

EXPERIMENTAL CUTANEOUS CANDIDIASIS IN RODENTS

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Epicutaneous application of viable *Candida albicans* or *Candida stellatoidea* under an occlusive dressing resulted in subcorneal microabscesses in newborn rats and mice. Neither heat-killed *C. albicans* nor occlusion alone resulted in epidermal micropustules. These results were dependent upon the number of organisms applied and the duration of application. Four additional species of *Candida* tested did not produce epidermal micropustules.

The microscopic pathology of the abscesses revealed penetration of the epidermis by *Candida pseudohyphae*; the tips of these hyphal processes were seen within the pustules when sections were stained by the periodic acid-Schiff method. The resident flora of the newborn mouse or rat was not substantially altered by occlusion with or without *C. albicans* during the interval tested.

This animal model of cutaneous candidiasis will permit greater manipulation and control in investigations of cutaneous candidiasis and the factors influencing neutrophil accumulation in the epidermis.

The accumulation of polymorphonuclear leukocytes, as subcorneal or intraepidermal microabscesses, is a pathologic feature of several human cutaneous diseases. The mechanism of cell accumulation in these diseases is unknown, although several reports have shown the experimental induction of epidermal micropustules following topical application of *Candida albicans* to human skin [1-3]. In order to study the factors which stimulate the directional migration of cells into the epidermis, we have established an experimental animal model in which epidermal micropustules are produced. In this paper we describe the time-dependent and dose-dependent production of subcorneal and intraepidermal microabscesses in newborn mice and rats, following epicutaneous application of yeast phase (blastospore) *C. albicans* under occlusion. We also describe the cutaneous pathogenicity of other *Candida* species as tested in this model.

MATERIALS AND METHODS

Candida Cultures and Suspensions

A pathogenic strain of *C. albicans* from a patient with mucocutaneous candidiasis was isolated and maintained

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on Sabouraud agar slants at room temperature. The following *Candida* species were also obtained from human sources and maintained on Sabouraud agar slants: *C. stellatoidea*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondi*, and two additional strains of *C. albicans*. Each species was identified by sugar assimilation and fermentation criteria as described by Silva-Hutner [4].

Viable *Candida* organisms for animal patch testing were transferred to Sabouraud broth and grown at room temperature for 1 to 5 days before application. Concentration and viability of organisms were determined by total cell counts on a Neubauer hemocytometer, using trypan blue dye in a final concentration of 0.08%. Greater than 99% of the organisms were viable blastospores. Appropriate concentrations were made by dilution with sterile Sabouraud broth. Heat killed *C. albicans*, produced by autoclaving Sabouraud broth cultures at 125°C at 15 lb/sq in. for 20 min, did not exclude trypan blue dye or grow on Sabouraud agar slants.

Patch Testing of Rodents

White/Swiss (W/S) mice and Simonson/albino (S/A) rats were obtained commercially as 14- to 15-day-pregnant animals from Simonson Laboratories, Gilroy, California. Newborn mice, 1 to 5 days old and newborn rats 1 to 4 days old, with no evidence of hair growth, were employed.

Patch testing was carried out with a 4-mm² porous nonwoven cotton patch (Webril, Kendall, Chicago, Illinois) saturated with a 10- μ l aliquot of the desired test culture suspension, backed by occlusive tape (Blenderm, 3M Co., St. Paul, Minnesota) and placed on the back of the test animal. The animals were isolated from their mothers (who frequently cannibalized offspring which were taped) and kept at room temperature for the desired time period. The patch was then removed, the animal sacrificed by ether, and skin cultures and tissue specimens for histologic examination obtained.

Histologic Studies

Patched areas were excised, fixed in 10% formalin for 24 hr, dehydrated and imbedded in paraffin, cut into 60

to 100 serial sections and stained with either hematoxylin and eosin (H & E) or the periodic acid-Schiff reaction (PAS). Sections were examined by light microscopy for epidermal microabscesses and subcorneal pustules. Focal dermal aggregates of polymorphonuclear cells (PMNs) alone were not considered to be lesions. PAS-stained sections were examined for yeast and filamentous forms of *Candida*. Control patches contained 10 μ l of sterile Sabouraud broth alone.

Microbiology Studies

Skin cultures were obtained before and after patching with either *Candida* cultures or sterile Sabouraud broth. Sabouraud agar slants were inoculated with a wire loop wiped over the moistened patched area of the test animal. All cultures were grown at room temperature for 96 hr.

