodies against different dermatophyte antigens seems to be of limited value in clinical practice, as the presence of these antibodies in individual patients is indicative of infection only in certain cases.

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