Effect of Hair Growth Cycles on Experimental Cutaneous Candidiasis in Mice

Peter G. Sohnle, M.D., Cathleen Collins-Lech, B.A., and Beth Hahn, B.S.
Department of Medicine, Medical College of Wisconsin, Milwaukee, and the Medical and Research Services, Veterans Administration Medical Center, Milwaukee, Wisconsin, U.S.A.

Experimental cutaneous Candida albicans infections were produced in mice by inoculating the organisms onto areas of shaved flank skin where the hair follicles were in either the anagen (growing) or telogen (resting) phase of the growth cycle. Infection with Candida occurred in a majority of animals inoculated on either anagen or telogen skin, and the rate of clearance of the organisms was equivalent for infections on the 2 types of skin. Some of the animals inoculated on anagen skin developed foci of Candida infection in the well-developed hair follicles, below the skin surface. Deep foci of infection were not found after inoculation of the telogen areas. The infections resulted in increases in epidermal thickness and sensitization of the animals to Candida antigens, but these responses were not different between animals inoculated on the 2 types of skin. The results of these experiments indicate that although Candida albicans can infect skin containing either active or resting hair follicles, foci of infection below the skin surface occur only when well-developed hair follicles are present. These findings may have relevance to the consequences of human cutaneous candidiasis. J Invest Dermatol 86:556–559, 1986

Candida albicans is a significant cause of cutaneous infections in humans. Histologic examination of naturally occurring cutaneous Candida infections in humans or of experimental cutaneous candidiasis in animals almost always shows the infection to be confined to the stratum corneum or upper epidermal cell layers [1,2]. Acute cutaneous Candida infections in both humans and animals characterize as their most characteristic histologic feature epidermal microabscesses containing neutrophils almost exclusively [1–4]. Since experimental cutaneous Candida infections may begin in the recesses around hair follicles [1], the stage of growth of the hair follicles could be important in this type of infection.

The growth of hairs in their follicles has been well described [5] and appears to be important in other cutaneous mycoses, particularly dermatophytosis. The first phase of the cycle (anagen) is characterized by rapid growth of the hair and a deep, well-formed hair follicle. At the end of this period is a short transitional period (catagen) in which the follicle is changed into a resting structure. During the next period (telogen), the follicle is inactive and hair growth does not occur. Since other changes, including thickening of the dermis and alterations of metabolic functions also occur in the surrounding skin, the term skin cycles has also been used to define these events [6]. There is a significant difference in the pattern of hair growth in humans as compared with that in certain animals such as rats, hamsters, and mice; in these animal species, hair growth occurs in waves so that all of the follicles in a particular area will be in the same stage; in humans, each follicle goes through its cycle independently of the neighboring follicles [5]. Therefore, animals such as mice can be used to assess the effect of hair growth cycles on particular cutaneous conditions since areas of the skin can be found in which all of the follicles will be in a single stage.

The stage of hair growth has been found to be very important in experimental dermatophytosis. Mice are susceptible to experimental inoculation with dermatophytic fungi only during the latter part of the anagen phase [7]. If inoculation is attempted on an area of skin where the follicles are in a different phase (such as telogen), the infection will not develop. Since only a relatively small area of skin in mice will be in the late anagen stage, these animals are not very susceptible to this group of pathogens; this characteristic may explain why early workers found mice to be relatively resistant to these infections [7]. Cutaneous candidiasis might be expected to differ from dermatophytosis with respect to the importance of the hair growth cycle since actual infection of the hair itself is not a prominent feature of the former infection. The present study was designed to use a mouse model of cutaneous candidiasis in order to assess the effect of the hair growth cycle on the development and course of this infection.

MATERIALS AND METHODS

Animals C57BL/6 mice, obtained from Harlan (Indianapolis, Indiana), were used in all of these experiments. The mice were housed in the Milwaukee VA Medical Center Animal Research Facility, which is fully approved by the American Association for Accreditation of Laboratory Animal Care. Female mice, 8–13 weeks old, were used in all experiments.

Organisms C. albicans was obtained as a clinical isolate and cultured for use in Sabouraud’s dextrose broth at 30°C for 70 h with constant stirring. Yeast phase organisms were harvested by centrifugation and washed with saline before use. The strain of C. albicans used for these studies is that which we have employed for previous work in the guinea pig model of cutaneous candidiasis [1,3,8].

