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Cutaneous Model of Invasive Aspergillosis▽

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Cutaneous models have proven useful in studies of the pathogenesis and treatment of Gram-positive bacterial infections. Because cutaneous invasive aspergillosis (IA) occurs in the clinical setting, we sought to develop a nonlethal murine cutaneous model of IA. We induced cutaneous IA in cyclophosphamide-treated nude BALB/c mice by subcutaneous injection of *Aspergillus fumigatus* conidia. Skin lesion areas correlated well with tissue fungal burdens, allowing dynamic visual monitoring of cutaneous infections. The cutaneous model accurately reflected alterations in *A. fumigatus* pathogenicity resulting from deletions of recognized virulence genes (*pabaA*, *sidA*, and *pksP*). Moreover, analysis of the roles of conidial and mycelial catalases revealed that the former is required for the initiation of cutaneous aspergillosis, whereas the latter contributes to its propagation. Finally, posaconazole treatment reduced skin lesion areas relative to those of untreated and fluconazole-treated controls. This novel cutaneous model system should be applicable to comparative studies of the pathogenesis, treatment, and tissue specificity of IA.

Studies of microbial pathogenicity and antimicrobial drug efficacy rely on the use of animal model hosts. For invasive aspergillosis (IA), current animal models are based on the introduction of a large inoculum of asexual spores (conidia) into an immunosuppressed rodent via the respiratory or intravenous route, resulting in a rapidly fatal pneumonic or disseminated infection (1, 12, 18, 22, 24). Pulmonary models that are not rapidly fatal have also been developed (5, 22, 23). Cutaneous infection models have been used extensively to study the pathogenesis and *in vivo* response to antibacterial agents of Gram-positive bacteria (8, 10). The potential benefits of these models include their technical simplicity and reproducibility and the ability to visually monitor the infected lesion over time. Moreover, because cutaneous models are nonlethal, they allow prolonged monitoring and repeated manipulations of infected animals.

Cutaneous aspergillosis occurs in 5 to 10% of IA cases, making the skin the second most frequently involved site after the lungs (9, 19). Cutaneous infection develops either as a result of systemic dissemination of pulmonary aspergillosis or, less commonly, because of cutaneous inoculation of conidia (primary cutaneous aspergillosis). The latter instance invariably involves trauma to the skin, such as an intravascular access site or extensive burns, usually in patients with significant underlying immunosuppression; outbreaks of cutaneous aspergillosis in neonates have been associated with contaminated dressings (27, 28). However, the efficacy of antifungal drugs and the expression of *Aspergillus* virulence factors in the setting of soft tissue infections have not been studied. We hypothesized that experimental soft tissue IA in murine thighs could serve as the basis for a simple and nonlethal model of IA. Here, we describe the optimization of this model and report on its performance as a platform for studies of virulence and antifungal drug efficacy. Importantly, the cutaneous model of IA is subacute, nonlethal, and highly reproducible and allows dynamic monitoring of fungal burden by determination of skin lesion dimensions.

(This study was presented in part at the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy-Infectious Diseases Society of America 46th Annual Meeting, Washington, DC, 25 to 28 October 2008 [4].)

MATERIALS AND METHODS

***Aspergillus* strains.** The *Aspergillus fumigatus* strains used in this study are summarized in Table 1. *A. fumigatus* strain 293 (*Af*293) was used in preliminary experiments to establish and optimize the cutaneous model and in antifungal drug treatment studies. *A. fumigatus* strains with deletion mutations of known virulence-associated genes and their parental wild-type strains were used in virulence studies. The Δ*sidA* mutant (deficient in the enzyme catalyzing the first committed step in hydroxamate-type siderophore biosynthesis [21]) was a gift from H. Haas, Innsbruck Biocenter, Austria; the Δ*pabaA* mutant (para amino benzoic acid auxotroph [7]) was a gift from E. M. Bignell, Imperial College School of Medicine, United Kingdom; and the Δ*pksP* mutant (deficient in polyketide synthase [25]) was a gift from K. A. Marr, Johns Hopkins University, Baltimore, MD. The production of *A. fumigatus* strains deficient in conidial catalase (*catA*) and mycelial catalases (*cat1* and *cat2*) was described previously (16).

All strains were grown on yeast extract-agar-glucose (YAG; yeast extract, 5 g/liter; glucose, 10 g/liter; agar, 15 g/liter; 1 M $MgCl_2$, 10 ml/liter; trace elements, 1 ml/liter) plates for 72 h in a humidified 37°C incubator. On the day of inoculation, conidia were collected in sterile saline with 0.08% Tween 20, washed twice, and filtered through 40-μm nylon filters (Bioscience Discovery Labware, Bedford, MA). Conidia were counted in a hemacytometer and resuspended in sterile saline at the desired concentrations (see below). The viability of conidia was determined by quantitative culture to be >95%.

