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Establishment of a Superficial Skin Infection Model in Mice by Using *Staphylococcus aureus* and *Streptococcus pyogenes*

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A new animal model for the purpose of studying superficial infections is presented. In this model an infection is established by disruption of the skin barrier by partial removal of the epidermal layer by tape stripping and subsequent application of the pathogens *Staphylococcus aureus* and *Streptococcus pyogenes*. The infection and the infection route are purely topical, in contrast to those used in previously described animal models in mice, such as the skin suture-wound model, where the infection is introduced into the deeper layers of the skin. Thus, the present model is considered more biologically relevant for the study of superficial skin infections in mice and humans. Established topical antibiotic treatments are shown to be effective. The procedures involved in the model are simple, a feature that increases throughput and reproducibility. This new model should be applicable to the evaluation of novel antimicrobial treatments of superficial infections caused by *S. aureus* and *S. pyogenes*.

An important stage in testing the potential of chemicals as antimicrobial drug candidates is establishment of their effectiveness in an animal model system (12, 30). A useful animal model system should be clinically relevant, experimentally robust, ethically acceptable, and convenient to perform and should provide reliable and reproducible results. We report here on a new model in mice that fulfills these criteria. The tape-stripping model has been developed to test the effectiveness of topical antibiotic treatments of superficial skin infections caused by *Staphylococcus aureus* or *Streptococcus pyogenes*. *S. aureus* and *S. pyogenes* are the most common causative agents of primary skin infections in humans (11, 17).

The existing mouse models for topical treatment of skin infections are the burnt skin model (1, 32, 36) and the skin suture-wound model (5, 14). Either the bacteria are introduced into the skin by injection in a traumatized skin area, as in the burnt skin model, or a bacterium-impregnated nylon suture is implanted into an artificial wound (a scalpel incision through all skin layers), which is then sewn or stapled shut, as in the skin suture-wound model. The area of infection in these models is usually dorsally located to hinder grooming or cleaning by the animal itself. Antibiotics dissolved in cream or ointment can be applied to the wound during the course of the experiment. At the experimental end point, the animal is killed, the infected area of the skin is cut out, and the number of bacteria in the sample is assayed (1, 5, 14, 32, 36). These mouse models for skin infections have some disadvantages in relation to superficial infections. The burnt skin model has been developed for studying issues related to infections in burn patients and is ethically unacceptable from an animal welfare perspective for the study of localized skin infections, such as impetigo and erysipelas. The skin suture-wound model involves cutting into the deeper layers of the skin and is thus not clinically relevant to purely topical conditions. Here we describe a new mouse model for superficial infections, the tape-stripping model. In this model a way of entry for the infectious agent is created by stripping off the fur and epidermis in a region on the back of the mouse by successive applications of an adhesive bandage.

In the development of this model, we used *S. aureus* and *S. pyogenes* as the infectious agents. Both bacteria are endemic in human populations and are regarded as opportunistic bacteria, usually causing infections in children, immunocompromised patients, or patients suffering from the effects of medical surgery (11, 22, 35). *S. aureus* can be isolated from throat or nasal swab samples from approximately one-third of the population (2, 16, 26, 33, 34) and is also commonly found in the skin flora, together with related species, such as *Staphylococcus epidermidis* (6, 7). Topical infections due to *S. aureus* and *S. pyogenes* are clinically relevant and cause a variety of serious symptoms, including toxic shock syndrome and skin lesions (17, 31), that can progress to sepsis and systemic shock if they are left untreated (3, 10, 24). These bacterial species are also the most common causes of impetigo in humans (13, 17). The antibiotic used to test the model was 2% fusidic acid in ointment (FAO; Fucidin ointment). In vitro FAO shows high levels of activity against several gram-positive organisms and species of staphylococci in particular (19, 20, 23).

MATERIALS AND METHODS

Bacterial strains and growth media. *Staphylococcus aureus* FDA486 is a laboratory strain previously used to study wound infections in rats (25). *Streptococcus pyogenes* 301 is a clinical dermal infection isolate obtained from the Univer-

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TABLE 1. Bacterial counts obtained in the various treatment groups

Group	Mean bacterial count (log_{10} CFU/wound) ± SD (no. of wounds)	
	S. aureus FDA486[a]	*S. pyogenes* 301[b]
Inoculum size	7.21 ± 0.08	7.28 ± 0.11
4 h postinfection	7.03 ± 0.37 (7)	6.48 ± 0.29 (16)
Untreated (day 4)	5.89 ± 0.64 (8)	3.21 ± 1.62 (16)
Placebo (day 4)	6.05 ± 0.31 (7)	6.15 ± 0.57 (16)
FAO (day 4)	4.68 ± 0.78 (12)	2.04 ± 1.31 (8)

