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Cutaneous Basophilic Hypersensitivity Response to Fungal Antigens in Guinea Pigs

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Guinea pigs infected with *Trichophyton mentagrophytes* developed a cutaneous fungal lesion and became skin test positive to fungal antigen (trichophytin). The cutaneous fungal lesion, while thought to be a cell-mediated response, differed histologically from the skin test site. Basophils were not demonstrated in biopsies of cutaneous fungal lesions, whereas basophils were numerous in biopsies of trichophytin skin test sites. When sensitization to trichophytin was accomplished by injection of hypha in complete Freund's adjuvant instead of infecting with live fungus, basophils could not be demonstrated in skin test sites. This report demonstrated that guinea pigs could be primed for cutaneous basophilic hypersensitivity (CBH) responses by infection with live fungus.

Guinea pigs experimentally infected with *Trichophyton mentagrophytes* develop inflammatory cutaneous fungal lesions, and also become sensitized to fungal skin test antigens (trichophytin) [1,2]. The host's hypersensitivity response was hypothesized to be responsible for the intense inflammatory response seen in the fungal lesions [3]. This hypothesis was supported by experiments in which a temporal correlation appeared between onset of intense inflammation in the lesion and conversion to positive skin test and lymphocyte transformation responses [4]. Therefore both fungal lesions and trichophytin skin tests are thought to be cell-mediated hypersensitivity responses.

Histological procedures which allowed basophils to be distinguished in biopsies of skin test sites have led to the description of a delayed inflammatory response called cutaneous basophilic hypersensitivity (CBH) [5–7]. This response differs from classical tuberculin type delayed hypersensitivity (DH) in several respects. CBH reactions are characterized by extensive infiltrations of basophils along with mononuclear cells, are relatively nonindurated, are often elicited only at early intervals after immunization, can be induced by immunization with antigens in incomplete Freund's adjuvant (IFA), and cannot be induced with immunization with antigens in complete Freund's adjuvant (CFA). It was not clear whether trichophytin skin test reactions were CBH or DH responses since earlier investigations did not employ procedures whereby basophils could be detected [8].

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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Abbreviations:
CBH:cutaneous basophilic hypersensitivity
DH:delayed hypersensitivity
IFA:incomplete Freund's adjuvant
CFA:complete Freund's adjuvant
PPD:purified protein derivative

The present study was undertaken to determine whether basophils were present in skin test sites elicited with trichophytin or in cutaneous fungal lesions elicited by *T. mentagrophytes*. Sensitization to trichophytin was accomplished by infection with live *T. mentagrophytes* spores or by immunization with CFA and hyphae. We hypothesized that animals with cutaneous fungal lesions had common antigens invoking the cell-mediated responses at skin test and fungal lesion sites, and therefore the histopathological appearances of these responses would be similar. We found that the histology of skin test sites and fungal lesion sites differed markedly, and that only the trichophytin skin test sites on infected animals were characteristic of CBH.

MATERIALS AND METHODS

Guinea Pig Infections

Chase-Moen guinea pigs (male or female) weighing 250–300 gm were used. These animals were bred in a closed colony at Letterman Army Institute of Research. Animals from this colony are skin test negative to trichophytin and culture negative for *T. mentagrophytes*. Only animals not previously skin tested were used in experiments. Infections were initiated with microconidiospores of *T. mentagrophytes* var. granulare ATCC 18748 by the method of Greenberg et al [2]. This consisted of spreading 100 spores on a shaved 3.8 cm^2 area of unabraded skin, covering the infection site with a moistened gauze pad, and then occluding the infection site. The occlusive materials were removed at the end of 3 days.

Antigens

Trichophytin was made following the procedure of Ottaviano et al [9] except that the dermatophyte was grown in a medium consisting of 4% glucose and 1% Pan Meade (Paines and Byrne, Greenford, England) rather than a defined medium. The final concentration of antigen was 100 μg/0.1 ml. Purified protein derivative (PPD) of tuberculin was purchased as the second strength (0.005 mg/0.1 ml) from Merck, Sharp, and Dohme.

Skin Testing

Antigens in 0.1 ml were injected intradermally with a 30-gauge needle at 3 sites in the parasacral area. Skin tests were observed and different sites were biopsied at 24, 48, or 72 hr.

Biopsies

Biopsies were all performed with a 6-mm dermal punch under 2% xylocaine anesthesia. The tissue was fixed and embedded according to the method of Dvorak et al [6] and stained with alkaline fuchsin. The cells directly beneath the epidermal-dermal junction were counted with the use of an oil immersion lens on the microscope. All cells in each adjoining field were examined until 250 cells were counted.