Cutaneous model optimization. Eight-week-old female nude (*nu*/*nu*) BALB/c mice (National Cancer Institute, Bethesda, MD) weighing 18 to 20 g were used

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TABLE 1. *A. fumigatus* strains used in this study

Strain	Description	Source
*Af*293	Clinical isolate	Nierman et al. (14)
B-5233	Wild type	Tsai et al. (25)
Δ*pksP* mutant	B-5233 Δ*pksP*::Hyg^r	Tsai et al. (25)
CEA17	Wild type	Schrettl et al. (21)
Δ*sidA* mutant	CEA17 Δ*sidA*::Hyg^r	Schrettl et al. (21)
*Af*237	Wild type	Brown et al. (7)
Δ*pabaA* mutant	*Af*237 Δ*pabaA*::Hyg^r	Brown et al. (7)
G10	Wild type	Monod et al. (13)
Δ*catA* mutant	G10 Δ*catA*::Phleo^r	Paris et al. (16)
Δ*cat1* Δ*cat2* mutant	G10 Δ*cat1*::Hyg^r Δ*cat2*::Phleo^r	Paris et al. (16)

in all experiments. All procedures were performed according to the highest standards for humane handling, care, and treatment of research animals and were approved by the M. D. Anderson Cancer Center Institutional Animal Care and Use Committee. Mice were housed in presterilized, filter-topped cages and provided with sterile food and drinking water containing tetracycline (1 g/liter).

Mice were anesthetized by inhalation of 2% isoflurane, and 100 μl of conidial suspension was injected subcutaneously (s.c.) with a 27-gauge needle into the right thigh. To determine the optimal parameters for this model, different combinations of immunosuppressive regimens and inoculum concentrations were tested for their ability to induce reproducible skin lesions.

The immunosuppressive regimens studied were as follows: 100 or 150 mg/kg of body weight cyclophosphamide, injected intraperitoneally (i.p.) 4 days and 1 day prior to inoculation and every 2 days thereafter starting on day 2 after inoculation; 250 mg/kg i.p. cyclophosphamide 1 day before inoculation, 200 mg/kg 2 days after inoculation, and 150 mg/kg 5 days after inoculation; and 250 mg/kg cortisone acetate injected s.c. 1 day before inoculation, alone or in combination with a cyclophosphamide regimen. The neutrophil count was determined to be less than 200 cells/μl in a subgroup of 5 mice who received 3 doses of cyclophosphamide at 100 mg/kg. Inoculums of 5×10^5, 5×10^6, and 5×10^7 conidia were tested with each of these drug regimens. Sham inoculation with 100 μl of sterile normal saline was performed as a negative control.

Skin lesions were measured daily using digital calipers (Fisher Scientific, Rochester, NY), and the longest and shortest diameters were recorded. The skin lesion area was approximated using the following ellipse area formula: lesion area = $\pi \times 0.5w \times 0.5l$, where w and l equal the width and length (in millimeters) of the lesion, respectively.

Tissue fungal burden. We assessed 3 different methods for the determination of the tissue fungal burden: quantitative cultures, real-time quantitative PCR (RT-qPCR), and tissue galactomannan content. Thigh tissue (including skin, subcutaneous tissue, and lateral thigh muscles) was excised on day 5 after inoculation and stored in sterile 2-ml collection tubes at −20°C until testing. Tissue was weighed and homogenized in 1 ml of sterile 0.9% saline with a bead beater (Biospec, Bartlesville, OK), and homogenates were centrifuged for 10 min at 20,800 × *g*. Quantitative cultures were performed by plating serial 10-fold dilutions of the tissue homogenates on YAG and counting CFU after 18 h of incubation at 37°C. The results were expressed as numbers of CFU per gram of tissue. DNA was extracted using the DNeasy tissue kit (Qiagen, Valencia, CA), and RT-qPCR was performed using the primers and probe previously described (6). For galactomannan assays, supernatants were diluted and assayed with the Platelia galactomannan enzyme immunoassay kit (Bio-Rad Laboratories, Redmond, WA) according to the manufacturer's instructions. Because of the galactomannan assay's nonlinear characteristics, tissue homogenates had to be diluted to achieve linear correlation with skin lesion areas. The dilution factor that yielded the best correlation with skin lesion areas (1:100) was determined in preliminary experiments using serial 10-fold dilutions of thigh homogenates. The assay's negative, cutoff, and positive controls, as well as uninfected thigh tissue, served as controls. Optical density at 450 nm was determined using a spectrophotometer (PowerWave HT; BioTek Instruments, Winooski, VT) and divided by the cutoff control reading to produce the galactomannan index (GMI). To correct for variations in tissue weight, the galactomannan index was normalized using the formula cGMI = (200 × GMI)/W, where cGMI is the tissue weight-corrected GMI and W is the sample weight in grams.

Histopathologic analysis. Infected thigh tissue was excised, fixed in 10% formalin, and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin or Grocott-Gomori methenamine silver (GMS).