[a] FA ≠ placebo ($P < 0.001$); 4 h = placebo ($P = 0.05$); 4 h ≠ untreated ($P = 0.01$); untreated = placebo ($P = 1.00$).
[b] FA ≠ placebo ($P < 0.001$); 4 h = placebo ($P = 1.00$); 4 h ≠ untreated ($P < 0.001$); untreated ≠ placebo ($P < 0.001$).

sity Hospital in Uppsala, Sweden. The Dry SPOT Streptococcal Grouping kit (DR0400 M; Oxoid Ltd., Basingstoke, United Kingdom) was used to determine the Lancefield type of the streptococcal strain. The streptococcal bacteria were grown overnight anaerobically at 37°C on defibrinated horse blood agar before they were tested according to the manufacturer's instructions. *S. pyogenes* 301 belongs to Lancefield serological group type A. The in vitro MICs of fusidic acid for these strains, as determined by Etest (AB BIODISK, Solna, Sweden), were as follows: *S. aureus* FDA486, 0.125 μg/ml and *S. pyogenes* 301, 4 μg/ml. *S. aureus* was grown in Luria broth and on Luria agar plates (Oxoid Ltd.). *S. pyogenes* was grown in Todd-Hewitt broth (Sigma Aldrich, Stockholm, Sweden) and on blood agar plates made by mixing 5% (wt/vol) defibrinated horse blood (National Veterinary Institute, Uppsala, Sweden) with Luria agar. *S. aureus* was grown aerobically at 37°C, and *S. pyogenes* was grown anaerobically (10% CO_2) at 37°C.

Tape-stripping infection model. Animal infection experiments were performed at the Microbiology and Tumor Biology Center, Karolinska Institute (Stockholm, Sweden), in accordance with institutional and national guidelines (ethical permit N154/02). Six- to 8-week-old female BALB/c mice (Taconic M&B, Ry, Denmark) were used for all experiments. The mice were anesthetized by intraperitoneal injection of 10 ml/kg of body weight of a 1:1:2 (vol/vol) mixture of Hypnorm (fentanyl/fluanisone; Janssen-Cilag Ltd., Saunderton, United Kingdom)-Dormicum (midazolam; Hoffman-La Roche AG, Basel, Switzerland)-distilled water. The fur was stripped from the mice with Tensoplast (Smith & Nephew Medical, Hull, United Kingdom), an elastic adhesive bandage. An area of ca. 2 cm^2 was tape stripped. In order to standardize the degree of barrier disruption elicited by the tape stripping, the transepidermal water loss (TEWL) was measured by using a DermaLab TEWL probe (Cortex Technology, Hadsund, Denmark). Measurements were made according to the guidelines of the Standardization Group of the European Dermatitis Society (27). TEWL is calculated automatically and is expressed in g/m^2 h. By tape stripping the back of the mice 7 to 10 times in succession, the TEWL reached approximately 70 g/m^2 h. Following this procedure, the skin became visibly damaged and was characterized by reddening and glistening but no regular bleeding. Microscopically, this procedure resulted in the controlled removal of most of the epidermal layer, with only a few basal epidermal cells remaining. After stripping of the skin, a bacterial infection was initiated by placing on the skin a 5-μl droplet containing 10^7 cells concentrated from an overnight bacterial culture in stationary phase. In each experiment, a group of mice were killed 4 h after infection to control the infectious dose (Table 1). The mice were treated with FAO (LEO Pharma, Ballerup, Denmark) on a regular basis, as described here. This dose gave a significant reduction in the numbers of CFU in preliminary dose-finding studies (0.5%, 1%, and 2% fusidic acid) and is the dose recommended by the manufacturer for human use. The first application of antibiotic to the stripped skin of the mice was at 4 h postinfection. Thereafter, beginning at 16 h after the first treatment, additional antibiotic applications were made twice daily (in the morning and the evening, with an 8-h interval) for a period of 4 days. For each treatment 25 to 30 mg of ointment was applied (estimated by weighing the pellet of ointment on a spatula). After each day the ointment tube was weighed to determine the average amount of ointment used for each mouse. Two infection control groups were included for each experiment: one consisted of untreated mice and the other consisted of mice treated with placebo ointment. The placebo ointment was identical to FAO except for the lack of the 2% fusidic acid. For all experiments in which untreated or topically treated groups were included, the experiments were terminated 18 h after the last topical treatment in order to avoid carryover effects in vitro. The addition of fucidinase (2.5 units per sample) to the homogenized samples did not influence the numbers of CFU, showing that 18 h is sufficient to avoid a carryover effect. Immediately after the mice were killed, the wounds, approximately 2 cm^2, were excised and homogenized together with 1 ml of phosphate-buffered saline in stomacher lab system bags by using a Stomacher 80 machine (Seward Ltd., Thetford, United Kingdom) set at 260 strokes per min for 120 s. The homogenates were washed once in phosphate-buffered saline to decrease the concentration of ointment. Suitable dilutions of the homogenates were plated on Luria agar (*S. aureus*) or blood agar (*S. pyogenes*) plates to determine the number of living bacteria (CFU). In order to investigate the reproducibility of the infection with *S. aureus* and *S. pyogenes*, three independent experiments that included untreated and placebo-treated groups were performed. In each experiment the mean CFU was calculated by using log_{10}-transformed data. Based on the averages of these three experiments, the mean, range, and coefficient of variation were calculated.

