

Chronic dermatophyte infections II. Antibody and cell-mediated immune responses

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SUMMARY

In patients infected with *Trichophyton rubrum*, the T lymphocyte responses (lymphocyte transformation) to trichophytin were reduced, compared with control subjects. The same levels were seen in patients with intractable chronic infection and those who responded well to treatment but the site of infection affected the immune response, lower levels being seen in patients with palmo-plantar infection than in those with groin infections. In infections caused by *T. mentagrophytes* by contrast, lymphocyte responses to specific antigens were significantly increased.

Host resistance to dermatophyte infection depends on a number of factors ranging from the proliferative response of epidermis to T lymphocyte mediated immunity. Non-specific serum factors (Roth *et al.*, 1959) including transferrin (King *et al.*, 1975) may inhibit fungal growth in the non-immune patient. There is also evidence of an increased turnover of epidermal cells beneath an area of skin invaded by a dermatophyte (Berk, Penneys & Weinstein, 1976), which presumably enhances shedding of the organism. The mechanisms regulating this proliferative response are not known. The role played by circulating antibodies in defence against dermatophytosis is difficult to assess as they may be absent in a significant proportion of infected patients (Pepys, Riddel & Clayton, 1959) apart from those with favus and some forms of chronic dermatophytosis (Grappel, Blank & Bishop, 1972). Antibody to *T. rubrum* does not appear to be bound *in situ* in the vicinity of an invading dermatophyte to account for its absence in serum (Hay & Saeed, 1981).

The resolution of lesions caused by dermatophyte fungi in both experimental (Poulain, Vernes & Biguet, 1978) and human infections such as scalp ringworm (Rasmussen & Ahmed, 1978) correlates well with the development of delayed type hypersensitivity to the organism. Jones, Reinhardt & Rinaldi (1974a) were able to show that this was also true of the anthropophilic organisms including *T. mentagrophytes* in experimentally induced infections of

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forearm skin in man. Conversely, failure to respond to dermatophyte infections has been correlated with poor *in vitro* cell mediated immune responses such as lymphocyte transformation (Hanifin, Ray & Lobitz, 1974; Jones, Reinhardt & Rinaldi, 1974b) or leucocyte migration inhibition (Hay & Brostoff, 1977) to specific antigen, other parameters of cell mediated immune responses being normal. However the mechanism by which T lymphocyte mediated responses act on a dermatophyte in epidermis is not known. It is possible that soluble inhibitory substances such as transferrin, antibody or lymphokines may diffuse into epidermis and affect the fungus itself or the epidermal cells (Artis & Jones, 1980).

We have now tried to correlate immunological reactions with the clinical behaviour of dermatophytosis in chronically infected patients.

PATIENTS

The clinical and mycological assessment of the patients is described elsewhere (Hay, 1981). In addition to the skin tests and leucocyte migration responses (Hay & Brostoff, 1977) described previously, blood was taken for total T and B lymphocyte counts, lymphocyte transformation and serology. Transformation assays were carried out using chronically infected *T. rubrum* patients, CTR, (20). Control groups were provided by patients (10) who had recovered from *T. rubrum* infections in similar sites, HTR, non-infected skin test negative individuals, C, (8) and patients with acute *T. mentagrophytes* infections, NTR (14). Subsequently other groups were studied: patients with palmar/plantar infections, P/P, (15), tinea cruris, GR, (15) and tinea corporis, TC, (7). All the infections in this latter study were caused by *T. rubrum* and the results of treatment were not taken into consideration.

METHODS

Antigens

Antigens used for both lymphocyte transformation and serological testing were prepared from isolates of *T. rubrum* (MRL 1570) and *T. mentagrophytes* var. *interdigitale* (MRL 1564). The organisms were grown in Sabouraud's broth at room temperature with continuous stirring. The hyphal mat was harvested after 1 week and disrupted in N saline in a tissue mill (Dynamill, Basel). Disrupted cells were allowed to sediment and the supernatant solution containing cytoplasmic antigen (CP antigen) was dialysed and lyophilized. The culture filtrate was retained and lyophilized (CF antigen).