Cutaneous Infections A shaved area of flank skin approximately 2 × 3 cm was inoculated directly with a paste containing approximately 2.5 × 10⁶ washed C. albicans yeast, which was...
rubbed into the skin with a cotton-tipped swab until the organisms were no longer grossly visible. No occlusive dressings were used. In some experiments, control sites on the opposite flank were inoculated with saline.

Areas of the skin in particular hair growth cycles were determined by their appearance after shaving. In black strains of mice such as the C57BL/6 animals used in these studies, anagen skin appears black, whereas telogen or catagen skin appears white [7]. For the purposes of these studies, no effort was made to separate telogen from catagen skin (these areas being referred to subsequently as telogen), or early from late anagen. Hair growth was measured in anagen and telogen areas by first shaving the skin of the proper location and then removing a sample of the previously shaved skin 2 weeks later from sacrificed mice; the skin samples were cut into strips approximately 1 mm thick and 1 cm long and mounted in water under a coverslip on a microscope slide. The length of the hair was measured with either an ocular micrometer under a microscope or with a ruler (when the hair was sufficiently long).

Clearance of the Infection Clearance of the infection was evaluated by removing periodically (generally every other day) from anesthetized mice 2 superficial pieces of skin approximately 2 × 3 mm each and culturing them on mycose l agar plates (Baltimore Biological Laboratories, Cockeysville, Maryland). The cultures were observed after 4 days of incubation for the presence of Candida colonies. The infection was considered cleared when both pieces of skin gave negative results.

Histology Skin samples removed from mice sacrificed at 1, 3, and 5 days post inoculation were fixed in 10% buffered formalin (pH 7.0). The tissue was processed for routine histology using standard procedures and stained with periodic acid-Schiff stain. An animal was considered to have become infected after inoculation if pseudohyphae could be seen in the stratum corneum or epidermal cell layers when the sections were examined by light microscopy. As a measure of epidermal proliferation, the thickness of the epidermal cell layer was determined by taking 6–10 measurements in a blind fashion with an ocular micrometer along a 1-cm length of skin. The stratum corneum and the epidermal microabscesses themselves were not included. Comparisons were made to skin sections from the opposite flank that had been inoculated only with saline. The depth of hair follicles was compared for anagen and telogen skin by measuring with an ocular micrometer the distance between the stratum corneum and the deepest part of the hair follicle at the center of each consecutive field along the section.

Measurement of Delayed Hypersensitivity in the Footpad One lot of heat-killed C. albicans yeast, which was kept frozen at -20°C, was used as the antigen in these experiments. Mice inoculated 14 days previously on either antigen or telogen areas were tested for delayed hypersensitivity by injecting 107 heat-killed organisms in 0.05 ml of saline into the right footpad and an equivalent volume of saline into the left footpad. In preliminary experiments, this number of Candida had been determined to cause no significant footpad swelling in unimmunized mice. Twenty-four hours after injection, the thickness of the feet was measured with a spring-loaded micrometer (Dyer Co., Lancaster, Pennsylvania), and the difference in swelling between the right and left feet was determined. A difference of 0.2 mm was considered a positive result in this test, based on past experiments with immunized and unimmunized mice. In previous experiments, we have evaluated animals at 4 h to check for possible Arthus reactions, but none was found.

Statistical Analysis Differences in hair growth, depth of hair follicles, epidermal thickness, and footpad responses were evaluated using the unpaired t-test. Comparison of the number of deep foci at 3 and 5 days between anagen and telogen areas and clearance of the infection at day 7 were made using the χ² test.

Table I. Growth of Hair in Anagen and Telogen Areas

<table>
<thead>
<tr>
<th></th>
<th>Anagen</th>
<th>Telogen</th>
<th>Statistical Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair growth</td>
<td>4.75 ± 0.17</td>
<td>0.70 ± 0.03</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Depth of hair</td>
<td>0.54 ± 0.04</td>
<td>0.22 ± 0.01</td>
<td>p &lt; 0.001</td>
</tr>
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</table>

*Statistical significance by the χ² test (NS = not significant).

Values represent regrowth of hair on the flank in mm 2 weeks after shaving (mean ± SE of data from 6 animals).

Values represent depth of hair follicles below the stratum corneum in mm (mean ± SE of data from 5 animals).

RESULTS

Characteristics of Anagen and Telogen Skin As shown in Table I, hair growth was much greater in anagen areas 2 weeks after shaving than it was in telogen areas. Hair growth moves in waves along the skin of mice and the patches of black skin (anagen) could be seen to move posteriorly after the course of 3–4 weeks. Microscopically, anagen skin was characterized by deep, fully formed hair follicles and a significantly thicker dermis than the telogen skin which had much smaller and shallower hair follicles (Table I).

