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CELL-MEDIATED REACTIVITY IN DERMATOPHYTOSIS: DIFFERENCES IN SKIN RESPONSES TO PURIFIED TRICHOPHYTIN IN TINEA PEDIS AND TINEA CRURIS

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Abstract. Cell-mediated immune responses were measured in 91 patients with dermatophytosis by means of delayed-type skin hypersensitivity to a purified trichophytin preparation (ethylene glycol method) and to tuberculin (purified protein derivative, PPD). The findings indicate that dermatophytes differ in their sensitizing capacity as measured by trichophytin skin sensitivity. *Trichophyton mentagrophytes* appeared to be a potent sensitizer compared with *Trichophyton rubrum* ($p<0.01$), whereas *Epidermophyton floccosum* appeared as a moderate sensitizer. The localization of infection also affected the cell-mediated response to trichophytin, i.e. the frequency of reactions was 44% in tinea cruris and 33% in tinea pedis, while tinea pedis with nail infections elicited delayed-type reactivity in 60% of cases. On the basis of the significant difference ($p<0.001$) in cell-mediated reactivity between chronically and non-chronically infected subjects as measured with trichophytin, it is concluded that the cell-mediated response is of importance for the development of host resistance to dermatophytic infections. This study provides further evidence in support of the view that a partial defect in the cell-mediated system may be responsible for establishment of chronic dermatophytosis.

Key words: Dermatomycosis; Delayed hypersensitivity; Skin test; Trichophytin

Interest in the immunology of ringworm has increased during the last decade (1, 3, 4, 5, 7). The puzzling problems of host resistance and what determines establishment of chronic infection still remain unresolved. Immunological factors are certainly of importance in respect of these aspects of the disease. Differences in cell-mediated reactivity between chronically and non-chronically infected subjects have been pointed out (8, 13). The importance of other clinical parameters was stressed in a previous study (10). Against this background, it was considered of interest to further investigate patients with dermatophytosis and to measure their delayed-type skin reactivity to purified trichophytin, and to tuberculin as a measure of cell-mediated immunity. The purpose of this study was to correlate cell-mediated immunity to the clinical parameters of ringworm, i.e. duration of infection (chronic/non-chronic infections), localization of infection and the type of dermatophyte involved.

MATERIAL AND METHODS

Patients

The subjects were 91 outpatients from the Department of Dermatology, Södersjukhuset, Stockholm, with mycologically verified dermatophytosis of the feet and groin. The study was carried out during 1977 and 1978 with patients who gave informed consent for skin testing. All patients were reviewed anamnestically and clinically and those with signs of immunological disturbances or immunosuppressive medication were excluded. Patients with a duration of infection of more than a year were considered chronic cases. Patients with a positive history of or actual allergic asthma, hay fever or atopic dermatitis were considered atopic subjects. The clinical investigation and mycological survey were carried out as earlier described (10).

The dermatophytes isolated at the Department of Dermatology at Södersjukhuset during 1978 are listed in Table III. These findings are representative of dermatophytosis seen at Södersjukhuset from a clinical and mycological point of view during the last decade.

Skin tests

All patients were tested on the volar aspect of their forearms with purified trichophytin (1 mg/ml) as earlier described (9, 10). The tests were read after 48 h as the means of perpendicular diameters of skin erythema and induration. Reactions with a mean diameter of at least 4 mm were considered positive. The patients were also tested with tuberculin (purified protein derivative, PPD, 2 test units) as a general test of delayed-type skin reactivity. Statistical analyses were done with the χ^2 test with Yates correction and, for small groups, Fisher's exact test.

Table I. *Survey of the clinical data on the patients with dermatophytosis verified by culture*

	Patients			Mean age	Atopic patients	Chronic infection	Dermatophytes isolated			
	M	F	Total				*T. rubrum*	*E. floccosum*	*T. mentagroph.*	Others
Tinea cruris	43	2 (4%)	45	33	4	8 (17%)	14	30	0	1
Tinea pedis	21	9 (30%)	30	49	4	15 (50%)	18	2	10	0
Tinea cruris and pedis	7	0	7	31	2	5	5	0	1	1
Toe-nail infection	8	2	10	57	0	9	9	0	0	1
Total	79	13	92		10	27	46	32	11	3

RESULTS

Clinical data

The clinical data of the patients tested are given in Table I. All the tinea cruris patients except 2 were men and *Epidermophyton floccosum* was isolated twice as often as *Trichophyton rubrum*. Eight patients were chronically infected, all of them with *T. rubrum*. Atopy was registered in 4 patients, 3 of whom were infected with *T. rubrum*. When comparing these data on the patients in the present study with the total data on patients in 1978 (Table III), parameters such as the sex ratio and the frequencies of isolated dermatophytes correspond closely. Thus, the patients with tinea cruris seem to be representative with respect to these parameters.