Lymphocyte transformation

A microculture system (Weir, 1978) was used to demonstrate lymphocyte transformation. Trichophyton antigen, *T. rubrum* (CP), unless otherwise specified, was used at two concentrations, 100 µg and 20 µg per ml. Phytohaemagglutinin (PHA), 3 µg/ml (Wellcome Reagents Ltd) and PPD 20 µg/ml (Evans Medical), were also incorporated into the assay plate.

Whole blood was sedimented over Ficoll-Hypaque (Flow Laboratories) at 1,000 *g* and the interface layer removed. The cells were washed three times in RPMI (Gibco, U.K.) and counted in a haemocytometer; 200 µl of RPMI and 10% fetal calf serum containing approximately 1×10^6 lymphocytes/ml were added to each well. The plates were incubated for 72 h and 1 µCi of ^3H thymidine (Radiochemicals, Amersham) added 12 h prior to harvesting. Wells containing fungal antigens or PPD were harvested at 96 h. The cells were harvested onto filter paper discs in an Ilacon harvester (Ilacon, U.K.) and after precipitation and drying the incorporation of

radioisotope was determined by scintillation counting. The counts were expressed as a stimulation index (mean of three values):

$$\text{Stimulation index} = \frac{\text{Mean counts per minute stimulated cultures}}{\text{Mean counts per minute unstimulated cultures}}$$

The concentrations of antigen used in the system were not inhibitory to lymphocytes from non-infected skin test negative subjects.

Total T and B lymphocyte counts

The lymphocytes were separated as described above. T cells were counted by the E-rosette method (Hudson & Hay, 1976). B cells were assessed by incubation of lymphocytes in the presence of anti-human immunoglobulin coupled to fluorescein (Wellcome Reagents Ltd) (Weir, 1978). Cells were counted using a fluorescence microscope. Total lymphocyte counts were checked using stained blood films and phase contrast microscopy.

Ten patients chronically infected with *T. rubrum*, CTR, and ten skin test negative control subjects, C, were studied. Both groups contained four individuals with a past history of asthma/hay fever.

Serology

Two methods of serological assessment were used. The microimmunodiffusion plate (Kaufman *et al.*, 1972) was set up using two types of *T. rubrum* antigen (CP and CF) at concentrations of 200 µg/ml. As a control rabbit anti-*T. rubrum* antiserum was included in each group of test wells. The second method involved counterimmunoelectrophoresis (Mackenzie, Proctor & Philpot, 1980) using CP alone. Plates were subjected to 4 V/cm for 90 min before washing and staining.

Fifty subjects (thirty-two CTR, eight HTR, five NTR, five C) were studied.

RESULTS

Lymphocyte transformation

The results of transformation to specific antigen in four groups of individuals are shown in Fig. 1. Lymphocyte stimulation indices (antigen concentration 100 µg/ml) for chronic *T. rubrum* (CTR), healed *T. rubrum* (HTR), normal controls (C) and *T. mentagrophytes* infected subjects (NTR) were 1.6 (± 1.0), 1.3 (± 0.4), 1.2 (± 0.4) and 9.0 (± 8.8). The mean stimulation index of the NTR group to *T. mentagrophytes* antigen was 7.2 (± 7.0). Stimulation indices for the same four groups using PHA were 23.6 (± 9.8), 25.6 (± 9.8), 25.3 (± 14.7) and 29.7 (± 14.8) respectively (Fig. 2), and for PPD, 8.05 (± 7.8), 7.5 (± 5.7), 6.2 (± 3.3) and 8.6 (± 6.1).

The differences between mean values for stimulation index to specific antigen in NTR patients and the CTR ($P > 0.01$) or HTR ($P > 0.01$) groups is significant. However there are no significant differences in the corresponding figures for transformation to PHA between CTR and HTR, ($P > 0.5$) or CTR and HTR groups ($P > 0.5$).