Suture-wound model. The established skin suture-wound model was carried out as described previously (14).

Histological examinations. In order to characterize the histopathology of the model, biopsy specimens were taken after the following treatments: immediately after tape stripping, 4 days after tape stripping, and 4 days after inoculation with *S. aureus* and *S. pyogenes* with and without treatment with placebo ointment. Immediately after the animals were killed, 5-mm punch biopsy specimens of excised skin were taken and immediately fixed in phosphate-buffered (pH 7.4) formalin (4%). The formalin-fixed biopsy specimens were embedded in paraffin and stained with hematoxylin and eosin. For identification of the bacteria, the biopsy specimens were stained with Gram's crystal violet solution (94448; Sigma-Aldrich). The following parameters and semiquantitative scoring system were used to describe the inflammatory response: for scoring of the inflammation (in the dermis, subcutis, muscular tissue, and connective tissues), 0, no inflammation present; 1, little inflammation present; 2, moderate inflammation present; and 3, severe inflammation present; for scoring of the presence of neutrophils, 0, no neutrophils present; 1, a few neutrophils present; 2, moderate occurrence of neutrophils; and 3, abundant occurrence of neutrophils; for scoring of the presence of mononuclear leukocytes, 0, no mononuclear leukocytes present; 1, a few mononuclear leukocytes present; 2, moderate occurrence of mononuclear leukocytes; and 3, abundant occurrence of mononuclear leukocytes; for scoring of presence of bacteria, 0, no bacteria; 1, scattered bacteria; 2, moderate numbers of bacteria; and 3, many large collections of bacteria. The observer was blinded to the treatments for all biopsy specimens.

Behavioral responses of mice. The mice were observed at least twice each day for signs of fatigue, stress, and aggressiveness. The mice were weighed before and after each experiment.

Statistical analysis. Statistical analysis of the log_{10}-transformed data was performed to ensure variance homogeneity and normality. Thus, an analysis of variance (generalized linear models [8]) was applied, followed by four predefined pairwise treatment comparisons adjusted for multiplicity by the Bonferroni method (18), yielding a statistical ranking. Furthermore, to ensure robustness in the analysis performed, the nonparametric Kruskal-Wallis approach (21) was used. The method showed no conclusive dissimilarities to the generalized linear models approach. All testing was performed on an overall 5% significance level, meaning that P values less than 0.05 were considered a statistically significant difference. All calculations and analyses were performed with SAS version 8.2 (SAS OnlineDoc; SAS Institute Inc., Cary, NC). Log_{10}-transformed data are presented as the mean and standard deviation (SD) in Table 1, whereas actual values and corresponding median values are presented in the figures.