Immunization

Guinea pigs were injected in each rear foot pad with 0.05 ml of a mixture of equal parts CFA (Difco) and lyophilized hyphae (3 mg in 0.1 ml saline). The hyphae were obtained from a 14 day old culture of *T. mentagrophytes*.

RESULTS

Biopsies of Skin Test Sites in Infected Animals

Eleven guinea pigs were infected with *T. mentagrophytes*, and the infection was allowed to progress through its natural course. These infected animals were divided into 3 groups and

each group was skin tested in 3 sites with trichophytin. One group of 3 animals was skin tested when the lesions were first barely visible (day 7 postinfection), and another group of 4 animals was skin tested after lesions were present (2 animals on day 13 and 2 animals on day 21 postinfection). Individual skin test sites were biopsed at 24, 48, or 72 hr after skin testing. The last group of 4 animals was skin tested after the lesions had healed (2 animals on day 28 and 2 animals on day 75 post infection), and skin test sites were biopsied 48 hours after skin testing.

Trichophytin skin tests were negative when lesions were first barely visible (day 7) and converted to positive responses after the lesions developed. Positive skin test responses were also elicited in animals with lesions which had healed (Table I).

Erythema developed within 1 hr after skin testing with trichophytin and peaked at 24 hr. The erythema decreased in size and intensity after 24 hr but was usually still visible at 72 hr.

It was noted that when trichophytin skin tests were negative, the cellular infiltrate contained few basophils (Table I). When trichophytin skin tests became positive (when fungal lesions were present), there was a marked increase in the number of basophils present in the infiltrate at 24, 48, and 72 hr after skin testing (Table I). The CBH response was not a transient phenomenon since it could still be elicited 75 days postinfection.

Biopsies of Fungal Lesions

Twenty-five animals were infected with *T. mentagrophytes*. The fungal lesions followed the clinical course previously described [2]. Animals were divided into 4 groups at intervals after infection when lesions were described as (1) slightly erythematous (day 7 postinfection), (2) erythematous and scaling (days 10–13 postinfection), (3) crusting (days 14–18 postinfection) and (4) healing (days 21–24 postinfection). Biopsies of the fungal lesions were taken and examined for the presence of basophils.

Basophils were not present in the dermal infiltrate at the site of the fungal lesion at any interval after infection. The results are presented in Table II.

Biopsies of Immunized Group

Four guinea pigs were immunized with hyphae in CFA. Animals were skin tested at 3 sites with trichophytin and 3 sites with PPD 3 weeks after immunization. Biopsies were taken at 24, 48, and 72 hr. Only an occasional basophil was present in

TABLE I. *Types of cells in trichophytin skin test sites elicited in guinea pigs infected with* T. mentagrophytes

Description of fungal lesion	No. of animals	Description of skin test	Hr after skin test	Mean No. of oil immersion fields counted	Differential cell counts in Dermal Infiltrate of skin test sites[a]			
					% Mononuclear	% Polymorphonuclear	% Mast	% Basophils
Lesion barely visible[b]	3	negative[c]	24	5.5	90 ± 6	8 ± 6	0	1 ± 1
			48	4.5	95 ± 5	1 ± 0	2 ± 1	2 ± 2
			72	5.5	95 ± 4	1 ± 0	0	2 ± 1
Lesion present[b]	4	positive[c]	24	3.2	75 ± 7	6 ± 5	1 ± 1	18 ± 8
			48	3.5	77 ± 11	3 ± 1	1 ± 1	21 ± 9
			72	4.0	81 ± 9	4 ± 3	0	15 ± 10
Lesion healed[b]	4	positive[c]	48	3.3	74 ± 9	2 ± 1	0	24 ± 7

[a] Results expressed as a percentage ± SD (250 cells were counted in each biopsy).

[b] The lesions were barely visible on day 7, and 3 animals were skin tested on that day. Lesions were present on days 13 and 21, and 2 animals on each day were skin tested. Lesions were healed on days 28 and 75, and 2 animals on each day were skin tested. Since sample sizes were small and results were comparable, the data was averaged for days 13 and 21 and also for days 28 and 75.

[c] Negative skin test denotes no erythema or induration. Positive skin test denotes marked erythema (greater than 10 mm in diameter) and no induration.