Virulence studies. To assess the sensitivity of the thigh infection model to alterations in the pathogenicity of *A. fumigatus*, we analyzed its response to infection with defined hypovirulent *A. fumigatus* deletion mutants and their parental wild-type strains. Isolates were inoculated in parallel in separate groups of immunosuppressed nude BALB/c mice (5 mice per group), as described above. Lesion sizes (monitored daily), tissue fungal burdens, and histopathologic findings on day 5 after inoculation were compared between the two groups. Experiments were repeated three times.

To determine if conidial or mycelial catalase production by *A. fumigatus* is required for cutaneous infection, we infected mice with a Δ*catA* or Δ*cat1* Δ*cat2* *A. fumigatus* strain and simultaneously injected an identical inoculum of parental wild-type spores (*A. fumigatus* strain G10) into the contralateral thigh (Table 1). We then compared the skin lesion areas and fungal burdens of thighs infected with catalase-deficient strains to those of thighs infected with wild-type strains.

Antifungal drug treatment. We evaluated the ability of treatment with posaconazole (Schering Plough, Kenilworth, NJ) to attenuate cutaneous lesion areas in our thigh infection model. The treatment group received 40 mg/kg posaconazole in an oral suspension daily. Two groups of mice served as negative controls: a nontreatment group and a group treated with the triazole fluconazole (Pfizer, New York, NY; oral suspension, 40 mg/kg daily), which is not active against mold. Fluconazole and posaconazole were diluted in 200 μl of normal saline and administered by oral gavage. Twenty mice were included in each treatment and control group.

Statistical analysis. Skin lesion areas and fungal burdens were compared between groups of mice using the unpaired Student t test. For mice inoculated simultaneously with catalase-negative and parental wild-type strains, the paired Student t test was used to compare lesion areas and fungal burdens. Because both skin lesion area and tissue galactomannan content values were normally distributed, we used Pearson's coefficient to calculate the linear correlation between these parameters. Graphs were plotted using Prism 4.0 software (Graph Pad Software, San Diego, CA). All statistical analyses were performed in SPSS 15.0 (SPSS, Inc., Chicago, IL). A two-tailed P value of <0.05 was considered statistically significant.

RESULTS

Cutaneous-model optimization. Our initial aim was to establish experimental parameters that yielded highly reproducible skin lesions while minimizing immunosuppressive-drug-related toxicity. Inoculation of nonimmunosuppressed BALB/c mice failed to induce thigh infections. Of the various immunosuppressive regimens evaluated, optimal results were obtained with 100 mg/kg of cyclophosphamide injected i.p. 4 days and 1 day prior to inoculation and 2, 4, and 6 days after inoculation. Larger doses of cyclophosphamide or the addition of cortisone acetate to this regimen resulted in greater toxicity (accelerated weight loss and mortality) without enhancing the performance of the model. Of the different inocula studied, the most consistent results were obtained by s.c. injection of 5×10^6 conidia in 100 μl saline. With the use of these parameters, all mice developed skin lesions at the site of infection within 48 h after inoculation. Lesions were typically erythematous macules with peripheral enhancement (Fig. 1A). Central eschars were apparent in about 20% of lesions. Skin lesions progressed slowly over 7 days of follow-up and were associated with minimal systemic morbidity and no mortality. Mice generally lost up to 10% of their body weight during the initial 4 days of immunosuppression; weight subsequently stabilized or increased. Histopathologic analysis revealed extension of fungal hyphae from subcutaneous tissue into the underlying thigh muscles and tissue infiltration by polymorphonuclear leukocytes (PMNLs) (Fig. 1B). Sham inoculations did not induce visible skin lesions.

Skin lesion area correlates with tissue galactomannan content. Of the 3 methods that we evaluated for the determination of fungal burden, only the tissue galactomannan content per-

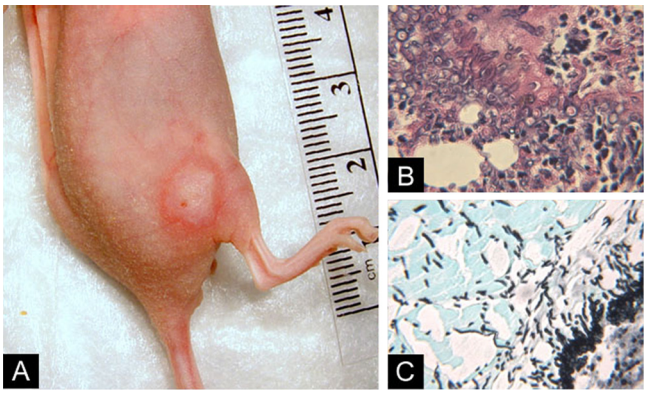

FIG. 1. Cutaneous model of IA. (A) A circumscribed skin lesion with peripheral enhancement appeared 48 h after subcutaneous injection of 5×10^6 *A. fumigatus* conidia in cyclophosphamide-treated nude BALB/c mice. (B, C) Histopathologic examination of thigh tissue sections revealed hyphal proliferation in the dermis and invasion of muscle tissue (hematoxylin and eosin, ×200 magnification [B]; GMS, ×200 magnification [C]).