Thirty patients with tinea pedis were included in this study (Table I). Of the dermatophytes isolated, 18 were *T. rubrum* and 10 *T. mentagrophytes*. Half of the patients were chronically infected with *T. rubrum*. On comparing these patients with the findings for all patients in 1978 (Table III), no significant differences were found with respect to the dermatophytes isolated. Chronic tinea pedis was found in half of the cases and *T. rubrum* was the causative organism in 12 of the 15 cases (Table I). Dermatophyte infestation of the toenails in patients with tinea pedis was verified by culture in a further 9 cases. These patients are considered as a separate group (results shown also in Tables I and III). Seven patients, comprising a separate group, were infected in both the groin and in the feet.

Delayed-type skin reactivity to trichophytin in chronically and non-chronically infected patients

The results of the skin tests with purified trichophytin are given in Table II. Delayed-type skin reactions were registered in 41% of all cases tested. Of patients with dermatophytosis of the skin, chronic patients were positive in 10% of cases, while more than half of the patients with non-chronic infections showed positive skin test ($p < 0.001$). Patients with toe nail infections, considered as a separate group, showed a high frequency of delayed-type skin reactions (66%) although chronically infected in 8 cases out of 9.

Delayed-type skin reactivity in relation to localization of infection

Table IV shows the findings for skin reactions when the dermatophytes are compared in detail with

Table II. *Frequencies of positive delayed skin reactions with trichophytin and tuberculin*

	Total		Dermatophytosis of skin				Toe-nail infection	
			Non-chronic		Chronic			
Test antigen	*n*	Positive	*n*	Positive	*n*	Positive	*n*	Positive
Trichophytin, 1 mg/ml	91	38 (41%)	54*	29 (53%)	28*	3 (10%)	9	6 (66%)
Tuberculin, PPD 2 TU	78	63 (80%)	45^{NS}	38 (84%)	24^{NS}	16 (66%)	9	8 (88%)

Statistical evaluation: * $p < 0.001$ (highly significant difference).

Table III. *Dermatophytes isolated at the Department of Dermatology, Södersjukhuset, Stockholm, during 1978*

Dermatophyte isolated	Total	Localization of infection					
		Tinea cruris		Tinea pedis		Nail infection	Others
		M	F	M	F		
Trichophyton rubrum	170	35	2	77	19	21	16
Epidermophyton floccosum	93	64	5	12	0	1	11
Trichophyton mentagrophytes	35	1	0	18	9	3	4
Others	5	0	0	0	0	1	4
Totals	303	100	7	107	28	26	35

respect to localization of infection. Of the patients with tinea cruris, skin reactions were registered in 44%, and of those with tinea pedis in 33%. The dermatophytes responsible for the delayed-type skin reactivity in tinea cruris were *E. floccosum* and *T. rubrum* at the same frequency, while in tinea pedis, *T. rubrum* sensitized only 6% of patients infected with this dermatophyte.

Delayed-type skin reactivity in relation to dermatophyte isolation

Table IV shows also the results of delayed-type trichophytin reactivity with respect to the type of dermatophyte isolated. 80% of the patients with delayed-type skin reactivity in tinea pedis were infected with *T. mentagrophytes*, while these patients comprised only 33% of this patient group. Thus, while there are small differences in the overall delayed-type skin reactivity between tinea cruris and tinea pedis patients (44% vs. 33%, respectively), the differences in the sensitizing abilities of the different dermatophytes are important.

Table IV highlights these differences. Of patients with *T. mentagrophytes* infection, 81% gave skin reactions compared with 43% and 30% of patients with *E. floccosum* and *T. rubrum* infections, respectively. If toe-nail infections are excluded, the delayed reactivity of *T. rubrum* patients was 21%. Significant statistical difference was registered between the *T. rubrum* and *T. mentagrophytes* groups ($p<0.01$, Table IV).