RESULTS

Gross Observations

Lesions were rarely evident on test animals, despite the microscopic presence of subcorneal pustules. A few animals developed small pustules visible to the unaided eye, and one displayed a small superficial erosion. Occasional lesions were seen under a dissecting microscope (10 \times magnification) or by epi-illumination microscopy. Lesions were seen as small superficial vesicles and pustules, scattered over the patched area. Numerous polymorphonuclear cells were seen when the vesicle contents were smeared and stained with Giemsa stain. Such macroscopic lesions were exceptional and occurred in animals patched with high doses for long time periods. In general, reliable identification of lesions could not be achieved by inspection alone.

Histologic Observations

The lesions formed by the occlusive patching of *C. albicans* were subcorneal and intraepidermal microabscesses which contained polymorphonuclear leukocytes (Figs. 1-3). These pustules contained from 3 or 4 to several hundred granulocytes. Larger lesions were formed when adjacent pustules coalesced to form superficial lakes of inflammatory

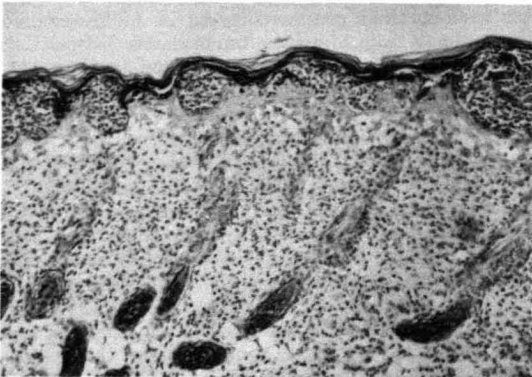


FIG. 1. Multiple microabscess formation in newborn mouse skin 24 hr following application of 6×10^6 *C. albicans* under occlusion (H & E, $\times 44$).

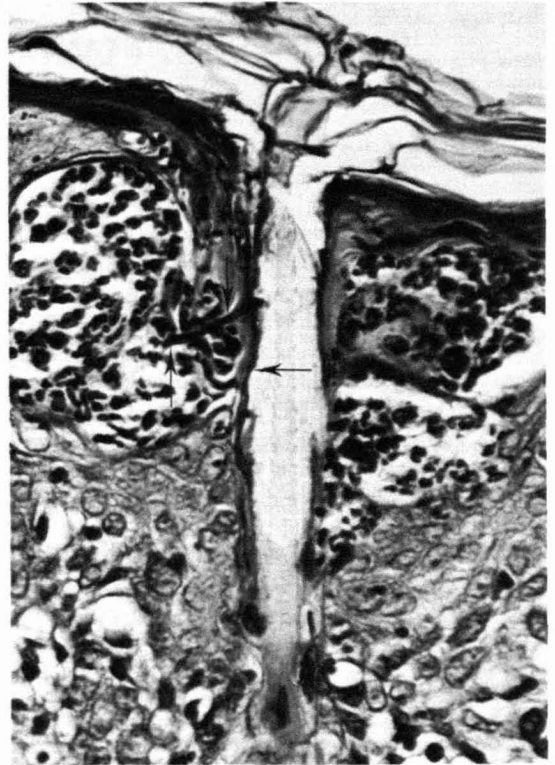


FIG. 2. Proliferation of *C. albicans* pseudohyphae within a hair follicle and its penetration of the stratum corneum at the site of microabscess formation (PAS, $\times 640$).

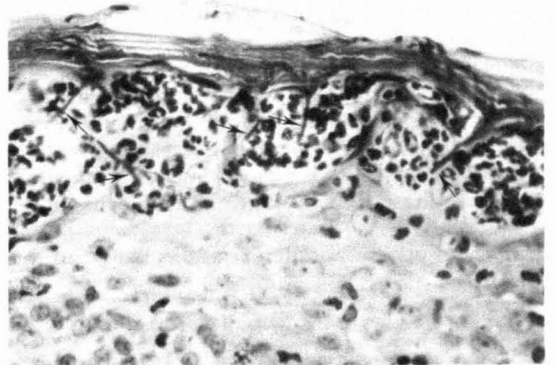


FIG. 3. Multiple microabscess formation, each associated with the presence of *C. albicans* pseudohyphae (PAS, $\times 640$).

cells. The keratinocytes surrounding the microabscesses appeared thin and flattened. Beneath these pustules, individual polymorphonuclear cells were seen invading the epidermis when serial sections were examined. Frequently, these pustules would develop about hair follicles, forming at the orifice and extending down the perifollicular tissue.

Dermal infiltrates of polymorphonuclear leukocytes were seen occasionally beneath epidermal lesions. These appeared often as aggregates close to

the epidermal-dermal interface. Only occasional marginating PMNs were seen in vessels.

