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Epidermal Cell Proliferation in Guinea Pigs with Experimental Dermatophytosis

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To elucidate the mechanisms underlying the self-healing process of experimental dermatophytosis produced in guinea pigs by an occlusive method with *Trichophyton mentagrophytes*, epidermal proliferative activity was evaluated by the in vivo tritiated thymidine-labeling technique performed at various intervals after the first and second infections. Determination of labeling indices disclosed that an increased epidermal proliferation correlated well with the severity of inflammatory changes, i.e., a peak activity was noted after 10 days in primary infection and at 2 days in reinfection, respectively, and was followed by subsequent spontaneous lesion clearance after 10 days. Application of a heat-killed spore suspension produced inflammatory changes with enhanced epidermopoiesis, similar to those induced by reinoculation of living spores, only in immune animals. The present results indicate that the dermatitic changes occurring in experimental dermatophytosis increase epidermopoiesis which facilitates elimination of the fungus from the stratum corneum and that host immune activity, particularly contact sensitivity to fungal antigen, exerts a crucial role to induce these changes.

In superficial fungal infections such as dermatophytosis and candidiasis, the causative fungi exist only in the stratum corneum and release various substances to induce the characteristic dermatitic changes. Their mode of growth, occurring predominantly in the nonviable portion of the skin, makes the primary body defense consisting of phagocytosis and killing of the causative organisms by the phagocytic system rather inefficient in their eradication. In experimental candidiasis of guinea pigs, Sohnle and Kirkpatrick [1] demonstrated that delayed type hypersensitivity to *Candida albicans* provokes increased epidermal proliferation which facilitates the elimination of the causative organisms from the skin with resultant desquamation. No such studies have been carried out in dermatophytosis in regard to the self-limiting clinical course.

The experimental dermatophytosis of guinea pigs induced by an occlusive method provides a reproducible self-healing model infection [2]. In such infected guinea pigs and also in human patients, it is possible to demonstrate contact sensitivity against trichophytin, the antigen released by dermatophytes. The trichophytin contact sensitivity plays an important role in aggravating dermatitic changes that is presumed to result in augmentation of epidermopoiesis [2,3]. Furthermore, transepidermal leukocyte chemotaxis toward the fungus-laden horny layer also occurs [4]. Using the model of experimental dermatophytosis of guinea pigs, we have shown that the leukocyte chemotaxis occurs due to complement activation as well as to release of fungus-derived chemotactic factors. Complement is activated by fungal components only through the alternative pathway in nonimmune animals, whereas complement activation occurs via both alternative and classic pathways in immune animals [4,5].

In the present study, using the infection model of self-limiting experimental dermatophytosis of guinea pigs, we have analyzed a temporal pattern of epidermal proliferation that facilitates the elimination of the causative organisms from the skin by resultant desquamation in both nonimmune and immune animals. We have studied the correlation between epidermopoiesis and inflammation by paying special attention to contact sensitivity to dermatophytes. Our results indicate that enhanced epidermal proliferation that occurs concurrently with inflammation plays an important role in lesion clearance and that the severity of inflammation is greatly influenced by the host immune state.

MATERIALS AND METHODS

Animals and Infection

Thirty female albino guinea pigs (Hartely strain) weighing 300–500 g were used. They were infected with a zoophilic strain of *T. mentagrophytes* under occlusive dressings using the inoculation technique reported previously [2]. Briefly, a spore suspension was applied to the manually depilated inoculation site on the back at a concentration of 20,000 spores/cm^2 under occlusion for 24 h. This produced reproducible self-healing infection with a lesion covering the entire inoculation site. To detect the presence of contact sensitivity to the dermatophyte the animals were also inoculated simultaneously with a heat-killed spore suspension, which was prepared by autoclaving at 120°C for 15 min, at a site symmetric to that inoculated with live spores. The severity of the inflammatory changes of the produced lesions were grossly graded on a subjective scale of 1 to 4 in the following manner: 1, weak erythematous spotty lesions; 2, diffuse erythematous lesions with or without scales; 3, infiltrated erythematous scaly-crusty lesions; 4, angry inflammatory lesions with serious weeping or thick crusts.

The animals, once recovered from the first infection, i.e., immune animals, were reinoculated in the same way 1 month later.