RESULTS

Establishment of staphylococcal infection. The number of CFU recoverable from the wound 4 h after application of 10^7 CFU of *S. aureus* FDA486 was 7.03 ± 0.37 log_{10} (Table 1). The different treatment regimens were begun after this initial 4-h period. There was no significant difference ($P = 0.05$) in the numbers of CFU per wound when 4 h versus 4 days of placebo treatment were compared (6.05 ± 0.31 log_{10}). This is evidence of the successful establishment of a staphylococcal infection in this model. There was a slightly greater reduction in the numbers of CFU per wound that was statistically significant ($P = 0.01$) when 4 h versus 4 days of no treatment (5.89 ± 0.64 log_{10}) were compared. The differences between the 4-day placebo

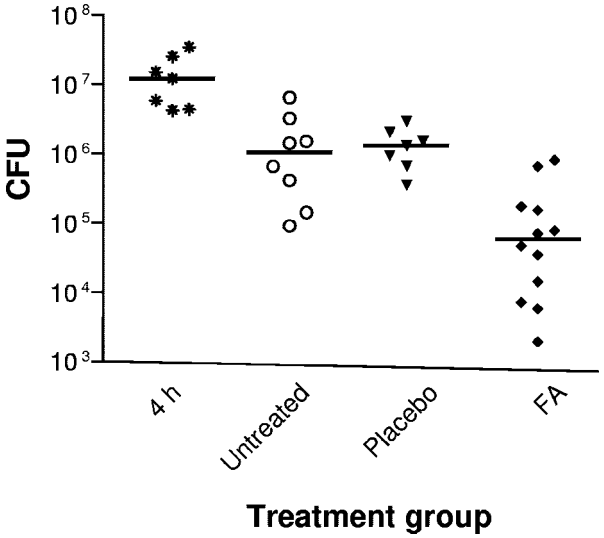


FIG. 1. Tape-stripped mice infected with *S. aureus* FDA486. The number of bacteria (CFU) extracted from each mouse is represented by the symbol for the corresponding experimental group. The median value of the data for each group is shown as a horizontal bar. The number of mice in each experimental group was as follows: 4 h postinfection, $n = 7$; untreated, $n = 8$; placebo, $n = 8$; FAO, $n = 12$.

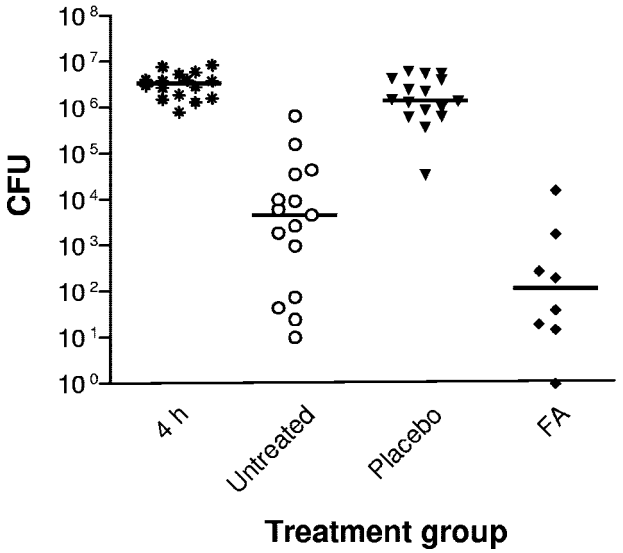


FIG. 2. Tape-stripped mice infected with *S. pyogenes* 301. The number of bacteria (CFU) extracted from each mouse is represented by the symbol for the corresponding experimental group. The median value of the data for each group is shown as a horizontal bar. The number of mice in each experimental group was as follows: 4 h postinfection, $n = 16$; untreated, $n = 16$; placebo, $n = 16$; FAO, $n = 8$.

treatment and 4 days of no treatment were not statistically significant ($P = 1.00$).

Effect of fusidic acid treatment on staphylococcal infection. Tape-stripped mice infected with *S. aureus* FDA486 were treated with FAO to test the efficacy of a topical antibiotic treatment in the new model (Fig. 1). Comparison of placebo (6.05 ± 0.31 $\log_{10}$) and FAO (4.68 ± 0.78 $\log_{10}$) treatment of the staphylococcal infection revealed a reduction in the numbers of CFU per wound that was statistically significant ($P < 0.001$).

Establishment of streptococcal infection. The numbers of CFU recoverable from the wound 4 h after application of approximately 10^7 CFU of *S. pyogenes* 301 was 6.48 ± 0.29 $\log_{10}$ (Table 1). Comparison of the numbers of CFU after 4 h and 4 days of placebo treatment (6.15 ± 0.57 $\log_{10}$) revealed no significant difference ($P = 1.00$). In contrast, comparison of the numbers of CFU at 4 h and 4 days without treatment (3.21 ± 1.62 $\log_{10}$) revealed a significant reduction ($P < 0.001$). Comparison of the placebo and the untreated groups 4 days after infection confirmed the significance of the reduction in the numbers of CFU in the latter group ($P < 0.001$).