Lesions permitted to mature for more than 24 hr developed subcorneal or intracorneal pustules only. These lesions occurred either between the stratum corneum and the granular layer, or entirely within the horny layer. If the patch was removed after 24 hr and the lesion permitted to resolve, newly forming stratum corneum beneath the pustules extruded the lesion upward, leading to exfoliation after 96 hr.

PAS stains were used to demonstrate the presence of fungal elements. Two morphologic phases of *Candida* were seen. The yeastlike blastospores could be seen on and within the stratum corneum, but were never seen within the pustules. The filamentous forms were also found at all levels of the horny layer. In addition, hyphae could be seen within the malpighian layer of the epidermis, and coming close to the dermal-epidermal junction. Microabscess formation was associated with the hyphal tips within the malpighian layer. Furthermore, hyphal proliferation was noted within pustules and hair follicles (Figs. 2, 3).

Dose Dependency

Both the rat and mouse models of cutaneous candidiasis exhibited a dose-dependent course. The results of epicutaneous application of *C. albicans* to rat and mice with various numbers of organisms for 24 hr are given in Table I. In some of the mice, lesions developed when as few as 10,000 organisms were applied, and all animals developed pustules with a dose of 50,000 blastospores. Rats appeared more responsive, developing lesions after the application of only 500 to 1,000 organisms. All rats responded to 10,000 blastospores.

Time Dependency

The development of lesions in both the rat and mouse followed a similar time course. Animals were patched with 6×10^5 organisms per patch for varied time intervals. The time response studies

TABLE I. Dose response in mice and rats to *C. albicans*

Number of organisms applied per animal	Animals with pustules/ animals tested ^a	
	Mice	Rats
100	ND ^b	0/6
500	ND	1/6
1,000	ND	3/6
2,500	0/9	6/7
5,000	0/6	4/5
10,000	4/6	6/6
25,000	5/6	6/6
50,000	6/6	ND
100,000	6/6	ND

^a Application time: 24 hr

^b ND: Not done

TABLE II. Time response in mice and rats to *C. albicans*

Application time (hr)	Animals with pustules/ animals tested ^a	
	Mice	Rats
3	0/6	0/6
6	0/7	0/6
9	1/6	0/6
12	2/6	2/6
15	5/6	2/6
18	5/6	5/6
21	6/6	6/6
24	6/6	9/9

^a Dose: 6×10^5 organisms per animal

are presented in Table II. In the mouse, lesions were detected as soon as 9 hr after patching, and all mice responded after 21 hr. Similarly, the rats first responded after 12 hr, and all developed pustules by 21 hr.

Occlusion

Occlusion beneath the patch was an essential requirement for the development of lesions in this model. Patches not secure to the test animal seldom produced lesions. Animal size and activity were factors contributing to the failure of occlusion. Smaller mice were the most difficult to patch properly, whereas patches regularly maintained good occlusion when placed on rats.

Resident Flora

Mouse skin was cultured on Sabouraud agar slants to determine gross alterations in resident flora following 24 hr of occlusive patching. The number of colonies was not altered significantly by 24-hr occlusion of the skin with a sterile Sabouraud broth patch. Sabouraud agar slants inoculated from unpatched animals grew 1 to 3 colonies. Animals patched with sterile Sabouraud broth for 24 hr produced 0 to 2 colonies. The *C. albicans*-patched animals grew out numerous colonies of pure *C. albicans* after 24 hr of occlusion.

Controls

Heat-killed cultures of *C. albicans* in Sabouraud broth failed to induce lesions in either rats or mice when 6×10^5 organisms were applied under occlusion for 24 hr. Sterile Sabouraud broth under occlusion for 24 hr failed repeatedly to produce lesions of any kind.

Pathogenicity of Other *Candida* Species

Two additional strains of *C. albicans* and five additional species of *Candida* (including *C. guilliermodi*, *C. krusei*, *C. parapsilosis*, *C. stellatoidea*, and *C. tropicalis*) were tested in the rat. All strains of *C. albicans* tested produced typical lesions within 24 hr. Of the other species tested, only *C. stellatoidea* produced pustules within 24 hr.

DISCUSSION

We have described a method for the experimental production of cutaneous *C. albicans* and *C. stellatoidea* infections in newborn rodents. Microscopic subcorneal pustules of polymorphonuclear leukocytes characterized the infections, preceded grossly observable lesions, closely resembled the histology of human cutaneous candidiasis, and developed as a function of the number of organisms applied and the duration of application. Careful examination of serial sections revealed hyphal elements within pustules, associating filamentous pseudohyphae and not blastospores with active infections.