Course of Experimental Cutaneous Candidiasis in Anagen and Telogen Areas Table II shows the number of animals inoculated on either type of skin which actually developed cutaneous candidiasis as judged by the presence of pseudohyphae invading the stratum corneum or epidermal cell layers. Most of the animals inoculated on either anagen or telogen skin did develop infection of the skin with Candida. Also shown in this table is the rate of clearance from the 2 types of skin, which was approximately equivalent.

One characteristic we have noted previously in this experimental model is that when telogen skin is inoculated, the infecting organisms become relocated to the surface of the skin by 3 days after inoculation [9]. As can be seen in Table III, a histologic examination of skin from anagen or telogen areas 3 or 5 days after inoculation revealed the presence of deep Candida foci in the anagen areas only. An example of one of these foci is shown in Fig 1. In each case, these foci were associated with and confined to the well-developed hair follicles of the anagen skin. In sections of inoculated anagen skin from 10 animals, 9 of these foci were found (in 4 individual animals) and 5 of these foci had an associated neutrophil infiltrate (Table III). No deep foci were seen in sections of anagen skin examined 14 days after inoculation, indicating that they had disappeared by that time.

Induction of Epidermal Proliferation by Candidiasis in Anagen and Telogen Skin Table IV shows the thickness of the epidermal cell layer (as a measure of epidermal proliferation) in anagen and telogen skin inoculated with Candida yast or with saline alone. By day 3, the epidermal cell layer had increased in thickness by more than 2-fold in the sites inoculated with the organisms, whereas inoculation with saline had little effect. There were no significant differences between anagen or telogen skin in this type of response. On microscopic examination of the sec-

Table II. Course of Experimental Cutaneous Candidiasis on Anagen and Telogen Areas

<table>
<thead>
<tr>
<th></th>
<th>Anagen</th>
<th>Telogen</th>
<th>Statistical Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals infected</td>
<td>8/10</td>
<td>16/18</td>
<td>NS</td>
</tr>
<tr>
<td>No. of animals cleared on day 7</td>
<td>13/32</td>
<td>16/32</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Statistical significance by the χ² test (NS = not significant).

Values represent number of animals infected (as judged histologically) over the total number inoculated.

Values represent number of animals cleared of the infection on day 7 over the total number inoculated.
tions taken at 3 days, the increased thickness of the epidermis could be seen to be due to an increased number of cells in this layer, rather than to edema or enlargement of individual cells.

Production of Delayed Hypersensitivity to Candida by Infection on Anagen or Telogen Skin The degree of sensitivity induced by experimental cutaneous candidiasis on either type of skin was evaluated using delayed hypersensitivity footpad testing to Candida antigens. Animals inoculated 2 weeks previously on anagen skin had footpad increments approximately equivalent to those of animals inoculated on telogen skin (0.42 ± 0.03 mm vs 0.38 ± 0.03 mm, not statistically significant).

DISCUSSION

The results of these studies indicate that there is some effect of the stage of the hair growth cycle on experimental cutaneous candidiasis, but that this effect is different from that which has been previously seen in studies of experimental dermatophytosis. Cutaneous candidiasis appeared to develop after inoculation of either anagen or telogen skin of the mouse. The rate of clearance from the 2 sites appeared to be approximately the same. However, because the culturing method was limited by its inability to distinguish (at early time points) the infecting organisms from remaining inoculum yeast, more rapid clearance of infections on anagen skin could have been missed. An equivalent enhancement of epidermal proliferation was elicited by infections on either type of skin and both resulted in sensitization of the animals Candida antigens. The major difference noted was that inoculation on anagen areas produced some deep foci of Candida infection, apparently representing folliculitis of the well-developed hair follicles present in the anagen skin.

The susceptibility of mice to experimental dermatophyte infections has been shown to be related to the strain used and the immune status of the animals [10,11]. In earlier studies of experimental dermatophytosis, Kligman [7] found that the infection could be produced only in late anagen. This finding was noted with several species of dermatophytes and with mice, rats, and hamsters, all of which have hair cycles confined to specific areas of the skin. In addition, this worker observed thickening of the epidermis and a folliculitis similar to that which we have noted in the present study. The major difference between experimental infections with Candida and those with the dermatophytes in animals with skin cycles appears to be that the former can infect telogen skin whereas the latter cannot. This observation suggests that actual infection of the hair, or at least of the follicle, is more important in dermatophytosis than it is in cutaneous candidiasis.

