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LYMPHOCYTE TRANSFORMATION IN VITRO IN ACUTE DERMATOPHYTOSIS: A FOLLOW-UP STUDY

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Abstract. Sixteen patients with recently acquired (= non-chronic) dermatophytosis were studied clinically and immunologically before, during and after treatment for 6 months. The methods applied were lymphocyte transformation in vitro (LTT) and intra-dermal test (IDT). Phytohæmagglutinin, pokeweed mitogen, purified protein derivate (PPD) and water-soluble extracts of *Trichophyton* (T) *rubrum*, *T. mentagrophytes* and *Candida albicans* were used as stimulators. In the LTT the patients in all four studies showed normal general immune responses. Compared with a control group of 22 healthy persons, lymphocytes of the patients showed significantly stronger stimulation with the *T. rubrum* extract ($0.01>p>0.001$) throughout the 6 months. The degree of stimulation after one month (the second study) was significantly stronger ($0.05>p>0.01$) than in the other studies. The stimulation in the last (fourth) study was the lowest (not significant). IDT showed a majority of delayed-type reactions (47–67%) in patients as compared with 7–13% in the controls, when using dermatophyte antigens. Three patients had an immediate-type reaction or mixed reactions. A correlation was found between the severity of lesions and the degree of stimulation in LTT ($p<0.02$ for *T. rubrum*, $p<0.007$ for *T. mentagrophytes*). In contrast, no relationship was found between IDT and clinical symptoms, and there was only a slight tendency toward correspondence between the results of IDT and LTT.

Key words: Dermatophytosis; Lymphocyte transformation *in vitro*; Follow-up study

In patients with dermatophytosis, specific cellular and humoral immunological responses are stimulated. The essential part of the primary immune response to dermatophytes is considered to be cell-mediated hypersensitivity (3). More or less specific humoral antibodies have been demonstrated by a great number of authors, but the importance of these antibodies is still obscure. They have been related to severe, inflamed infections and chronic cases (3, 9).

This study aimed at evaluating the degree and duration of the cell-mediated immunity in patients with acute dermatophytosis.

The lymphocyte transformation test (LTT) in vitro has been found to be useful in the evaluation of hypersensitivity in dermatophytosis (1, 4, 5, 7, 10) but is not found to have been used previously in any longitudinal study such as the present one.

PATIENTS

Sixteen patients, 14 males and 2 females, were included in the study. All suffered from a recently acquired (less than 3 months) dermatophytosis caused by *Trichophyton* (T) *rubrum* (R), *T. mentagrophytes* (M), *T. verrucosum* (V), *Epidermophyton* (E.) *floccosum* or *Microsporum* (M.) *canis*. In half of the patients the lesions were mildly inflamed and/or minor areas were infected. In the other half the inflammation was moderate or severe and/or the affected area larger (Table I). Four of these had a vesiculous reaction on the hands. Nine of the patients had reported mycotic infection in the past, but all on other locations.

In all patients, topical treatment (miconazole nitrate cream 1%) was applied twice daily for at least 4 weeks. In 7 patients with severe infection, griseofulvin (500–1000 mg daily) was given for 1–3 months.

Two of the 16 patients were lost to follow-up after the introductory study, while 3 more patients were lost at a later state. Ten patients passed all four examinations. A control group of 21 females and one male without dermatophytosis or history of fungal disease was involved in the study.

The patients were examined (i) at the time of diagnosis, (ii) one month, (iii) 3 months and (iv) 6 months later.

METHODS

1. Identification of dermatophytes was made microscopically (KOH 20%) and by culturing of skin scales on Sabouraud dextrose agar.

2. LTT was carried out as described earlier (8, 10). 100000 mononuclear cells per culture tube were cultured in 0.5 ml RPMI–1640 medium (Gibco) with 15% pooled human serum, 25 mM HEPES buffer, 15 IU heparin, 24000 IU penicillin, 500 μg streptomycin base and 1.2 mmol glutamine. After the addition of 50 μl mitogenic solution the cells were cultivated in 5% Co_2 in humidified air at 37°C. Mitogens used were phytohæmagglutinin (PHA) (Difco), pokeweed-mitogen (PWM) (Gibco),

Table 1. *Review of patients 1–16, agent, site, and severity of dermatophytosis and most important results of lymphocyte transformation (LTT) and intradermal test (IDT) at the second visit*

++ = severe inflammation or large area involved, + = modest inflammation or small area involved. Icpm = increment, counts per minute. D-type = delayed-type hypersensitivity. TR = *T. rubrum* extract. TM = *T. mentagrophytes* extract