Trichophytin Skin Test

The test was performed in 3 animals every day by injecting 0.1 ml of 0.01% purified trichophytin intradermally and observing the response at 24 h, as described previously [2], until the development of positive reactions. Uninfected animals showed no delayed-hypersensitivity reactions.

Autoradiography

The tritiated thymidine autoradiographic study was performed in normal skin before inoculation in 8 animals as a control, and in lesional skin of at least 3 animals at 2, 7, 11, 14, 17, and 26 days after the first inoculation and at 1, 2, 4, 7, 12, and 17 days after reinoculation. The animals were injected with 0.1 ml tritiated thymidine (New England Nuclear, 1000 μCi/ml, 23.7 Ci/mol) intradermally. Three-millimeter skin punch biopsies were taken from the sites 1 h later. The specimens were prepared for autoradiography as reported previously [5]. A cell was considered labeled if it had more than 7 grains over the nucleus and the results were expressed as the number of labeled epidermal cells per 1000 basal cells (labeling index, LI). All basal cells on a section were counted except for those associated with hair follicles.

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Abbreviations:
LI: labeling index

RESULTS

Clinical Changes in Experimental T. mentagrophytes *Infection*

Clinical courses of *T. mentagrophytes* infection were very similar in each animal, as shown schematically in Fig 1. The inoculation site first became erythematous at 1–2 days after infection and the intensity of erythema increased gradually until 8 days, at which time, almost concurrently with the demonstrability of delayed-hypersensitivity reactions to trichophytin in every animal, the lesions became infiltrated suddenly and covered by thick silvery scales. Climax inflammatory changes were observed between 9–14 days, after which, sloughing of the crusty scales with massive fungal elements occurred and healing began to take place. By week 4 the lesions were replaced by smooth alopecic scar.

The animals that had recovered from the primary infection were reinoculated with *T. mentagrophytes* in the same way. They showed a more rapid onset of inflammatory changes, which were observable even at the time of removal of the occlusion after 24 h, reached a peak within 2 days, and began to subside 4 days after infection. The infection was completed within 10 days.

An autoclave-killed spore suspension did not produce any changes on the skin of inexperienced animals. However, it induced an erythematous reaction identical to that produced by the living spore suspension on the skin of the immune animals with positive trichophytin test reactions, at the time of removal of the occlusion. Although the inflammatory changes were similar to those noted with inoculation of the living spores until 3 days postinoculation, resolution of the lesions took place more rapidly at the inoculation site of the heat-killed spore suspension, being almost completed after 8 days.

Labeling Indices of the Epidermis

In the epidermis of the control skin only a small number of basal cells showed uptake of thymidine (Fig 2). As shown in Fig 3, the time course of LI in infected skin closely paralleled the grade of inflammatory changes. The LI increased in the initial stage slowly but later rapidly after the first inoculation, reaching a maximum at 11 days when the thickness of the epidermis was also at the maximum (Fig 2), and thereafter began to decrease gradually. However the epidermis studied in the clinically alopecic scar at 26 days postinfection was still