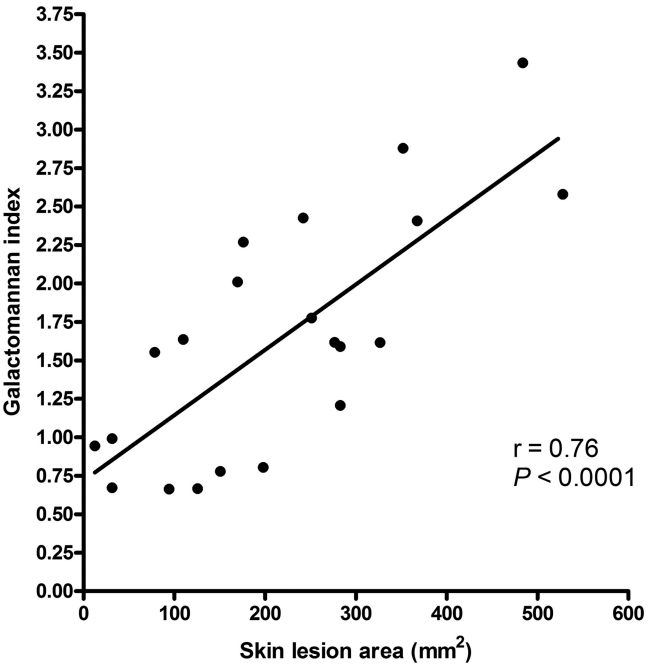

FIG. 2. Correlation between skin lesion area and tissue galactomannan content. A significant linear correlation between skin lesion area and thigh tissue galactomannan content is described by a Pearson's coefficient of 0.76 ($P < 0.0001$).

formed well in our model. Quantitative cultures yielded a mean fungal burden of 7.4×10^5 CFU/g tissue (95% confidence interval, 5.0×10^5 to 9.9×10^5 CFU/g); control mice inoculated with sterile saline had a mean fungal burden of 0 CFU/g tissue. However, numbers of CFU/g correlated poorly with skin lesion size (Pearson's coefficient, 0.14; $P = 0.5$). RT-qPCR was affected by frequent false-negative results, which we attributed to the known PCR-inhibitory effect of myoglobin (2). In contrast, we found a linear correlation between skin lesion areas and the corresponding fungal burdens in mouse thighs as determined by the tissue galactomannan content (Pearson's coefficient, 0.76; $P < 0.0001$) (Fig. 2).

Hypovirulent *Aspergillus fumigatus* mutants produce attenuated skin lesions. To determine the sensitivity of the cutaneous model to alterations in *A. fumigatus* virulence, we compared skin lesions and fungal burdens between *A. fumigatus* strains with deletion mutations in genes encoding well-described virulence factors (*pksP*, *pabaA*, and *sidA*) and their respective parental wild-type strains (Table 1). All three *A. fumigatus* deletion mutants (Δ*pksP*, Δ*pabaA*, and Δ*sidA* mutants) were associated with skin lesion areas significantly smaller than those of mice infected with the wild-type strains (Fig. 3). Specifically, the mean skin lesion area on day 3 after inoculation was 39% smaller in Δ*pksP* mutant-infected mice ($P = 0.007$), 69% smaller in Δ*pabaA* mutant-infected mice ($P = 0.001$), and 47% smaller in Δ*sidA* mutant-infected mice ($P = 0.03$) than in wild-type-infected mice. On GMS-stained tissue sections from thighs infected with hypovirulent strains, fungal elements were few and clustered within subcutaneous tissue, with only limited penetration into the underlying muscle tissue. In the case of Δ*pabaA* mutant-infected mice, almost no germination of conidia was observed (Fig. 3). In contrast, widespread distribution and extension of hyphal elements into muscle tissue was observed in mice infected with the parental wild-type strains. The tissue galactomannan content was lower in mice infected with the Δ*pabaA* mutant than that in mice infected with the parental wild type ($P = 0.01$). The difference in galactomannan content bordered on statistical significance for the mice infected with the Δ*pksP* mutant ($P = 0.07$) and was nonsignificant for the mice infected with the Δ*sidA* mutant ($P = 0.3$) (Fig. 3).

Catalase production by *A. fumigatus* spores and hyphae is required for initiation and propagation of cutaneous IA. Some genes of potential importance in the pathogenicity of *A. fumigatus* appear to be dispensable for experimental invasive pulmonary aspergillosis. For example, conidial catalase (encoded by *catA*) and mycelial catalases (encoded by *cat1* and *cat2*) were hypothesized to facilitate IA by enhancing resistance to oxidative stress. However, deletion of *catA*, while increasing the susceptibility of conidia to H_2O_2, has not been clearly shown to impair the pathogenicity of *A. fumigatus* in an inhalational model system (16). Conversely, deletion of both mycelial catalases had a minor effect on hyphal H_2O_2 susceptibility yet impaired *A. fumigatus* virulence in the inhalational model (16). We sought to determine whether the cutaneous IA model could be used to elucidate the pathogenic roles of *A. fumigatus* catalases.