Effect of fusidic acid treatment on streptococcal infection. Comparison of placebo and fusidic acid treatments for the streptococcal infection (Fig. 2; Table 1) revealed a very significant effect ($P < 0.001$) of the antibiotic in clearing the infection. Thus, the mean CFU count after the 4-day FAO treatment (2.04 ± 1.31 $\log_{10}$) was reduced more than 4 $\log_{10}$ compared with that after the 4-day placebo treatment (6.15 ± 0.57 $\log_{10}$). Statistical comparison of the 4-day FAO treatment group and the 4-day untreated group showed that there was no significant difference ($P > 0.05$).

Reproducibility of the model. The reproducibility of the model was assessed in three independent experiments, each of which included both *S. aureus* and *S. pyogenes*, with untreated and placebo-treated mice. The coefficients of variance (CVs) for the untreated mice were 18% (mean, 6.09 $\log_{10}$; SD, 1.10 $\log_{10}$) for those infected with *S. aureus* and 36% (mean, 3.96 $\log_{10}$; SD, 1.41 $\log_{10}$) for those infected with *S. pyogenes*. The CVs for the placebo-treated mice were 30% (mean, 6.21 $\log_{10}$; SD, 1.86) for those infected with *S. aureus* and 12% (mean, 6.62 $\log_{10}$; SD, 0.81 $\log_{10}$) for those infected with *S. pyogenes*.

Histological examination of infected and noninfected mice. The results for the scores of the inflammatory response, infiltrating neutrophils and mononuclear leukocytes, and the presence of bacteria are presented in Table 2. The tape-stripping procedure removed most of the epidermis, although a single layer of epidermal cells or scattered epidermal cells remained. Most of the remaining epidermal cells were necrotic. A minimal inflammatory response was evident immediately after tape stripping, and except for the missing epidermis, the skin appeared normal. Four days after tape stripping an acute subcutaneous phlegmonous inflammation with fibrin deposition and edema was observed with the infiltration of a few neutrophils. The inflammation was most pronounced in the subcutaneous and connective tissues. Inoculation of both *S. aureus* and *S. pyogenes* induced a late stage of subcutaneous fibrinoid necrosis after 4 days, with a few neutrophils mainly seen as nuclear dust deep in the subcutis (Fig. 3). In addition to neutrophils, the inflammatory cell infiltrate consisted of mononuclear cells, including lymphocytes and histiocytes. The inflammatory response was most intense in the subcutaneous tissues. For both mice infected with *S. aureus* and mice infected with *S. pyogenes*, the presence of bacteria (Fig. 4) was less evident in the untreated mice than in placebo-treated mice and the skin appeared dry compared to the skin of the placebo-treated mice. In some of the infected animals, fibrosis and remarkable regeneratory changes were observed in the subcutaneous tissue.

TABLE 2. Scores for inflammatory response, infiltrating neutrophils and mononuclear leukocytes, and presence of bacteria

Group, day, and parameter	Mean[a]	SD	Untreated		Placebo	
			Mean	SD	Mean	SD
Tape-stripped skin						
Day 0 (n = 8)						
Inflammation score						
Dermal	0.0	0.0				
Subcutaneous	0.1	0.4				
Muscular	0.0	0.0				
Connective tissue	0.0	0.0				
Neutrophils	0.0	0.0				
Mononuclear leukocytes[b]	0.0	0.0				
Presence of bacteria	0.0	0.0				
Day 4 (n = 8)						
Inflammation score						
Dermal	0.8	1.4				
Subcutaneous	2.6	0.5				
Muscular	0.3	0.7				
Connective tissue	1.3	1.3				
Neutrophils	0.3	0.3				
Mononuclear leukocytes[b]	0.0	0.0				
Presence of bacteria	0.0	0.0				
S. aureus, day 4[c]						
Inflammation score						
Dermal			1.3	1.2	0.0	0.0
Subcutaneous			2.5	0.8	2.4	0.9
Muscular			0.4	0.5	1.6	1.1
Connective tissue			0.4	0.5	1.1	0.5
Neutrophils			0.6	0.6	0.5	0.4
Mononuclear leukocytes[b]			0.4	0.2	1.2	1.7
Presence of bacteria			0.6	0.7	3.0	0.0
S. pyogenes, day 4[d]						
Inflammation score						
Dermal			0.0	0.0	1.2	1.3
Subcutaneous			2.8	0.5	2.2	1.3
Muscular			0.3	0.5	1.6	1.1
Connective tissue			0.5	1.0	1.1	0.5
Neutrophils			0.3	0.5	0.7	0.3
Mononuclear leukocytes[b]			0.3	0.3	1.7	0.7
Presence of bacteria			0.3	0.3	2.4	1.3

[a] Scoring quantification is described in Materials and Methods.
[b] Mononuclear leukocytes include lymphocytes and histiocytes.
[c] Eight mice were in the untreated group and six mice were in the placebo group.
[d] Five mice were in the untreated group and five mice were in the placebo group.