In view of the similar responses obtained from patients with chronic and healed dermatophytosis caused by *T. rubrum* lymphocyte transformation values for patients infected in different sites with this organism were compared (Fig. 3). Stimulation indices to trichophytin (*T. rubrum* 100 µg/ml) in individuals with palmar/plantar infections (P/P), tinea cruris (GR) and tinea corporis (TC), were 1.2 (± 0.5), 2.6 (± 1.2) and 2.8 (± 2.4) respectively. There is a significant difference between P/P and GR patients ($P > 0.01$).

A valid statistical comparison between P/P and TC groups is not possible in view of the

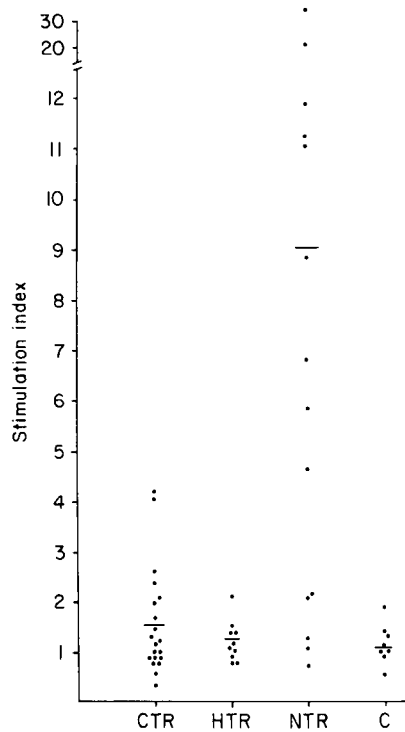


FIGURE 1. Lymphocyte transformation responses to *T. rubrum* antigen in treatment responsive and non-responsive patients. Chronic *T. rubrum*—CTR; healed *T. rubrum*—HTR; non-*T. rubrum*—NTR; normal controls—C (100 μ g *T. rubrum* cyto antigen/ml).

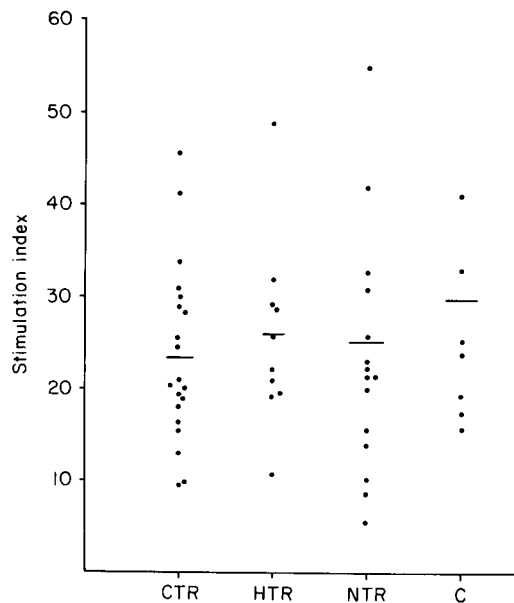


FIGURE 2. Lymphocyte transformation responses to PHA in treatment responsive and non-responsive patients. Abbreviations as for Fig. 1 (phytohaemagglutinin 3 μ g/ml).