Deletion of the spore-specific catalase gene (*catA*) was associated with a 77% reduction in the mean skin lesion area ($P = 0.002$) and significantly lower tissue galactomannan content than that associated with the parental wild-type strain. Examination of GMS-stained tissue sections 5 days after inoculation with the Δ*catA* mutant revealed ungerminated spores and no hyphal elements (Fig. 4). A reduction in the mean skin lesion area (57% smaller than that seen in parental wild-type-strain-infected mice) was observed in thighs infected with the mycelial-catalase-deficient Δ*cat1* Δ*cat2* double mutant ($P = 0.01$); however, the galactomannan content in these thighs was not significantly lower than that in wild-type-strain-infected

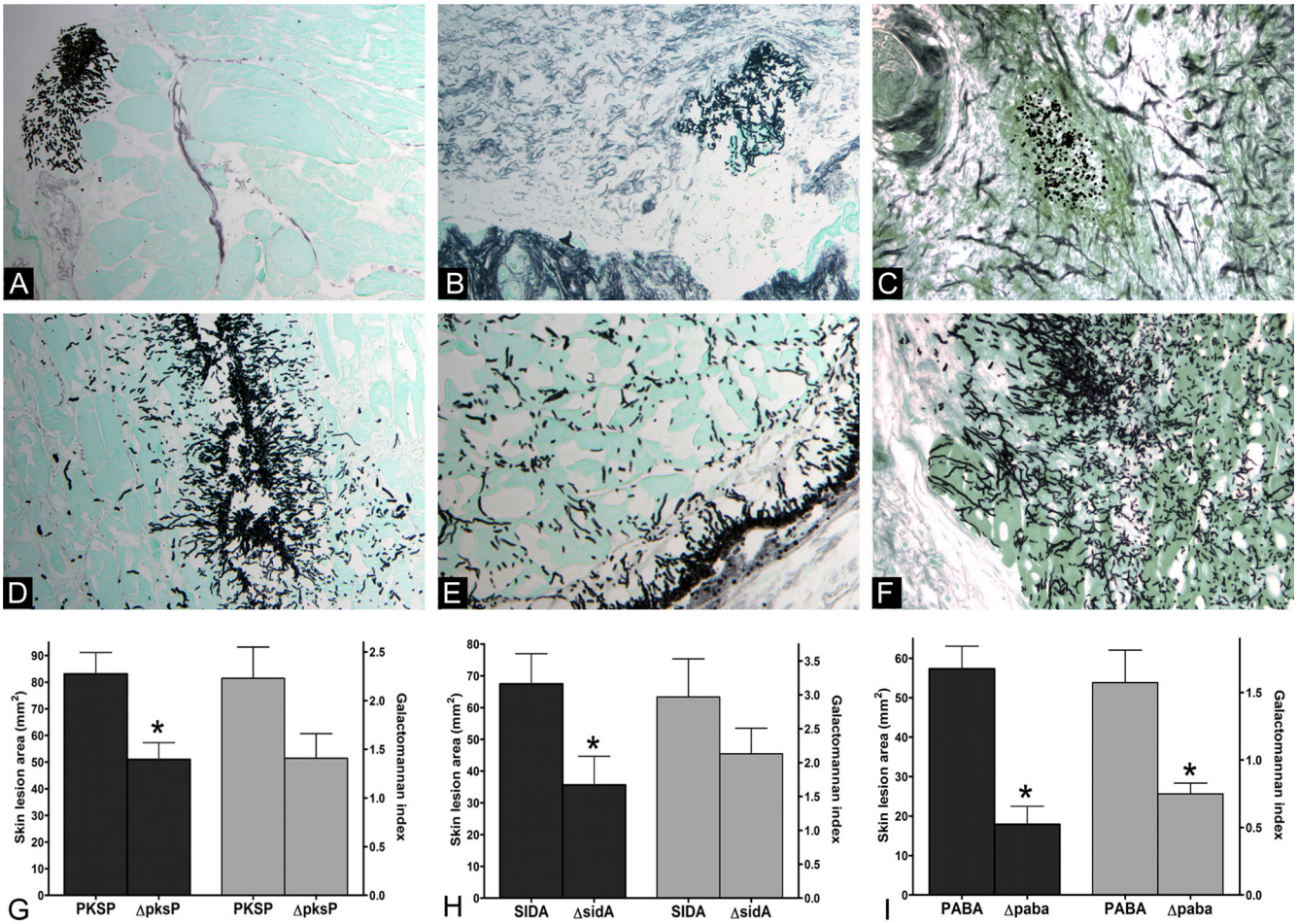

FIG. 3. Features of murine thigh infection with hypovirulent *A. fumigatus* mutants and their parental wild-type strains. (A to F) GMS-stained thigh tissue sections of mice infected with *A. fumigatus* isolates with deletion mutations in known virulence-associated genes and parental wild-type strains are shown (original magnification, ×200). (A to C) In tissue infected with the Δ*pksP*, Δ*sidA*, and Δ*pabaA* mutants, fungal elements were few, clustered, and limited to subcutaneous tissue. (C) Δ*pabaA* conidia failed to germinate. (D to F) In contrast, the parental wild types produced widespread infection with deep invasion of muscle tissue. (G to I) The corresponding differences in skin lesion areas (black bars) and tissue galactomannan content (gray bars) are shown. Bars and error bars represent means and standard errors, respectively (15 mice per *A. fumigatus* strain). *, $P < 0.05$.

