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CELL MEDIATED CROSS-REACTIVITY *IN VIVO* AND *IN VITRO* TO PURIFIED DERMATOPHYTE ANTIGEN PREPARATIONS IN SENSITIZED GUINEA PIGS

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Abstract. Guinea pigs were sensitized to either *Trichophyton rubrum*, *Trichophyton mentagrophytes*, or *Epidermophyton floccosum*. Cross-reactivity experiments were performed *in vivo* and *in vitro* with purified antigen preparations prepared from the respective dermatophyte according to the ethylene glycol method. All sensitized animals responded with delayed-type skin reactions to all three antigen preparations, although the strongest reactivity observed was to the homologous antigen. Spleen and blood lymphocytes from many (but not all) animals were stimulated by these antigens and the response to the homologous antigen was always more pronounced. The findings indicate the presence of a common group-specific dermatophyte antigen as well as the possibility of species-specific antigens in the experimental system studied.

Key words: Dermatophytes; Delayed hypersensitivity; Lymphocyte transformation; Skin test; Trichophytin

The dermatophytes of the genera *Trichophyton*, *Epidermophyton* and *Microsporon* are related, from a biological and clinical point of view, and their antigenic properties have been considered to overlap. This opinion has been partly substantiated by clinical use of trichophytin preparations from one dermatophyte species for skin test purposes. However, so-called polyvalent trichophytins produced from different dermatophytes have also been employed, but these do not permit of any conclusion as to the cross-reactivity of skin reactions. Furthermore, the results from certain serological investigations suggest the presence of species-specific antigens having few antigenic determinants in common (2, 5, 13).

From the clinical point of view it is of interest to establish whether the dermatophytes have common group-specific antigens to which cellular immunological reactivity is expressed. For this reason guinea pigs were immunized with various dermatophytes and cell-mediated responses to the respective antigen preparation were measured *in vivo* and *in vitro*.

MATERIAL AND METHODS

Preparation of antigens. Strains of *Trichophyton rubrum* (TR), *Trichophyton mentagrophytes asteroides* (TM) and *Epidermophyton floccosum* (EF) were isolated from active human skin lesions, cultured on Sabbouraud's glucose agar and identified according to standard criteria (14). Each dermatophyte strain was then cultured for 4 days on liquid medium and purified antigen preparations were obtained from the dried mycelia according to the ethylene–glycol method as described elsewhere (9). The protein content was measured *ad modum* Lowry et al. (12) using bovine serum albumin as the standard and showed the following values: TR 11%, TM 14% and EF 20% per dry weight.

Immunization of animals. Groups of white male guinea pigs were sensitized with saline suspension of the dried mycelia of TR, TM and EF in Freund's complete adjuvant, respectively. Details of the immunization have been published elsewhere (9).

Skin test. Groups of 5 sensitized guinea pigs were tested 6 weeks after sensitization with 0.1 ml of TR, TM, or EF antigen preparation (1 mg/ml) intracutaneously in the abdominal wall. The same volume of saline was given as a control (for details, see 9). Prior to the skin test, dermatophyte infestation was excluded by means of culture from the animals. Skin tests were read after 24 and 48 h; perpendicular diameters of the induration were measured and mean values calculated. Reactions with a mean diameter of at least 5 mm were considered to be positive. Five unsensitized guinea pigs served as a control group.

Lymphocyte stimulation test (LST). Groups of sensitized guinea pigs (12 to TR and EF respectively, 10 to TM) were sacrificed at 4–8 weeks and lymphocytes were collected from the spleen. Blood lymphocytes were collected from 7 guinea pigs sensitized to TR and EF respectively. LST was performed as described elsewhere (9, 10). Lymphocytes were incubated in Eagle's medium with 10% pooled guinea pig serum for 7 days. The antigen preparations were titrated in preliminary experiments and the concentrations giving maximum stimulation were used in the present experiments. Spleen lymphocytes from all

Table I. *Delayed-type cutaneous hypersensitivity to purified trichophytin antigen preparations (1 mg/ml)*

TR=*Trichophyton rubrum*, TM=*Trichophyton mentagrophytes*, EF=*Epidermophyton floccosum*, *M*=mean values, S.D.=standard deviation

Animal group and no.	Skin induration in mm at 24 h		
	TR	TM	EF
Sensitized to TR			
1	10	9	10.5
2	11.5	12	12
3	15	11	11.5
4	16	11.5	12
5	13	8	11
M	13.1	10.3	11.4
S.D.	2.45	1.71	0.65
Sensitized to TM			
1	10.5	12	12
2	7	10.5	11
3	8.5	10.5	8
4	10	13.5	14.5
5	8	11	9
M	8.8	11.5	10.9
S.D.	1.44	1.27	2.55
Sensitized to EF			
1	6.5	10	9.5
2	5.5	8.5	12
3	5.5	8	11.5
4	6.5	11.5	14.5
5	6.5	11.5	10
M	*6.1	9.9	*11.5
S.D.	0.54	1.63	1.96

* Statistically significant difference ($0.025>p>0.01$).

sensitized animals were exposed to all three antigens in two concentrations, whenever possible. LST experiments with blood lymphocytes were performed with TR and EF-sensitized animals only. The results were recorded as lymphocyte stimulation index (LSI) i.e. the ratio between counts per minute (cpm) obtained with and without anigen. Lymphocytes from 8 unsensitized guinea pigs served as controls.