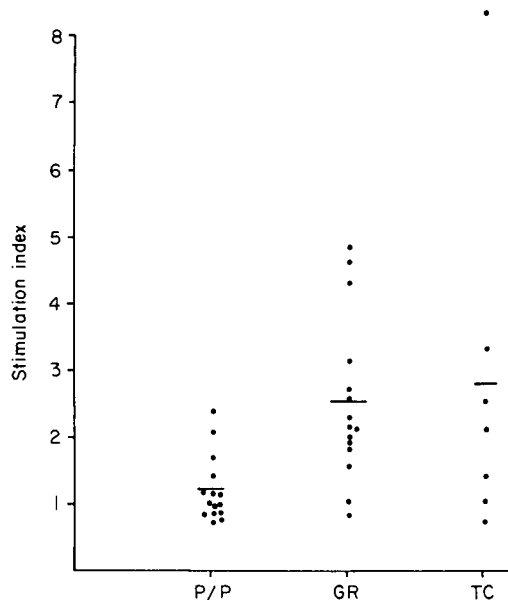


FIGURE 3. Lymphocyte transformation responses to *T. rubrum* antigen. The influence of site of infection. Palmar or plantar—P/P; tinea cruris—GR; tinea corporis—TC (100 µg/ml *T. rubrum* cyto antigen).

distribution of stimulation indices and the small number of patients within the latter group (Fig. 3). However, stimulation indices to PHA in P/P, GR and TC patients were $23.2 (\pm 10.5)$, $23.5 (\pm 12.1)$ and $28.7 (\pm 26.4)$ respectively. There is no statistical difference between these figures ($P > 0.5$ for all values). Three patients with tinea corporis were receiving systemic corticosteroid therapy. Their mean stimulation index to PHA was $9.5 (\pm 9.4)$ as opposed to $43.1 (\pm 2.6)$ for the remaining four patients of this group.

To exclude the possible influence of fluctuation of spontaneous activity in the lymphocyte transformation test, values for mean ^3H thymidine incorporation (c.p.m.) into unstimulated cells were recorded as follows: CTR (1396 ± 620), HTR (1306 ± 580), NTR (1465 ± 635), C (1330 ± 628), P/P (1281 ± 580), GR (1313 ± 555) and TC (1199 ± 603). These values do not differ significantly.

Serological tests

In immunodiffusion, thirteen (28%) out of forty-five sera examined showed precipitins to *T.*

TABLE 1. T and B lymphocyte populations in chronically infected patients

		B lymphocytes		T lymphocytes	
		%	Cells mm^{-3}	%	Cells mm^{-3}
Controls	(10)	17.2	296	57.1	1074
(C)		± 4	± 112	± 9	± 344
Chronic <i>T. rubrum</i>	(10)	14.7	258	62.0	1311
(CTR)		± 5	± 59	± 11	± 489

rubrum cytoplasmic antigen. Nine positive sera were from CTR patients, two from HTR and one from a patient with chronic *M. canis* (NTR) infection. One normal control was positive. Fifteen sera showed positive responses to culture filtrate antigen.

In counterimmunoelectrophoresis, eighteen sera (38%) developed precipitin lines to the cytoplasmic antigen (twelve CTR, three HTR, two NTR, one C).

Total T and B cell counts

The results are shown in Table 1.

There are no statistically significant differences between percentage or total T and B lymphocyte counts from controls or chronically infected patients— $P > 0.1$ (T cells) and $P > 0.1$ (B cells) for both parameters.

DISCUSSION

This study confirms that there is T lymphocyte hyporesponsiveness to trichophytin, but not to PHA or PPD in patients with chronic dermatophytosis (CTR) caused by *T. rubrum*. We found no evidence to support the existence of a quantitative abnormality of T lymphocytes in this group. By contrast, lymphocytes from patients infected with *T. mentagrophytes* (NTR) showed significantly higher stimulation indices to trichophytin derived from either organism. As in a previous study (Hay & Brostoff, 1977) patients infected with *T. rubrum* in palmar or plantar skin sites had similar responses irrespective of the response to treatment. However patients with groin infections caused by this organism (GR) had low but significantly higher responses than those with palmar or plantar infections (P/P). In our experience *T. rubrum* rarely caused tinea corporis (TC) without invading other sites. In the few TC patients we have studied the lymphocyte transformation responses were similar to the GR group. We have already reported a small sub-group of patients with resistant tinea corporis caused by *T. rubrum* associated with underlying conditions, such as SLE or corticosteroid therapy, which may contribute to defective immune responses (Hay, 1981). Poor stimulation responses to PHA in lymphocytes from three such patients has been shown in the present study.