thighs. Compared with the wild-type strain, the Δ*cat1* Δ*cat2* mutant formed shorter, more stunted hyphae (Fig. 4), indicating that hyphal catalase production is required for filamentous growth in tissue.

Posaconazole treatment reduces skin lesion size. A unique feature of the cutaneous model is that it enables measurement of changes in skin lesion dimensions over time, reflecting the extent of *A. fumigatus* growth and tissue penetration. This characteristic offers the potential to dynamically monitor the *in vivo* activities of antifungal drugs. We compared the evolution of skin lesions in mice treated orally with posaconazole (40 mg/kg daily) with those of untreated mice and mice treated with the drug fluconazole, which is not active against mold. A significant reduction in the mean skin lesion area was observed in posaconazole-treated mice compared with those of mice in both control groups (Fig. 5). On days 2 through 5 after inoculation, the mean skin lesion area was 47% to 49% smaller in posaconazole-treated mice than in the control mice ($P < 0.001$).

DISCUSSION

We developed and optimized a murine model of cutaneous IA. This model has several characteristics that qualify it as a potentially useful addition to currently available IA animal models (18): it is highly reproducible, owing at least in part to the precise delivery of a predetermined inoculum by subcutaneous injection; it is technically simple to perform; and it does not require excessively expensive equipment or materials. Moreover, some unique features of this cutaneous model have not been available in previous IA models; specifically, the ability to infer the tissue fungal burden from the dimensions of skin lesions allows visual assessment of the infection process. As we have shown, this feature can be exploited to dynamically monitor the course of infection and response to antifungal treatment. In addition, because this is a subacute, nonlethal model, it may facilitate the study of *Aspergillus* virulence factors and responses to pharmacotherapy with greater resolution