Statistical analysis. The Friedman two-way analysis of variance by ranks was used and, when significant, multiple comparison procedure was employed (Table I). The results from the LSI experiments were evaluated by the Mann-Whitney test (3).

RESULTS

Intradermal test

The results of the skin tests are summarized in Table I. All sensitized animals reacted to the test antigens after 24 h with a significant cutaneous infiltration. The reactions persisted after 48 h. None of the controls reacted.

All guinea pigs sensitized with TR showed significant positive reactions to the purified TR antigen (Table I) as well as to the EF and TM antigen preparations. The mean diameter of the latter two reactions was somewhat smaller than that to TR but no statistically significant differences between the groups were registered. Guinea pigs sensitized to TM showed a pattern similar to TR-sensitized animals (Table I). The strongest reactions occurred to TM antigen but all TM-sensitized animals displayed positive reactions to both TR and EF antigen preparations although no statistically significant difference was registered. Also, in EF-sensitized group, all three antigen preparations elicited positive reactions (Table I). By analogy with the other sensitized groups of guinea pigs the EF-antigen preparation displayed the strongest reactivity, the mean diameter of skin indurations being almost twice as large as that obtained with TR antigen. A statistically significant difference was also present between the reactions to these two antigens ($0.025>p>0.01$).

Lymphocyte stimulation test (LST)

The results of the stimulation experiments are set out in Table II. The responses are expressed as means of lymphocyte stimulation indices (LSI). All mean LSI values for the optimal concentrations of the homologous and heterologous antigens (TR, TM and EF) in the three experimental groups exceeded one, whereas the corresponding indices in the control group were below that number. The differences between the sensitized and non-sensitized guinea pigs were statistically significant in most cases, i.e. LSI values were significantly higher in the sensitized groups. The highest LSI values were always registered with the homologous antigens. Within the sensitized groups, LSI varied substantially between the individual guinea pigs for the homologous as well as for the heterologous antigen. Fig. 1 gives an example of this variation in TR-sensitized animals.

Blood lymphocytes

The results of the stimulation experiments with TR-sensitized lymphocytes are shown in Fig. 2. EF-sensitized lymphocytes reacted correspondingly. Blood lymphocytes from TM-sensitized guinea pigs were not examined. The results are analogous to those obtained with spleen lymphocytes, i.e.

Table II. *Differences between LSI values (spleen lymphocytes) obtained with different trichophytin antigen preparations in sensitized and control guinea pigs, respectively*

LSI = Lymphocyte stimulation index. TR = *Trichophyton rubrum*. TM = *Trichophyton mentagrophytes*. EF = *Epidermophyton floccosum*. *n* = number of animals. *M* = mean LSI values. S.D. = standard deviation

	Antigen preparations					
	TR, μg/ml		TM, μg/ml		EF, μg/ml	
Guinea pigs	1	0.1	100	10	10	1
Sensitized to TR						
n	12	10	12	12	12	12
M	2.47	2.76	1.0	1.43	0.9	1.83
S.D.	1.80	2.04	0.6	0.83	0.76	1.69
P	<0.002	<0.002	<0.01	<0.01	NS	<0.002
Sensitized to TM						
n	7	5	10	10	10	7
M	0.97	1.16	1.46	2.39	1.48	1.47
S.D.	0.40	0.19	1.25	3.09	2.14	0.64
P	NS	<0.05	NS	<0.01	NS	<0.01
Sensitized to EF						
n	12	10	12	12	12	12
M	1.47	1.53	0.99	1.20	1.45	3.78
S.D.	1.14	1.18	0.65	0.71	1.51	3.61
P	<0.002	<0.05	<0.05	<0.05	<0.05	<0.002
Control animals						
n	6	6	8	8	6	6
M	0.63	0.88	0.44	0.57	0.29	0.65
S.D.	0.09	0.16	0.22	0.27	0.11	0.08

P = statistical difference between sensitized and control group $p<0.05$ statistically significant. NS = not significant.