Patient	Dermatophyte	Site of infection	Severity of infection	LTT Icpm TR 1:2000	LTT Icpm TM 1:100	IDT D-type TR 1:1000	IDT D-type TM 1:1000
1	*T. rubrum*	Trunk	++	1 583	6 752	Pos.	Pos.
2	*T. rubrum*	Groin	++	4 943	2 048	Pos.	Pos.
3	*T. rubrum*	Groin	+	970	1 549	Pos.	Neg.
4	*T. rubrum*	Feet	+	1 322	4 144	Pos.	Pos.
5	*T. rubrum*	Feet	+	1 082	1 068	Neg.	Neg.
6	*T. rubrum* + *T. mentagroph.*	Feet	+	326	1 676	Pos.	Neg.
7	*T. mentagroph.*	Feet	++	1 783	6 597	Pos.	Neg.
8	*T. mentagroph.*	Feet	++	853	3 870	Neg.	Neg.
9	*T. mentagroph.*	Feet	++	1 034	3 917	Pos.	Pos.
10	*T. mentagroph.*	Feet	++	3 281	10 717	Pos.	Pos.
11	*T. mentagroph.*	Feet	+	348	1 941	N.D.	N.D.
12	*T. verrucosum.*	Neck + arms	+	159	137	Pos.	Pos.
13	*T. verrucosum.*	Beard	++	5 836	4 758	Pos.	Pos.
14	*M. canis*	Neck + arms	++	1 566	2 428	Neg.	Neg.
15	*E. floccosum*	Groin	+	282	904	Neg.	Neg.
16	*E. floccosum*	Groin	+	232	678	Neg.	Neg.

Tuberculin (PPD) (Serum Institute of Denmark) and extracts of TR and TM. The procedure for the production of dermatophyte extracts has been described elsewhere (2). The stock solutions are water soluble and contain 10 mg protein per ml. In the LTT the TR extract was diluted with medium 1:1000 and 1:2000 and the TM extract 1:50 and 1:100. Cultures with PHA, ConA and PWM were incubated for 96 hours and cultures with PPD, TR and TM for 120 hours. To each tube 50 μl containing 0.05 μCi of [^{14}C] thymidine (specific activity of 0.25 mCi/mg; NEN Chemicals) was added 24 hours before harvesting in a Skatron semi-automatic harvester. All cultures were set up in triplicate. The radioactivity was counted in a liquid scintillation counter. The stimulation was expressed as increment counts per minute (Icpm), i.e. median counts per minute (cpm) in stimulated cultures minus median cpm for unstimulated control cultures. The LTT was performed on 31 different occasions, each one including 2–4 patients and 2 control individuals.

3. Intradermal testing (IDT) was carried out after one and 6 months with PPD, *Candida albicans* (CA) 1:100, TR 1:1000 and TM 1:1000. An immediate reaction was considered present when an urticarial reaction lager than 10 mm diameter was seen after 20 min. A delayed reaction was defined as a papular infiltration more than 7 mm in diameter after 48 hours. The fungal antigen extracts were equivalent to commercial products (*Candida albicans* 1:100, Dermatophytin 1:30 (Hollister-Stier)).

4. The Mann-Whitney Wilcoxon rank-sum test was used for the comparisons between patients and controls, and within the control group. For comparison of the results from four different investigation days, Friedman's multisample test was used. Fisher's exact test was used for comparison of frequencies.

RESULTS

1. *Lymphocyte transformation test*

The control group consisting of 22 healthy persons, several of whom were examined several times, was evaluated throughout the investigation time of 1½ years. This period was divided into three sequen-

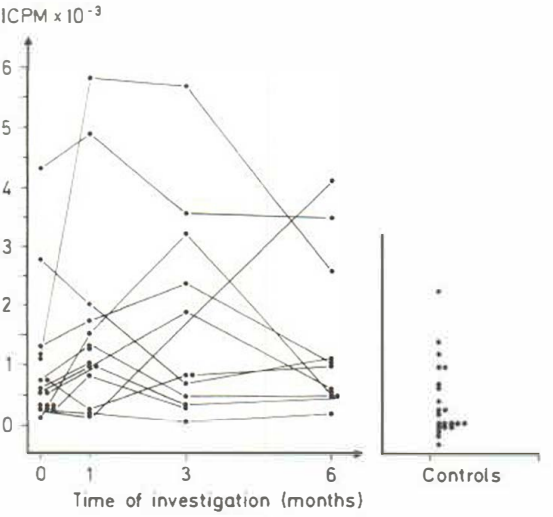

Fig. 1. Lymphocyte transformation in vitro in acute dermatophytosis by *T. rubrum* (dilution 1:2000). Follow-up study.

Table II. *Results of intradermal test*

One patient with TM and 2 with TR infection reacted with both immediate and delayed hypersensitivity. They are included in the group of delayed response

Antigen	Group	*n*	Immediate		Delayed		Negative	
			n	%	*n*	%	*n*	%
PPD	Patients	15	0		15	100	0	
	Controls	15	0		14	93	1	7
C. albicans	Patients	15	1	7	11	73	3	20
1 : 100	Controls	15	1	7	13	87	1	7
T. rubrum	Patients	15	0		10	67	5	33
1 : 1 000	Controls	15	0		2	13	13	87
T. mentagrophytes	Patients	15	1	7	7	47	7	47
1 : 1 000	Controls	10	0		1	10	9	90

tial periods. The results from each period were compared with the results from the other two periods, individually or combined, each control person being represented only by the first value from the period for each stimulant. No significant differences between the results in these three periods of time were found concerning any of the stimulants. Therefore, one standard control group was considered adequate for each of the stimulants in the comparisons with the patient groups. This standard control group consisted of the first value for each control individual, giving 22 control values for each stimulant.