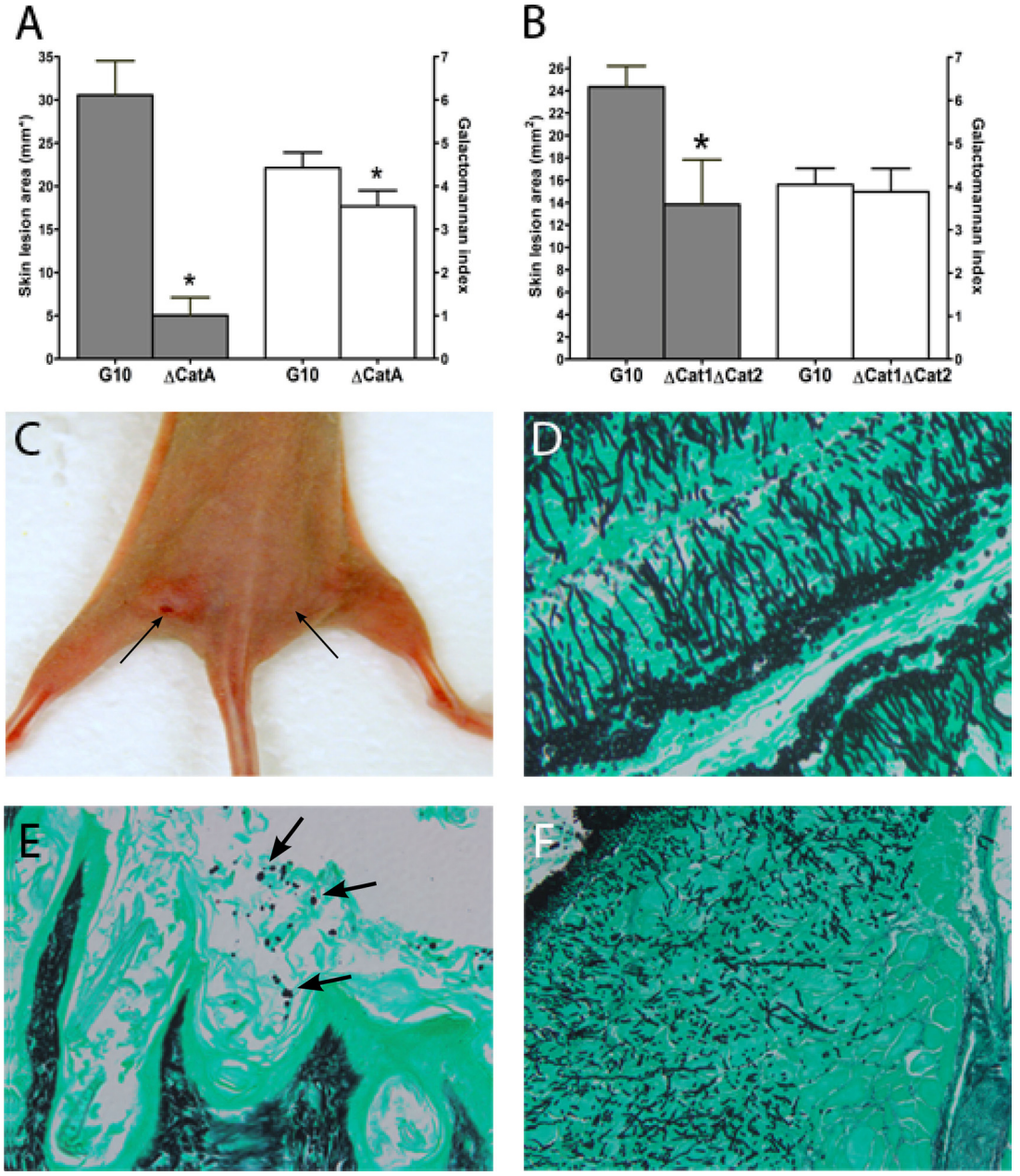

FIG. 4. Importance of catalase production by *A. fumigatus* spores and hyphae for cutaneous IA. Nude BALB/c mice were inoculated simultaneously with catalase-deficient mutants and their parental wild-type strains in opposite thighs. (A and C) Deletion of the spore-specific *catA* gene was associated with significant reductions in the mean skin lesion area and galactomannan content compared with those after infection with the wild-type strain. (C) Left arrow, site of strain G10 inoculation; right arrow, site of Δ*catA* mutant inoculation. (E) Failure of Δ*catA* spores (arrows) to germinate and produce invasive hyphae can be seen in GMS-stained tissue. (B) Deletion of the mycelium-specific catalase genes *cat1* and *cat2* was associated with a small reduction in skin lesion area and a nonsignificant reduction in tissue galactomannan content compared with those of mice infected with the parental wild-type strain. (D and F) Histopathologic analysis revealed that Δ*cat1* Δ*cat2* conidia germinated but formed shorter hyphal elements (F) than the wild-type strain (D) (original magnification, ×200). Bars and error bars represent means and standard errors, respectively (10 mice per *A. fumigatus* strain). *, $P < 0.05$.

than that of models in which an overwhelming, rapidly fatal infection is induced.

A further advantage of the cutaneous model is that, because the site of infection is localized and easily accessible, it is amenable to specialized assays and manipulations. For example, we recently utilized this model to assess angiogenesis in *A. fumigatus*-infected tissue. To that end, a proangiogenic extract of basement membrane (Matrigel) was injected subcutaneously at the site of invasive aspergillosis, where it polymerized and formed solid plugs. Seven days after infection, the plugs were excised and the extent of endothelial cell migration and network formation was determined (3). Thus, the accessibility and subacute nature of the cutaneous model allowed us to monitor angiogenesis *in situ* and to describe the antiangiogenic activity of *A. fumigatus in vivo*.

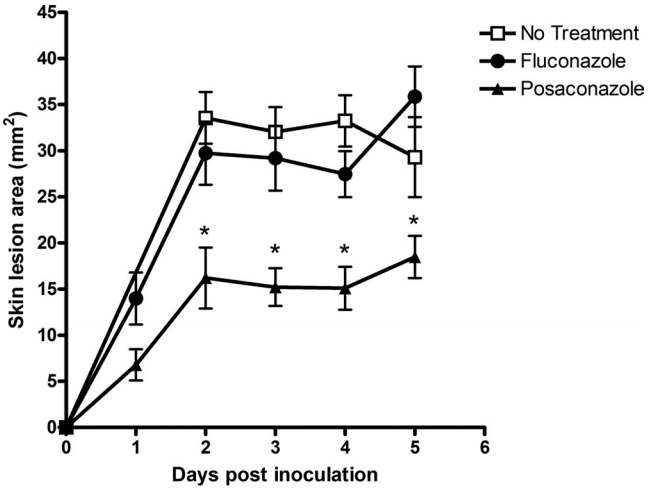

FIG. 5. Posaconazole treatment reduces skin lesion areas. Compared with no drug treatment or fluconazole treatment (40 mg/kg/day), treatment with posaconazole (40 mg/kg/day) resulted in a 47% to 49% reduction in the mean skin lesion area on days 2 through 5 after inoculation with strain *Af*293 ($P < 0.001$). Each datum point represents the mean skin lesion area (±standard error); 20 mice were used for each treatment group. *, $P < 0.001$.