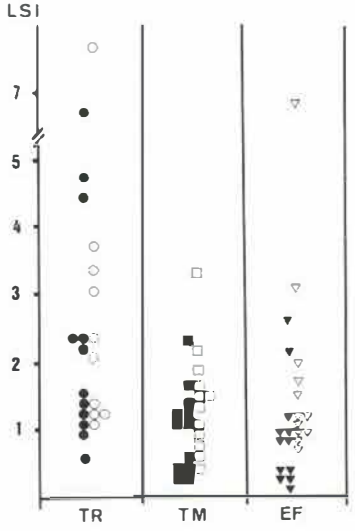

Fig. 1. Stimulation of spleen lymphocytes from sensitized guinea pigs in the presence of purified trichophytin antigen (expressed as LSI). TR = *T. rubrum* antigen preparation: ●, 1 μg/ml; ○, 0.1 μg/ml. TM = *T. mentagrophytes* antigen preparation: ■, 100 μg/ml; □, 10 μg/ml. EF = *E. floccosum* antigen preparation: ▼, 10 μg/ml; ▽, 1 μg/ml. LSI = Lymphocyte stimulation index.

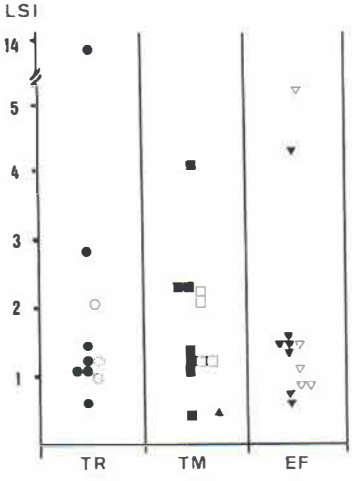

Fig. 2. Stimulation of blood lymphocytes from sensitized guinea pigs in the presence of purified trichophytin antigen (expressed as LSI). TR = *T. rubrum* antigen preparation: ●, 1 μg/ml; ○, 0.1 μg/ml. TM = *T. mentagrophytes* antigen preparation: ■, 100 μg/ml; □, 10 μg/ml. EF = *E. floccosum* antigen preparation: ▼, 10 μg/ml; ▽, 1 μg/ml. LSI = Lymphocyte stimulation index.

mean stimulation indices were higher than those in the control group.

DISCUSSION

The question of cross-reactivity between different dermatophyte species is still under debate. Results from most serological studies indicate the importance of species-specific dermatophyte antigens (2, 5). Investigations of cell-mediated reactivity, on the other hand, present a more complex picture. Most of these reports, especially on delayed-type skin reactions, favour the concept of group-specific dermatophyte antigens, i.e. of significant cross-reactivity (1, 4, 7). Some *in vitro* studies, however, have suggested important antigenic differences between dermatophyte species (15, 16). Furthermore, Keeny et al. reported species-specific reactions with serological methods and substantial cross-reactivity with skin test (11).

This conflicting evidence could possibly be explained by basic methodological differences between the above-mentioned investigations. In the first place, different antigen preparations were used, having properties which are not entirely comparable. Furthermore, the methods employed differed both qualitatively and quantitatively from each other. Such differences are highly relevant for measurements of immunological specificity. Finally, the degree of specificity that can be measured in delayed-type skin reactions cannot be compared to that observed with sensitive serological methods.

Our results must be interpreted against this background of facts and conflicting evidence. We found that animals sensitized to one of the three dermatophytes (TR, TM and EF) reacted positively to antigen preparations from all three dermatophytes, as assessed by skin test and LST. These results indicate cross-reactivity between the dermatophytes when measured with cell-mediated responses. The differences in the amplitude of reactivities suggest, on the other hand, the presence of additional species-specific antigens, although the reactivity to these antigens seemed to be rather weak in the experimental system studied.

The results of skin tests show that all animals reacted positively and that homologous antigen tended to elicit stronger skin reactivity. The lymphocyte stimulation experiments are rather more difficult to interpret. The activity varied between animals, some of them being negative, and was generally on a low level. The reason for this weak stimulability in LST, which was also reported by Hunjan et al. (8) is not known but it may be connected with some suppressive effect mediated by cells, T-suppressor lymphocytes, macrophages, or a suppressive factor released by these cells. It has recently been demonstrated that guinea pigs with experimental dermatophyte infection have inhibiting serum factors that depress blastogenic activity (6). It is possible that such factors are also present in dermatophyte-immunized animals. It can also be mentioned in this context that the dissociation between depressed lymphocyte stimulation and rather strong skin sensitivity, as seen in this study, is similar to that demonstrated by us in patients with *T. rubrum* infection (10).

In conclusion, it seems that the dermatophytes studied are antigenically related through a common main antigen. Possible species-specific antigen characteristics are indicated by different amplitudes in homo- and heterologous systems. Provided the present experimental conditions can be applied to humans it would be sufficient to use antigen preparations from one dermatophyte species for clinical testing purposes.

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