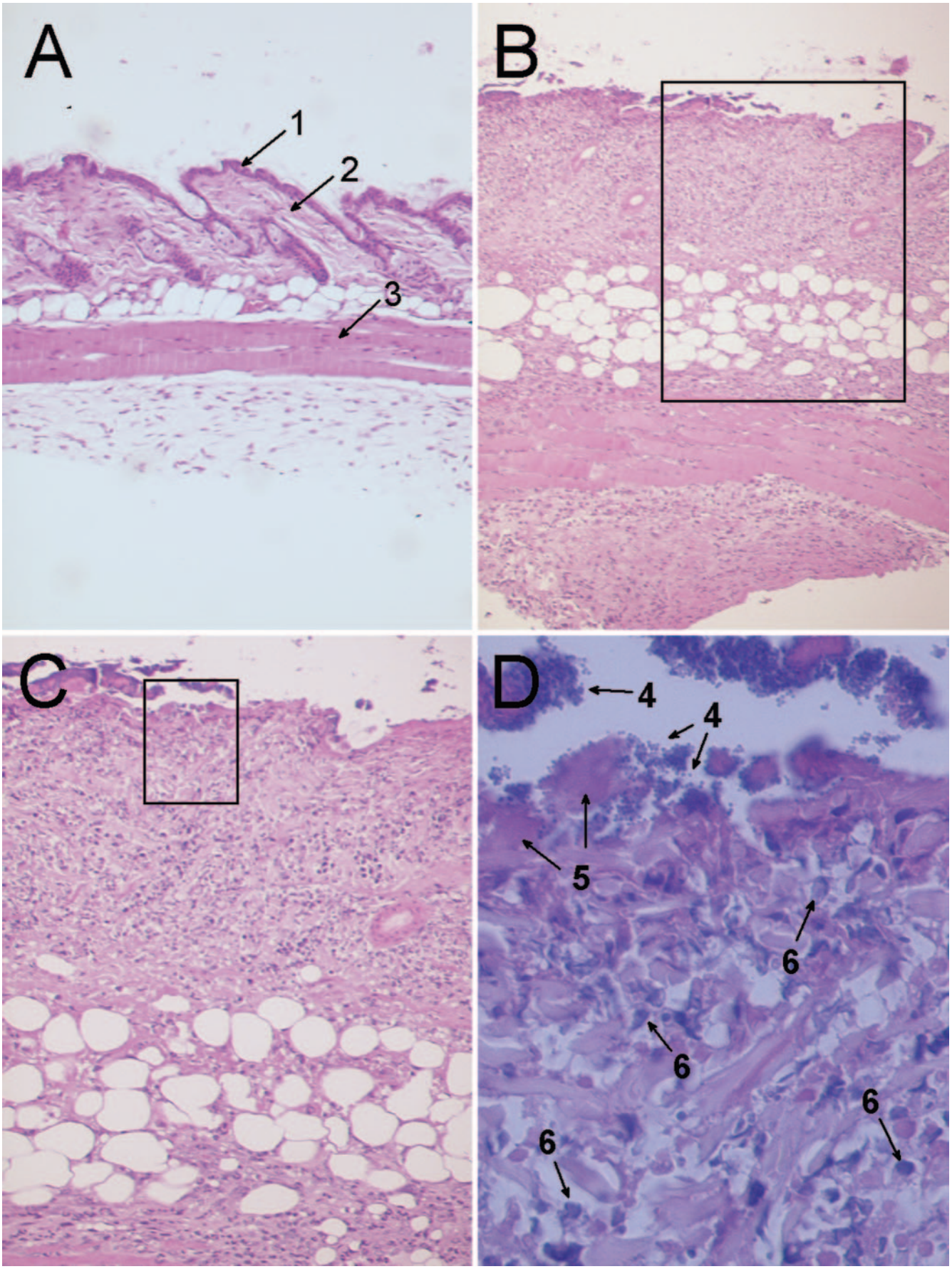
Behavioral responses of the mice. No abnormal behavioral patterns, such as fatigue, stress, or aggressiveness, were observed among the mice at any time during the course of these experiments. The mice gained, on average, 0.5 g of weight during the experiment, with no differences between the various test groups. A small fraction (2 of 48) of the mice displayed signs of deeper infections, with necrotized tissue and softening of the backbone skeletal structure (data not shown).