In most clinical cases, IA occurs after inhalation of airborne conidia (11). Once they traverse the upper airways, most conidia are phagocytosed by resident alveolar macrophages and destroyed by oxidative mechanisms (20). Subcutaneously injected conidia likely first encounter different subsets of immune cells, such as Langerhans cells and dendritic cells. One indication of the importance of these differences is that corticosteroids were not required in our cutaneous model, whereas their use is needed to downregulate alveolar macrophage function in inhalational-IA models. Nevertheless, we found concordance between the expression of the virulence-associated *A. fumigatus* genes *sidA*, *pabaA*, and *pksP* in the cutaneous model and that in previously described inhalational (7, 21) and disseminated (25) models. Thus, although the cutaneous model does not mimic invasive pulmonary aspergillosis, our results indicate that it provides useful information on the pathogenicity of *Aspergillus* strains.

Cutaneous aspergillosis occurs in immunocompromised patients either as a primary infection, often following localized trauma, or as a secondary event arising from spread from adjacent infected tissue or hematogenous dissemination from a distant site (27). Interestingly, the *Aspergillus* species that cause primary cutaneous aspergillosis differ significantly from those encountered in pulmonary aspergillosis. For example, *Aspergillus flavus*, an uncommon cause of invasive pulmonary aspergillosis, has been associated with 44% of primary cutaneous aspergillosis cases in non-HIV-infected patients (17, 27). Similarly, of the 21 cases of invasive *Aspergillus ustus* infection reported in the medical literature since 1971, 10 occurred in patients with primary cutaneous aspergillosis (15). In contrast, *A. fumigatus* was recovered in only 26% of primary cutaneous aspergillosis cases (17, 27). These associations suggest the existence of tissue-specific *Aspergillus* virulence factors that have thus far not been characterized.

Extracellular catalase production by *A. fumigatus* has been hypothesized to facilitate invasive infection by detoxifying H_2O_2 produced by PMNLs. Δ*catA* conidia are hypersensitive to H_2O_2 but not more susceptible than the parental wild-type strain to attack by macrophages (16). Thus, the nonattenuated virulence of the Δ*catA* mutant in an inhalational model (16) can be explained by the fact that *catA* does not appear to protect conidia from the oxidative burst of alveolar macrophages. In contrast, we found that deletion of *catA* prevented *A. fumigatus* conidia from germinating in subcutaneous tissue and initiating invasive infection. This finding is consistent with the predominance of PMNLs in *A. fumigatus* skin lesions, suggesting that conidial catalase plays a central role in allowing conidia to evade oxidative damage by PMNLs. Deletion of the mycelial catalase genes *cat1* and *cat2* was previously shown to result in a slight increase in mycelial susceptibility to H_2O_2 and restricted hyphal growth in lung tissue (16). Similarly, Δ*cat1* Δ*cat2 A. fumigatus* was associated with smaller skin lesions and limited hyphal elongation in our cutaneous model. Together, these observations suggest that conidial catalase is an organ-specific *A. fumigatus* virulence factor whose pathogenic role is evident in the presence of a PMNL-biased immune response. More generally, organ-specific model systems such as our cutaneous model may be used to dissect the relative importance of *A. fumigatus* virulence factors in different organs.

The optimal method for determining tissue fungal burden in animal models of IA is the subject of debate. Quantitative cultures of hyphal organisms may have limited reproducibility, because hyphal fragmentation secondary to tissue grinding can produce spuriously high colony counts (18). Specifically, the poor correlation that we observed between the number of CFU/g tissue and skin lesion size suggests that the vigorous homogenization necessary to process thigh tissue affected the reproducibility of quantitative cultures. Surrogate biomarkers, such as RT-qPCR and a galactomannan immunoassay, have been successfully utilized to measure fungal burden in tissues (6, 26). Measurement of thigh galactomannan content as described here correlated well with the skin lesion area and appeared to be an adequate method of validating tissue fungal burden.

Our study has certain limitations. Chiefly, the applicability of any virulence factor identified using the cutaneous model to the pathogenesis of invasive pulmonary aspergillosis will need to be verified using an inhalational model. However, because the endpoint of the cutaneous model is the area of skin lesions rather than mortality, it is well suited for the rapid screening of *A. fumigatus* mutant libraries to detect phenotypic changes using a relatively small number of animals. Further testing could then be performed with a pulmonary model. Second, we did not study the non-*fumigatus Aspergillus* species commonly associated with cutaneous aspergillosis in our model. Nevertheless, our results show that cutaneous aspergillosis with *A. fumigatus* can be easily induced in cyclophosphamide-treated BALB/c mice and that virulence data obtained with the cutaneous model parallels that of pulmonary models. Lastly, because local immunological defenses and pharmacokinetics may differ significantly between soft tissues and the lungs, the activities of antifungal drugs observed in the cutaneous model should be validated in a pulmonary model before conclusions are made about efficacy against invasive pulmonary aspergillosis.

We conclude that the cutaneous model of IA is a useful

addition to current animal models of inhalational and disseminated IA. This subacute, localized model is robust, its results are reproducible, and it allows dynamic monitoring of the infection site.

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