Rats and mice exhibited similar dose requirements of 10,000 and 50,000 organisms, respectively. Although some organisms will be caught in the interstices of the applied gauze, it was possible to approximate the number of organisms required for pustule formation. At present, we are unable to explain the apparent higher susceptibility of rats. Species variation, epidermal thickness, or the easier maintenance of occlusion in rats may be contributing factors.

The development of lesions in both mice and rats required a period of approximately 21 hr. Pustules were not seen earlier than 9 to 12 hr, even when supraoptimal doses were applied. We presume that this time period is required for the transformation of blastospores into pseudohyphae, their penetration of the cornified layer, the generation or activation of chemoattractants, and the emigration of granulocytes from vessel lumens to the epidermis.

Not only hyphal proliferation, but penetration into the malpighian layer appeared necessary for the development of lesions. Hyphae of pathogenic species were able to penetrate viable intact epidermis and produce pustules. Nonpathogenic species developed hyphae confined to the stratum corneum, but failed to produce lesions. Penetration of the malpighian layer by viable pseudohyphae appeared to be the initial stimulus for the inflammatory response.

The predilection for perifollicular involvement in this model was striking, and may reflect greater ease of entry at these sites. The microenvironment of the follicle may also provide the same benefits as occlusion, i.e., moisture and hydration of the epidermis. The requirement of occlusion has been noted in experimental *C. albicans* infections in man [3].

Experimental cutaneous candidiasis has been studied only in man. Smith [1], studying prickly heat, first produced experimental *C. albicans* lesions. Later, Kärcher [2] induced vesiculopustular lesions in human skin using viable *C. albicans*. Maibach and Kligman [3] applied *C. albicans* to human skin under occlusion, and discrete pustules developed within 36 to 72 hr. Subcorneal pustules were seen histologically. Although similar histopathologic changes were said to be induced by a

water-soluble extract of *C. albicans* cell-wall fraction, its composition has not been elucidated fully.

This animal model differs from human studies. The rodent model lesion is a microscopic pustule detected by histologic methods (often in the absence of gross changes) and is fully formed within 21 hr. In the human model, the lesion is detected by gross observation and requires 36 to 72 hr to reach macroscopic proportions. Presumably, the lesions in this animal model represent earlier pathologic changes than the human model. The relative thinness of the rodent epidermis may also reduce the time required for lesions to develop. Fungal elements were occasionally present in human studies, but were confined to the cornified layer. In the rodent model, fungal pseudohyphae were regularly present and intimately associated with lesions. They were not confined to the stratum corneum and often penetrated the malpighian layer. These findings may represent early features of candidiasis present before host defense mechanisms have expelled the invading organisms. Others have described dermal and perivascular lymphocytes after 3 days in human experimental candidiasis. In newborn rodents, lymphocytes were rarely observed. This difference is unexplained, except for the very acute nature of the animal lesions and the immaturity of newborn rodents. Finally, the high frequency of perifollicular lesions in animals is not noted in human studies.

The data and observations suggest a sequence of events leading to the development of subcorneal pustules in candidiasis. The *C. albicans* applied to rodents as blastospores transform and proliferate as filamentous pseudohyphae under occlusive patches. Pseudohyphae penetrate the cornified layer and enter the malpighian layer by an unknown mechanism. The activation or release of chemotactic principles leads to selective migration of polymorphonuclear granulocytes into the epidermis, which accumulate as subcorneal and intraepidermal micropustules about hyphae.

This directional migration of granulocytes into the epidermis is being studied in greater detail. Denning and Davies [5] have described the in vitro chemotaxis of polymorphonuclear cells by *C. albicans* and shown it to be dependent on a heat-labile serum factor. We have recently found that *Candida* will activate serum complement in vitro [6]. Complement activation did not require antibody or early-reacting components of the classical pathway, suggesting alternative or properdin pathway utilization. Since yeastlike organisms, e.g., zymosan, activate the properdin pathway [7], complement activation may be one mechanism whereby chemoattractants are generated by penetrating pseudohyphae.

This study presents an animal model of cutaneous candidiasis which will permit greater latitude in experimental design, manipulation, and control than is feasible in existing human models. Application of this model to rodents, genetically deficient

in immune mediators or experimentally depleted of humoral or cellular constituents, may be helpful in elucidating the inflammatory process in candidiasis. Similar applications might assist studies of antifungal agents, pathogenicity of *Candida* species, and neutrophil migration in the epidermis.

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