TABLE II. *Types of cells in fungal lesions*

Description of fungal lesion[b]	No. of animals	Mean No. of oil immersion fields counted	Differential Cell Counts in Dermal Infiltrate of Fungal Lesion[a]			
			% Mononuclear cells	% Polymorphonuclear cells	% Mast Cells	% Basophils
Slight erythema	3	4.5	92 ± 7	5 ± 2	0	1 ± 2
Marked erythema and scaling	8	4.0	99 ± 1	1 ± 1	1 ± 1	0
Crusting	10	4.0	85 ± 7	15 ± 6	0	0
Healing	4	4.2	97 ± 4	3 ± 4	0	1 ± 1

[a] Results expressed as a percentage ± SD (250 cells were counted in each biopsy).

[b] Slight erythema was visible on day 7. Marked erythema and scaling occurred from days 10–13, crusting occurred from days 14–18, and healing occurred from days 21–24 post infection. On each of these days, 2 animals were skin tested. The results are presented as the mean of each group.

TABLE III. *Types of cells in skin test sites elicited in guinea pigs immunized with hyphae in CFA*[a]

Skin test antigen	Description of skin test	Hr after skin test	Mean No. of oil immersion fields counted	Differential Cell Counts in Dermal Infiltrate of skin test site[b]			
				% Mononuclear Cells	% Polymorphonuclear cells	% Mast cells	% Basophils
Trichophytin	positive	24	4.0	93 ± 3	2 ± 2	0	4 ± 1
		48	4.0	95 ± 2	2 ± 2	0	3 ± 3
		72	5.2	93 ± 2	1 ± 1	0	5 ± 3
PPD[c]	positive	24	3.0	89 ± 8	8 ± 8	0	2 ± 1
		48	3.2	92 ± 7	4 ± 2	0	5 ± 5
		72	3.2	98 ± 2	1 ± 1	0	1 ± 1

[a] Four animals were skin tested 3 weeks after immunization.

[b] Results expressed as a percentage ± standard deviation (250 cells were counted in each biopsy).

[c] Purified protein derivative of tuberculin.

the dermal infiltrate of either the trichophytin or PPD skin test sites. The results are presented in Table III.

DISCUSSION

Guinea pigs experimentally infected with *T. mentagrophytes* develop cutaneous fungal lesions, and they have positive skin test reactions to trichophytin. In this paper, we demonstrated that these infected animals reacted to skin testing with trichophytin in a manner that was compatible with the description of cutaneous basophilic hypersensitivity [7]. In contrast, the animals sensitized by immunization with hyphae and CFA responded to trichophytin skin testing with a classical PPD delayed type hypersensitivity response.

Basophils could not be demonstrated in the dermal infiltrate at the sites of the cutaneous fungal lesions. Either basophils were not attracted to the fungal lesion sites, or they degranulated and could not be recognized. Degranulation could have been caused by the overwhelming amount of fungus seen in the lesions [10]. This idea of degranulation of the basophils is supported by the findings of DeBernarda et al [11] who demonstrated that the number of recognizable basophils decreased in CBH reaction sites 30 min after injection of additional antigen. Degranulation of basophils was also shown to occur in cutaneous *Candida albicans* infections in guinea pigs if lesions were biopsied later than 24 hr after infection [12].

Our results may not be entirely in agreement with results reported for cutaneous *Candida albicans* infections in guinea pigs [12]. Basophils were seen in the lesions caused by candida, but were not visible in our lesions caused by *T. mentagrophytes*. As already stated, however, the basophils may have degranulated prior to biopsy in our study. Basophils were not present in candida skin test sites of nonimmune animals (immune animals were not tested), while basophils were seen in our trichophytin skin test-positive sites. The candida-infected animals were biopsied before cell-mediated immunity could develop, while our *T. mentagrophytes* infected animals were biopsied before as well as after cell-mediated immunity developed. We saw basophils only after cell-mediated immunity developed. Therefore the apparent differences in the results of these 2 studies can probably be explained by differences in methodology.

Our guinea pigs infected with *T. metagrophytes* had persistent CBH responses. CBH responses have been shown to persist if significant antibody responses are not initiated [7]. Investigators do not agree on the nature of the antibody response in dermatophyte infected animals [1]. Part of the problem in studying antibody responses to fungal infections arises from the absence of specificity with fungal antigens. We have performed ring precipitation tests with trichophytin on sera of infected and control guinea pigs and found positive responses in both groups (unpublished data); therefore, we cannot determine whether or not the persistent CBH response was associated with the lack of significant antibody production.

Animals are often primed for CBH responses at skin test sites by immunization [7]. However, investigators have shown that guinea pigs can be primed for CBH responses at skin test sites after infection with vaccinia virus, or at the site of a lesion caused by *Candida albicans* [12, 13]. Our study is therefore one of the few reports demonstrating that a microbial infection can prime an animal for a CBH skin test response.

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