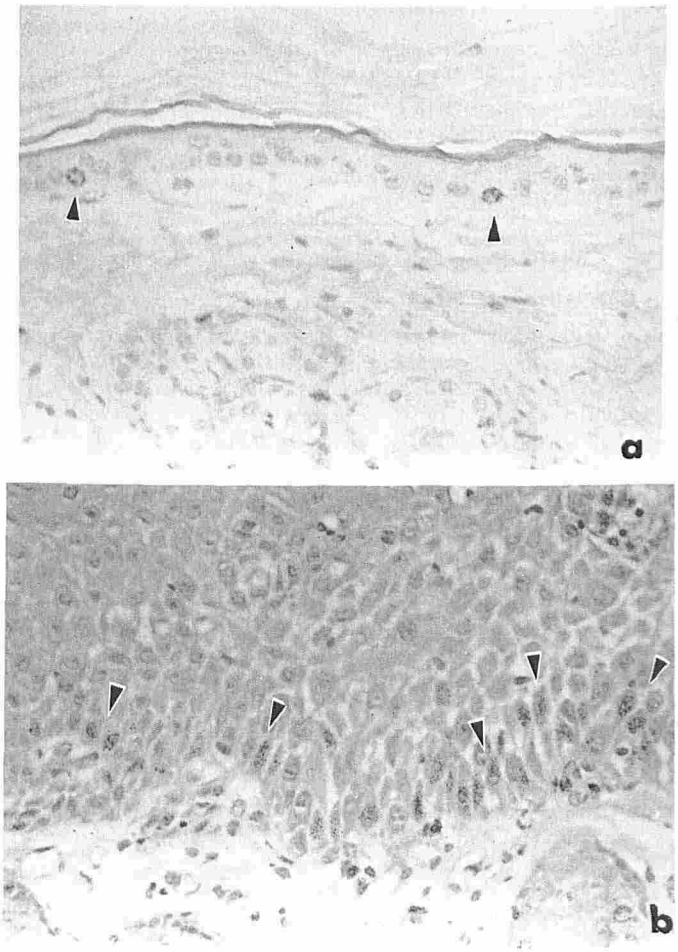

FIG 2. *a*, Autoradiographic preparation of normal guinea pig skin with a few epidermal basal cells labeling with tritiated thymidine (*arrowheads*). *b*, lesion 14 days postinfection now showing a large number of basal or suprabasal cells (*arrowheads*) in a highly acanthotic epidermis incorporating tritiated thymidine (H & E, × 400).

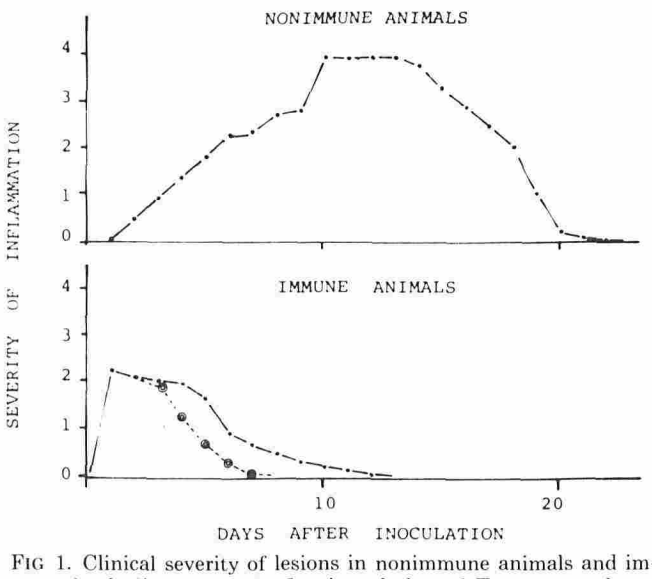

FIG 1. Clinical severity of lesions in nonimmune animals and immune animals (*lower graph*) after inoculation of *T. mentagrophytes*. *Dotted line with double circles in the lower graph* denotes the lesions induced by the application of heat-killed spores in immune animals.

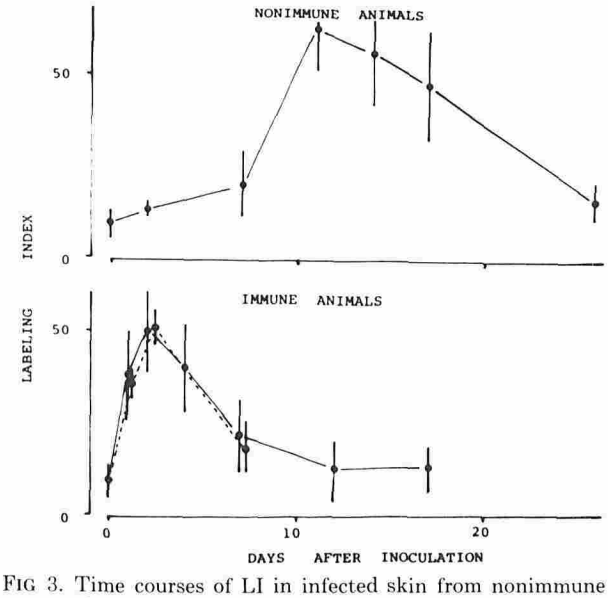

FIG 3. Time courses of LI in infected skin from nonimmune and immune animals. Each point represents mean and standard deviation. *Dotted line with double circles* shows LI in the skin lesions induced by inoculation of the autoclave-killed spore suspension.

highly acanthotic and showed a significant higher level than that of the controls ($p < 0.02$); histologically there was still a moderate inflammatory cell infiltration in the dermis.