DISCUSSION

We have established a new model for superficial skin infections caused by *S. aureus* and *S. pyogenes* which we suggest to be a relevant and useful model for localized skin infections in humans. In contrast to previously described models for skin infection (1, 5, 14, 32, 36), the infection route in our model is topical. Partial removal of the epidermal layer of the skin allowed both *S. aureus* and *S. pyogenes* to colonize the skin and to elicit a profound inflammatory response. TEWL, which is a measure of skin barrier integrity, was used in order to ensure that the same degree of barrier disruption was achieved in all the mice and, thereby, to increase the reproducibility of the model.

The infection was maintained throughout the 4-day duration of the experiment (placebo groups). Thus, in the placebo groups, from an initial infection with 10^7 bacteria of either species, the number of CFU recovered from each wound dropped by less than 1 $\log_{10}$ over the course of the 4-day treatment. Topical treatment with FAO significantly reduced the numbers of *S. aureus* and *S. pyogenes* CFU recoverable after the 4-day treatment (Fig. 1 and 2; Table 1), showing that an established topical treatment is effective in the model. For both *S. aureus* and *S. pyogenes*, the numbers of recoverable CFU were different when the mice were left untreated for 4 days and when the mice were left untreated for 4 h (Table 1). For *S. aureus* this number was significantly higher (1.2 $\log_{10}$; $P < 0.001$) than that for the fusidic acid-treated group. For *S. pyogenes* the number of bacteria was also 1.2 $\log_{10}$ higher in the untreated groups than in the fusidic acid-treated animals, although this difference was not significantly different ($P > 0.05$). A plausible explanation for this is that *S. pyogenes* grows best under microanaerobic conditions (17) and that such conditions are more closely approximated by the presence of ointment in this assay. The genetic background of the BALB/c mice may also explain the reduced number of streptococci in the untreated group, as BALB/c mice have been shown to be much more resistant to group A streptococci than C3H/HeN mice (15). However, the use of more susceptible mouse strains may lead to an unacceptably high mortality rate, as observed in C3H/HeN when they are exposed to streptococci. In our model, BALB/c mice were used because the dominating agent in superficial infections, *S. aureus* (11, 17), colonized these mice well. BALB/c mice are also considered relevant because

FIG. 3. Histological appearance of normal dorsal skin of mice (A; magnification, ×100) and *Staphylococcus aureus*-infected skin lesion (B, magnification, ×100; C [boxed area in panel B], magnification, ×200; D [boxed area in panel C], magnification, ×1,000) on day 4. Biopsy specimens were taken immediately after the termination of the experiment, fixed in formalin, and embedded in paraffin. The biopsy specimens were stained with hematoxylin and eosin. The inflammatory cell infiltrate consists of mononuclear cells, including lymphocytes, histiocytes, and, to lesser extent, neutrophils. The inflammatory response is associated with marked fibrosis, edema, and fibrin deposition. Coccoid bacteria are present. The epidermal layer is absent in the infected lesions. Numbered arrows indicate the following: 1, epidermis; 2, dermis; 3, muscular layer; 4, bacteria; 5, fibrin deposition; 6, inflammatory cell infiltrate.