That the deep Candida foci did not result in prolonged period of positive cultures from the infected anagen skin could have been due to inefficiency of our culturing technique for sampling sites below the epidermis or perhaps to elimination of the organisms by the neutrophilic infiltrate attracted to the site. Candida abscesses produced by injecting the organisms into the dermis of guinea pigs or into the thigh muscle of mice have been found to be self-limited in healthy animals [12,13]. Also, human neutrophils have been found to be capable of damaging Candida pseudohyphal forms in vitro; this damage appears to be mediated by the cell's oxidative metabolic processes and the myeloperoxidase system [14,15].

Although the development of deep Candida foci in the follicles of the anagen skin did not appear to affect the rate of clearance

### Table III. Candida Foci Below the Skin Surface in Animals Inoculated on Anagen or Telogen Skin

<table>
<thead>
<tr>
<th></th>
<th>Anagen</th>
<th>Telogen</th>
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</thead>
<tbody>
<tr>
<td>Animals with foci/total</td>
<td>4/10</td>
<td>0/18</td>
</tr>
<tr>
<td>Foci/number of animals</td>
<td>9/10</td>
<td>0/18</td>
</tr>
<tr>
<td>Foci with polymorphonuclear leukocytes/total foci</td>
<td>5/9</td>
<td>0/0</td>
</tr>
</tbody>
</table>

Values represent number of Candida foci found below the surface of the skin on a 1-cm skin section taken from each animal at either 3 or 5 days after inoculation. The difference in number of animals with deep Candida foci resulting from inoculations on either anagen or telogen areas is significantly (\( p < 0.01 \)) different by the \( \chi^2 \) test.

### Table IV. Increases in Epidermal Thickness Produced by Experimental Cutaneous Candidiasis in Anagen and Telogen Areas

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anagen</td>
<td>Telogen</td>
</tr>
<tr>
<td>Inoculated with Candida</td>
<td>22.6 ± 1.3</td>
<td>21.2 ± 1.5</td>
</tr>
<tr>
<td>Inoculated with saline</td>
<td>15.8 ± 0.9</td>
<td>17.2 ± 0.6</td>
</tr>
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</table>

The differences in epidermal thickness at 3 days in Candida-infected skin between anagen and telogen areas is not statistically significant by the unpaired \( t \)-test. Values represent the thickness of the epidermal cell layer in microns as measured with an ocular micrometer (mean ± SE of data from 5 mice in each group).

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Figure 1. Photomicrograph of a focus of Candida infection of a hair follicle 3 days after inoculation of anagen skin. Candida pseudohyphae (arrowhead) are present in the area of the hair itself. Note that the organisms and resulting neutrophilic microabscesses are confined to the follicle. Periodic acid-Schiff stain, × 400 original magnification.
or the degree to which the mice were sensitized, it should be noted that these animals were healthy and apparently able to clear the organisms from deeper sites. The result could be different in a host with defective defense mechanisms, particularly leukopenia; in this situation, a deep Candida focus from infection of a completely developed anagen hair follicle might be a site for more severe or even disseminated Candida infection. Recently, eczema gangrenosum was found to have begun as Pseudomonas aeruginosa folliculitis in 6 immunosuppressed patients, although dissemination of the organism to the blood stream was not documented in these patients [16]. Destruction of the hair follicle itself with resulting alopecia is also a possibility in folliculitis caused by a highly inflammatory organism such as C. albicans. Human patients with chronic mucocutaneous candidiasis sometimes have alopecia [17-19]. The possibility exists that this condition could have an immunologic basis, especially if one considers that the vitiligo some of these patients have may be due to antimalanocyte antibodies [20]. However, the patchy nature of the alopecia in some patients [17] suggests that loss of the hair follicles from the infection itself could also be at fault.

In summary, C. albicans appears to be capable of infecting either telogen or anagen skin in animals having hair cycles. In contrast, the dermatophytes have previously been shown to produce infections only when inoculated onto skin in the late anagen stage. Candida infections on the 2 types of skin are quite similar except that deep foci of infection, apparently representing folliculitis, develop only when anagen skin is inoculated. These foci might possibly be important in infections in immunocompromised patients or perhaps in the development of alopecia after cutaneous Candida infections.

REFERENCES

11. Calderone RA, Hay RJ: Cell-mediated immunity in experimental murine dermatophytosis. II. Adoptive transfer of immunity to dermatophyte infection by lymphoid cells from donors with acute or chronic infections. Immunology 53:465-472, 1984