Delayed-type skin reactivity to PPD

Of the 91 patients tested with trichophytin, 78 were also tested with tuberculin (Table II). Patients giving negative skin tests with 2 test units of PPD

Table IV. *Delayed skin reactivity to trichophytin in patients with dermatophytosis with respect to localization of infection and dermatophyte isolated*

Dermatophyte isolated	Localization of infection									
	Tinea cruris		Tinea pedis		Tinea cruris and pedis		Tinea pedis with toe-nail infection		Total	
	n	Pos.	*n*	Pos.	*n*	Pos.	*n*	Pos.	*n*	Pos.
Trichophyton rubrum	14	6 (42%)	18	1 (5%)	5	1	9	6 (66%)	46*	14 (30%)
									37[a]	8 (21%)
Epidermophyton floccosum	30	13 (43%)	2	1	0		0		32	14 (43%)
Trichophyton mentagrophytes	0		10	8 (80%)	1	1	0		11*	9 (81%)
T. rubrum and E. floccosum	1	1	0		1	0	0		2	1
Total	45	20 (44%)	30	10 (33%)	7	2 (28%)	9	6 (66%)	91	38 (41%)

[a] *T. rubrum* infections of skin (toe-nails excluded).
Statistical difference: * $p<0.01$ (significant).

were not further tested with higher concentrations of tuberculin. Patients with non-chronic dermatophytoses showed a somewhat higher frequency of positive tests than those with chronic infections (84 and 66%, respectively), but this difference is not statistically significant.

DISCUSSION

A simple clinical way of determining cell-mediated immunity is to apply a skin test with a reliable antigen. In previous studies (9, 10) it was shown that purified trichophytin provokes reproducible skin reactions, compared with a commercial antigen. It was suggested that differences in cell-mediated immunity were present, depending on the type of infecting dermatophyte and the localization of the infection, and that skin tests might reflect the immune status of an individual with dermatophytosis (10). In the present study, these findings were highlighted against a background of two different clinical types of infection, namely tinea cruris and tinea pedis. The main findings previously reported were reproduced.

Concerning the dermatophytes involved, their varying ability to stimulate cell-mediated reactions has been explained by the degrees of inflammation produced (12). Accordingly, *T. mentagrophytes* was the most potent sensitizer, producing sensitization in 81% of patients infected with this dermatophyte. *T. rubrum*, which produces infections having a mild clinical course, was the weakest sensitizer, with positive skin reactions in 30% of infected patients. *E. floccosum* occupies an intermediate position, with positivity in 44% of cases, a finding suggested in a previous study and now confirmed. As it is generally believed that the sensitizing capacity is correlated to cellular immune defence, it seems likely that the skin reactivity is of clinical importance when infections are caused by *T. mentagrophytes* and *E. floccosum*, but of less significance in *T. rubrum* infections.

The type of dermatophyte involved is only one part of the multifactorial background of clinical immunity. The localization of infection is another factor considered in this study. Differences in skin reactivity between tinea cruris and tinea pedis are not especially pronunced, as shown in Table IV However, when considering the groups in more detail, interesting differences can be seen. If the tinea pedis group is considered to be infected solely with *T. rubrum*, which is the commonest cause, only one positive delayed-type skin reaction remains (5%). However, if the same comparison is made with tinea cruris, considering only *E. floccosum* infections, positive skin reactions occur in 43% of cases. Thus, in the model cases of tinea cruris and tinea pedis, there are clearcut immunological differences as measured with the trichophytin skin test.

What is more surprising is that *T. rubrum* in the groin gave the same frequency of delayed-type skin reactions as *E. floccosum*. These results emphasize the importance of the site of infection when discussing sensitizing capacity. That the same dermatophyte species (*T. rubrum*) in two different localizations gave such diverse skin reactivities (5% and 43% respectively) cannot be explained by pure coincidence. The hypothesis previously offered that local skin factors might influence the degree of exposure of the sensitizing antigen to the immune system is one explanation (10). The penetration of antigen would, according to this assumption, occur easily in the groin with its thin moist skin, while it would be more difficult for the same dermatophytic antigen to penetrate through the thick skin of the feet. The penetration could also be enhanced by the presence of follicles in the groin region. In fact, the important role of hair and hair follicle invasion for the immune response has been pointed out recently (2, 6). Nevertheless, it is still possible for dermatophyte with a high sensitizing capacity to penetrate a thick barrier. This is exemplified in this study by *T. mentagrophytes* which stimulated cell-mediated reactions in 80% of patients with tinea pedis. Thus, the degree of cell-mediated response from a clinical standpoint is modified by at least two factors, namely the sensitizing capacity of the dermatophyte involved and the site of infection and by these two factors in combination.