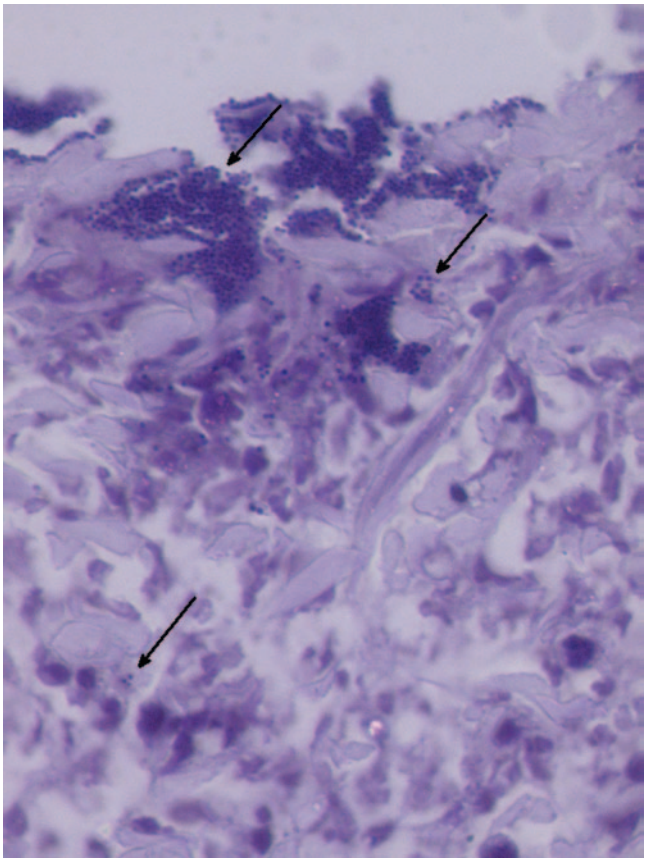


FIG. 4. Staining of gram-positive bacteria in *Staphylococcus aureus*-infected skin (magnification, ×1,000) on day 4. Biopsy specimens were taken immediately after the termination of the experiment, fixed in formalin, embedded in paraffin, and stained with Gram's crystal violet solution. Coccoid bacteria are present in the superficial layers of the dermis (arrows).

they respond immunologically to the superantigens (staphylococcal enterotoxin B) produced by *S. aureus* (29).

A pronounced and significant reduction in the numbers of CFU was achieved following 4 days of treatment with FAO. However, complete eradication of the staphylococci and streptococci was not reached after 4 days of treatment. This was expected, as superficial infections normally should be treated for 7 to 10 days in order to obtain a successful outcome (13). The fact that the infection was not cleared after this time allows the model to be used to compare various antimicrobial treatments with FAO.

Histologically, inoculation with *S. aureus* and *S. pyogenes* induced a pronounced acute inflammatory response characterized by the presence of neutrophils, lymphocytes, histiocytes, and fibrin deposition. The inflammatory response included most of the layers of the skin. Considering the histology of the infection caused by the staphylococci and streptococci, it most resembles that of human erysipelas, except that our model lacks the epidermal layer. Erysipelas is an acute bacterial infection of the dermis and subcutaneous tissues that is associated with clinical inflammation. Erysipelas is generally caused by group A streptococci (4). The model has less resemblance to the histology of impetigo, which is a contagious superficial pyogenic infection of the skin caused by *S. aureus* and *S. pyogenes*. In impetigo, the epidermis splits just below the stratum granulosum and large subcorneal pustules are formed, and these may also contain bacteria. The upper dermis contains an inflammatory infiltrate of neutrophils and lymphocytes (9). In our murine model, the formation of subcorneal pustules does not occur. We propose that our model would be a relevant disease model for localized skin structure infections caused by *S. aureus* or *S. pyogenes*, which can occur following skin barrier disruption.

The disruption of the barrier by using tape stripping resulted in a homogeneous removal of the upper epidermal layers in all the biopsy specimens examined immediately after tape stripping. Considering the numbers of CFU, the reproducibility of the model is acceptable, in that it has CVs that ranged from 12% to 36%, depending on the strain and the treatment.

The tape-stripping model presented here is relatively painless and nonintrusive for the animal. It is technically quick and simple to perform, with only a few uncomplicated steps involved in preparing the animals for treatment. In the process of validating this new model, we also performed some preliminary experiments using the established skin suture-wound model (14). In our hands, the throughput time per mouse (the total time taken to prepare a wound and inoculate it with bacteria) was approximately 2 min for the tape-stripping model, whereas it was at least 20 min for the skin suture-wound model. The reduction in the time required to process each animal by use of the tape-stripping model relative to that required by use of the skin suture-wound model provides a significant advantage when one is dealing with many animals. Considering the reduction of the numbers of CFU following treatment with FAO, the efficacy of fusidic acid is in agreement with those detected in previous investigations of FAO, e.g., by use of the skin suture-wound model (14, 28). Also, the variability in the model appears to be comparable to that detected in previous studies with the skin suture-wound model (14).

In conclusion, partial removal of the epidermal layer of BALB/c mouse skin by tape stripping allows *S. aureus* and *S. pyogenes* to colonize the skin, and this colonization is associated with an inflammatory host response, as determined by histology. Infections with both *S. aureus* and *S. pyogenes* can be treated by topical administration of FAO. The model is simple and reproducible and can be used for the evaluation of new antibiotic treatments for superficial skin infections. The model may also be advantageous for studies of the mechanisms involved in superficial skin infections.

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