Only nine (28%) of twelve (37%) patients with chronic *T. rubrum* infection were shown to have precipitating antibody by immunodiffusion and counterimmunoelectrophoresis respectively. A similar proportion of patients who had recovered from the infection had demonstrable specific antibody, 25% and 38% respectively. The chronically infected group did not therefore exhibit a higher level of antibody responses.

Despite differences in the methods of antigen preparation, chronic infection with dermatophytes, most commonly *T. rubrum*, has been associated in this and other studies with poor *in vitro* T lymphocyte function (Hanifin *et al.*, 1974). There are a number of explanations for this phenomenon. These include primary or secondary defects in immune function, e.g. T lymphocyte dysfunction (Hay, 1981). A high incidence of atopy is also probably significant in contributing to immunological hyporeactivity (Lobitz, Honeyman & Winkler, 1972). Histamine mediated responses, such as immediate type hypersensitivity to the infecting organism, may depress T lymphocyte function (Wang & Zweimann, 1978) and suppression of immediate type hypersensitivity on intradermal testing with trichophytin using an antihistamine may uncover a delayed reaction (Jones *et al.*, 1974b). This has not been confirmed by other workers (Hay, 1979). In addition, other groups have shown that lack of stimulating serum factors (Allen *et al.*, 1979) or the presence of serum blocking factors including antibody (Walters, Chick & Halliday, 1978) may contribute to chronicity in dermatophytosis. Alternatively modulation of T

TABLE 2. *Trichophyton* spp.—the spectrum of clinical and immune responses

	<i>T. verrucosum</i>	<i>T. mentagrophytes</i> var. <i>interdigitale</i>	<i>T. rubrum</i>
Inflammation	Yes	Yes	Little
Course	Recurrence rare	Periodic recurrence	Often chronic
Delayed type hypersensitivity	+	+	— (or immediate +)

lymphocyte responses, for instance by suppressor cells or disruption of lymphocyte recirculation (Bullock, 1976) may occur.

The site of infection may also influence the host response and, irrespective of the result of treatment, patients with palmar or plantar dermatophytosis showed poorer lymphocyte transformation to specific antigen than those with groin infections. Similarly a higher proportion of positive responses in both antibody production (Attapattu, 1977) and delayed type hypersensitivity to trichophytin (Kaaman, 1978) have been noted in patients with groin as opposed to peripheral (foot) infections. However in contrast to related organisms (Table 2) *T. rubrum* stimulates a weak inflammatory reaction in the patient and infections have a tendency to become chronic. The organism may therefore be less effective than related species in stimulating T lymphocytes, a proposition which is supported by the demonstration in this study of differences in lymphocyte transformation seen in patients infected with *T. rubrum* (CTR or HTR) and *T. mentagrophytes* (NTR). The effect of this differing capacity for stimulation of immune responses would be minimized in the presence of impaired host defences, for instance, in disorders such as chronic mucocutaneous candidosis, or conditions where the fundamental structure of the epidermis was altered, such as tylosis. In both these examples chronic infections with organisms other than *T. rubrum* have been demonstrated (Hay, 1981).

This interpretation of the factors affecting chronicity in dermatophytosis is an oversimplification of a complex interaction between immunogenicity and invasiveness of the infecting organism, the site of infection and the patient's immune responses. Changes in the latter by underlying disease processes, immunosuppressive therapy or by the induction of some form of immune tolerance may certainly favour the establishment of a chronic infection. However this investigation has shown that the patient's immune responses may vary according to the site of infection and the species of invading organisms. *T. rubrum* would appear to have an important biological advantage if it can evade effective host retaliation in certain sites such as plantar skin. This factor could explain the rapid increase in the incidence of *T. rubrum* infections in Europe and the U.S.A. in the last 30 years and their prominence as causes of chronic dermatophytosis.

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