The high frequency of delayed-type skin reactions in tinea pedis with toe-nail infection seems more difficult to understand. All cases except one were of the longstanding chronic type and the causative dermatophyte was *T. rubrum*. From a clinical point of view, nails offer specific problems concerning diagnosis and treatment. Moreover the immunological processes might operate under special conditions. The dermatophytes infiltrate the nail plate and remain there for long periods. This creates good conditions for antigenic com-

ponents to stimulate the immune system and may result in sensitization of the individual and a higher frequency of delayed-type skin reactivity. Nevertheless the nail matrix seems to be a poor site for an immunological defence reaction, thereby leaving the dermatophyte untouched by a cell-mediated immune response. Thus, a situation may be created with strong sensitization, as shown by positive delayed-type skin tests, while the cellular immune response may for some reason be inhibited and accordingly, the infection become chronic.

The central problem of interest, i.e. host resistance to infection in clinical immunological terms, is exemplified by comparison of the chronically infected subjects with non-chronically infected patients. During recent years, more and more evidence has pointed to defects in cell-mediated immunity as a cause of chronic infection (4, 7, 13). In previous studies, it was suggested that a partial immunological defect could explain the clinical findings (10). It was also shown that patients with chronic *T. rubrum* infections were skin anergic, yet reactive in vitro as measured with the lymphocyte stimulation test to the same antigen preparation (11). These findings supported the idea of some defect in the cell-mediated immune system. In the present study, cell-mediated immunity as gauged by trichophyton skin reactivity rarely occurred in chronically infected patients, while more than half of the non-chronically infected patients showed positive skin reactions, a difference which is statistically significant. The low frequency of delayed-type skin reactions in chronically infected patients may be the result of some suppressive factor modifying the immune response. But tuberculin reactivity showed no significant differences between chronically and non-chronically infected patients. The PPD findings indicate that the defect in cellmediated immunity may be specific for dermatophytes.

In conclusion, the factors of importance in stimulation of cell-mediated responses of clinical significance are the dermatophyte involved and the site of infection. The present study provides further evidence in support of the view that cell-mediated immunity plays an important part in host resistance to dermatophyte infections and that chronic infections may be explained by partial defects in the individual's cell-mediated immune system.

REFERENCES

1. Artis, W. M. & Jones, H. E.: The effect of human lymphokine on the growth of *Trichophyton mentagrophytes*. J Invest Dermatol *74:* 131, 1980.
2. Chittasobhon, N. & Smith, J. M. B.: The production of experimental dermatophyte lesions in guinea pigs. J Invest Dermatol *73:* 198, 1979.
3. Grappel, S. F., Bishop, C. T. & Blank, F.: Immunology of dermatophytes and dermatophytosis. Bacteriol Rev *38:* 222, 1974.
4. Hanifin, J. M., Ray, L. F. & Lobitz, W. C., Jr: Immunological reactivity in dermatophytosis. Br J Dermatol *90:* 1, 1974.
5. Helander, I.: Cell-mediated immunity in dermatophytosis. Thesis, Turku, Finland, 1975.
6. Hutton, R. D. & Kerbs, S.: Experimental *Trichophyton mentagrophytes* infection in hairless and haired dogs. Lab Anim Sci *28:* 216, 1978.
7. Jones, H. E., Reinhardt, J. H. & Rinaldi, M. G.: A clinical, mycological and immunological survey for dermatophytosis. Arch Dermatol *108:* 61, 1973.
8. — Immunologic susceptibility to chronic dermatophytosis. Arch Dermatol *110:* 213, 1974.
9. Kaaman, T., von Stedingk, L. V. & Wasserman, J.: An evaluation of delayed hypersensitivity in guinea pigs to different trichophytin preparations. Acta Dermatovener (Stockholm) *56:* 283, 1976.
10. Kaaman, T.: The clinical significance of cutaneous reactions to trichophytin in dermatophytosis. Acta Dermatovener (Stockholm) *58:* 139, 1978.
11. Kaaman, T., Petrini, B. & Wasserman, J.: *In vivo* and *in vitro* immune responses to trichophytin in dermatophytosis. Acta Dermatovener (Stockholm) *59:* 229, 1979.
12. Kerbs, S., Greenberg, J. H. & Jesrani, K.: Temporal correlation of lymphocyte blastogenesis, skin test responses and erythema during dermatophyte infections. Clin Exp Immunol *27:* 526–530, 1977.
13. Sorensen, G. W. & Jones, H. E.: Immediate and delayed hypersensitivity in chronic dermatophytosis. Arch Dermatol *112:* 40, 1976.

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