In contrast in nonimmune animals, the LI in immune animals that recovered from the first infection, showed a rapid increase which reached a maximum at 2 days postinfection. It remained at significantly higher levels than that of the controls until 7 days and gradually returned to normal. LI at the inoculation site of the heat-killed suspension also showed a similar pattern of change as noted in the lesions produced by the living spores.

DISCUSSION

We demonstrated that accelerated epidermopoiesis in the skin of guinea pigs infected by *T. mentagrophytes* correlates well with the severity of inflammatory reactions and that within 10 days of the peak of the dermatitic changes spontaneous regression of the lesions occurs; the severity of the inflammatory changes is variable depending upon the immunologic state of the host. Therefore, it is reasonable to presume that the enhanced epidermopoiesis gives rise to the self-limiting course of the infection, since elevated LI, which is generally considered proportional to increased epidermal turnover [6], facilitates elimination of the organism with resultant desquamation. In studies of experimental *Trichophyton verrucosum* infections in cattle, Lepper and Anger [7] noted that its epidermal turnover time was reduced to 12 days in reinoculated areas as compared with 18 days in normal skin.

Our time course study in experimental dermatophytosis showed that the epidermal proliferative activity correlated well with the severity of inflammation. In the lesions of tinea corporis of human patients the most inflammatory portion is always noted along the eczematous ring that advances peripherally, pursuing a part that still retains fungus-laden stratum corneum. In their spatial study of the same lesions of tinea corporis, Berk et al [8] found that LIs at the rim were much higher than those elsewhere in the lesions and of normal skin. In contrast to such annular and rather resistant lesions noted in tinea corporis of human patients, those of the experimental infection in guinea pigs are always uniformly inflammatory and self-healing. Possibly the horny layer of the guinea pigs, which is much thinner, more fragile, and more functionally deficient than that of humans [9], allows quicker penetration of toxic and allergic materials into the skin which elicits inflammatory changes more efficiently with subsequent desquamation of the whole fungus-laden stratum corneum. The fact that, despite the topographic closeness to the prevalent areas such as the groin, it is rare for dermatophytosis to occur in the genitalia of humans where the horny layer is very thin may also be explained by analogy.

Contact allergen that permeates through the stratum corneum is taken up by the intraepidermal antigen-presenting cells, i.e., Langerhans cells, to be presented to T lymphocytes for the development of contact sensitivity. Langerhans cells are reported to be more efficient in inducing T-cell responses to trichophytin than are macrophages [10]. We observed that the lesional skin of the nonimmune animals began to show highly inflammatory changes associated with a remarkably elevated LI, concurrently with the development of immunoreactivity 8 days postinfection. Moreover, it is noteworthy that even the heat-killed spore suspension induces exactly the same rapidly developing erythematous response as that produced by the live spores in immune animals, reflecting their contact sensitivity to trichophytin [2,11]; the subsequent clinical course of such lesions is somewhat shorter than that of the actual infection, probably because of the lack of fungal growth. These findings provide evidence to support the concept that the contact sensitivity to fungal antigens plays an important role in the production of markedly inflammatory changes as well as the spontaneous regression noted in dermatophytosis [2,3].

Since exudation of serum in the dermatitic lesions takes place, ensuing complement activation with resultant leukocyte chemotaxis will further augment these changes [4,5]. However, it is unlikely that the alternative pathway activation evokable even in "virgin" infections plays the role of the initiator of the inflammation, since the application of heat-killed spore suspension produced no changes in nonimmune animals.

In experimental cutaneous candidiasis of guinea pigs, another common inflammatory superficial fungal infection of the skin, Sohnle and Kirkpatrick [1] observed much higher LIs in the skin of immune animals than in nonimmune animals throughout the time period of infection, which lasted over 10 days in both groups.

It is of interest that the skin that appeared to have already recovered from the primary infection still showed acanthotic epidermis associated with much higher LI than that of the control skin as well as a moderate inflammatory cell infiltrate in the dermis. This is probably due to prolonged local immune reactions that persist in the dermis long after elimination of the causative fungus from the skin. This may also be related to the localized nature of resistance to dermatophyte aggression during second-set response [12].

In conclusion, both irritant dermatitis induced by toxic substances derived from dermatophytes, in association with transepidermal leukocyte chemotaxis due to complement activation, and contact sensitivity reactions to fungal antigens all together exert their effects to increase epidermopoiesis that results in rapid elimination of causative organisms from the skin.

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