LTT using PHA, PWM and PPD as mitogen showed normal stimulation of lymphocytes from both the control group and the patients at all investigation dates, with no significant differences between the groups.

TR and TM extracts in all dilutions were capable of stimulating lymphocytes to a higher degree in patients than in controls at all four examinations. Significant differences were found with TR diluted 1 : 2 000 on all examination dates ($0.001<p<0.05$) (Fig. 1) and with TR diluted 1 : 1 000 at the first and second visit ($p<0.05$). When using TM diluted 1 : 100, a significant difference between lymphocytes from patients and controls was only found at the last examination ($p<0.05$); the other comparisons showed no significant differences.

The values obtained from the 10 patients who passed all four examinations during the 6-month follow-up study were evaluated with Friedman's multisample test. PHA, PWM and PPD stimulation showed no significant variation from date to date. Stimulation with TR gave the highest stimulation at the second investigation and this observation was significant for TR diluted 1 : 2 000 ($0.05>p>0.01$). Both dilutions of TR showed the lowest responses at the 6-month examination, and so did both TM dilutions.

The responsiveness in the LTT was related to the clinical manifestations. A pronounced correlation was found between strong stimulation and inflamed or widespread lesions and vice versa (Table I). Strong stimulation Icpm was evaluated in relation to the response of the control persons. Thus an Icpm of more than 1 000 was considered strong stimulation when using TR diluted 1 : 2 000, whereas an Icpm of more than 2 000 was considered strong in respect of TM diluted 1 : 100. When using Fisher's exact test, the correlation between a high Icpm and severe infection was found significant ($p=0.02$ for TR and $p=0.007$ for TM).

2. *Intradermal test*

IDT was performed in 15 patients and 15 control persons (Table II). All except one of the controls showed delayed-type hypersensitivity to PPD. The reactions to *C. albicans* were similar in the two groups. Significant differences are noted in the results when using TR and TM antigen extracts, indicating delayed-type hypersensitivity in the infected persons (67 %) vis-à-vis the controls (13 %) when using TR ($p=0.004$), and in 47 % versus 7 % when using TM ($p=0.02$) (Table II). Three patients (21 %), i.e. 2 with TR and one with TM infection, displayed an immediate-type or mixed reaction with the fungal antigens.

A review of the location of the dermatophytosis, dermatophytes and skin reactivity is shown in Ta-

ble I. When using TR extract, negative skin tests were seen mainly in patients with dermatophytes other than TR, and not associated with the site of infection. Retesting of the 5 skin test negative patients 5 months later revealed delayed reactivity in one patient, while the rest remained negative. In the skin test positive patients, retesting revealed no changes except for one TR-infected patient who became negative. The use of TM extract gave similar results (Table I). No correlation was observed between the severity of infection and the presence of delayed-type hypersensitivity, either for TR or for TM extracts.

Finally, we looked at the relationship between the degree of stimulation (Icpm) in LTT and the results of IDT, and found a tendency, though not significant, toward a correlation between the two parameters.

DISCUSSION

The results obtained by studying patients with acute dermatophytosis using LTT confirm previous results (10) that lymphocytes from the patients are stimulated to a higher degree than those from control individuals using TR and TM extracts. The degree of stimulation is significantly related to the degree of severity of clinical symptoms.

The study was designed as a follow-up of the patients for 6 months, when all were cured. The observation that the degree of stimulation was significantly higher at the second investigation one month after the first visit as compared with the results from the remaining three visits indicates a certain delay until maximal stimulation capability is obtained. The finding of a still significantly stronger stimulation in patients at the last visit corresponds well with the concept of a certain acquired, long lasting immunity in dermatophytosis. In an earlier study Svejgaard et al. (9) demonstrated precipitating antibodies in patients with acute, inflammatory dermatophytosis. In a follow-up study of these patients for 6 months, precipitating antibodies were not demonstrated after 3 months. Thus, demonstrable antibodies disappear before cell-mediated immunity as detected by LTT, which stresses the importance of a well functioning cell-mediated immune response in dermatophyte-infected persons.

The results of IDT show the preponderance of delayed-type skin reactions in acute dermatophytosis. The presence could be related to dermatophyte species and inflamed/widespread reaction, but not to location, which is in contrast to the observation of Kaaman (6).

In conclusion, the immunological findings in this group of otherwise healthy patient suffering from recently acquired uncomplicated dermatophytosis support the assumption that cell-mediated immunity plays a major role in the defence mechanisms. Furthermore, the ability of the sensitized lymphocytes to react with their specific antigen continues for at least 6 months, indicating a certain long-lasting immunity. Finally, the results of LTT as compared with those of IDT indicate that the former method reflects more reliably the clinical and immunological state of the patient.

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