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Systemic dissemination and cutaneous damage in a mouse model of staphylococcal skin infections

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Abstract

Serious staphylococcal infections frequently begin in the skin. The present study used a mouse model of such infections to evaluate the ability of *Staphylococcus aureus* to disseminate from the skin and to determine if cutaneous damage from the infections was required for dissemination. The mice were inoculated with *S. aureus* onto flank skin prepared by a tape-stripping method that caused minimal disruption of the epidermal keratinocyte layers. After these inoculations the staphylococci were found to disseminate to the spleen and kidneys of almost all animals within 6 h. Induction of leucopenia did not affect this process. Cutaneous damage was prominent in these experimental infections and included loss of the epidermis, neutrophil infiltration into the epidermis, and complete necrosis of the dermis. The latter also occurred in cyclophosphamide-treated animals, indicating that the organisms themselves and not the host inflammatory responses were responsible. Dermal necrosis did not develop until 48 h after inoculation, a time by which dissemination had already occurred. Therefore, in this mouse model system *S. aureus* is capable of penetrating the epidermal keratinocyte layers and disseminating rapidly after inoculation; the experimental infections do produce significant dermal damage, but the latter develops after dissemination has already taken place.

Keywords

Staphylococcus aureus; Cutaneous infections; Keratinocytes; Epidermis; Dermis; Bacterial dissemination

1. Introduction

Staphylococcus aureus is a major human pathogen and is particularly adept at invading and infecting the skin. Staphylococcal infections have assumed increased importance recently because of the emergence of community associated methicillin resistant *S. aureus* strains; of the infections caused by these organisms, the majority involve skin and soft tissue [1], and some of these can produce severe necrotizing fasciitis [2]. Systemic dissemination and metastatic infections are also common with staphylococcal infections. Nasal carriage of *S. aureus* probably represents the ultimate source of the organisms causing bacteremia and it has been speculated that they may reach the bloodstream after colonizing impaired skin [3]. However, we know relatively little about the relationship between staphylococcal skin infections and bacterial dissemination from this site.

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A number of experimental models of staphylococcal skin infections have been described and these show that the organisms can readily invade the epidermis and dermis to produce localized infections [4–11]. These models have generally not been used to study the relationship between staphylococcal skin infections and systemic dissemination, although in one system positive blood cultures were found after skin infection in leukopenic (but not nonleukopenic) mice [9, 10]. On the other hand, staphylococci can cause a variety of pathologic changes in the skin, including impetigo, furuncles, subcutaneous abscesses, scalded skin syndrome, and necrotizing fasciitis [2,12]; it seems reasonable that blood vessels in the damaged dermis of *S. aureus* infected skin might be compromised, thereby allowing entry of the organisms into the bloodstream.

We have recently developed an experimental model of staphylococcal skin infections in mice and in the present study have used this system to investigate the relationship between cutaneous damage and systemic dissemination of the organisms. The experimental infection system employed skin preparation by a tape-stripping method that caused minimal disruption of the epidermal keratinocyte layers, along with cultures of spleen and kidney as a sensitive method to demonstrate systemic dissemination of the organisms. The goals of these studies were to determine the incidence and timing of bacterial dissemination after inoculation of *S. aureus* onto minimally damaged skin, and to relate dissemination to the cutaneous changes caused by the local infection.

2. Results

2.1. Dissemination after cutaneous inoculation

In order to determine if the inoculated organisms could penetrate a relatively intact epidermis and disseminate systemically, cultures of spleen and kidneys were performed after the cutaneous inoculations (Fig. 1). The spleen and kidney cultures were negative after 1 h in all animals, indicating that the inoculation and retrieval procedures did not result in contamination of the organ specimens from the inoculated skin. However, by 6 h almost all of the spleen and kidney cultures had become positive, including 6 of 6 for each organ with *S. aureus* strain #25923 in C57BL/6 mice and 6 of 6 in spleen and 5 of 6 in kidney for this strain in Balb/c mice; in addition, at 6 and 24 h with all the bacterial and mouse strain combinations tested, 46 of 46 spleen cultures and 44 of 46 kidney cultures were positive. The cultures generally remained positive for the 96 h period, except for Balb/c mice, in which spleen and kidney cultures both became negative at 96 h.

The number of CFU recovered from the skin increased between 1 and 48 h, indicating proliferation of the organisms in the skin during this period. Also, studies were done with inoculations of *S. aureus* strain #25923 onto C57BL/6 mouse skin that had been shaved but not tape-stripped beforehand; the results of these experiments showed a decrease in organisms retrieved from spleen and kidney ($\log_{10}$ values of 0.3 ± 0.4 CFU in spleen and 1.0 ± 0.6 in kidney; both significantly less compared to tape-stripped skin, at $P < .001$ in each case by ANOVA and Tukey's tests). In general, treatment with cyclophosphamide to render the animals leukopenic did not significantly affect the results or produce increases in the numbers of bacteria recovered from the skin, spleen, or kidney sites. Similarly, studies with a second strain of *S. aureus* (Newman) demonstrated results like those obtained using the standard strain (ATCC #25923), and experiments using Balb/c instead of C57BL/6 mice also demonstrated equivalent findings, except as noted above.

2.2. Location of organisms in the skin

As early as 6 h after the epicutaneous inoculations onto tape-stripped skin, bacteria were seen to have invaded from the stratum corneum inoculation site into the epidermal keratinocytes

and the dermis (Fig. 2 and Fig. 3). Studies with the Newman strain also showed penetration into the epidermal keratinocyte layers and dermis at 24 and 48 h. In some cases bacteria were found within the epidermal keratinocyte layers in the absence of an inflammatory cell infiltrate (Fig. 3B). In addition, bacteria were sometimes found in the dermis (Fig. 3C) and occasionally were associated with dermal blood vessels there (Fig. 3d). The latter observation was infrequent (5 such sites found in 35 sections from 11 animals inoculated 24 h beforehand, with 4 of the 5 sites found in cyclophosphamide-treated mice). In general, distribution of the organisms in infected skin was approximately similar in Balb/c mice and in cyclophosphamide-treated C57BL/6 mice as compared to untreated C57BL/6 mice. It should be noted that loss of the epidermis at later time points reduced the numbers of fields counted that listed organisms present in this layer. Similarly, conversion of the dermis (and often the overlying epidermis) into a separate crust by the dermal necrosis process discussed below caused redistribution of the organisms from infected epidermis and dermis into crusts located above the skin surface.

Infections of hair follicle outlets were fairly frequent at 6 or 24 h after inoculation in the experimental infections (Fig. 4). However, relatively few of the hair follicle outlet infections resulted in inflammatory cell infiltration into the follicular keratinocytes. Also, deep hair follicle infections did occur at 6 or 24 h after inoculation, but they were relatively infrequent. Generally, the deep hair follicle infections observed were not associated with either infiltration of inflammatory cells or disruption of follicular integrity.

Some experiments were done with heat-killed *S. aureus* strain #25923 in C57BL/6 mice in order to determine if such organisms could be found to penetrate into deeper layers of the skin. In 3 such experiments with 6 mice in total, the percent of fields showing organisms at 24 h were as follows: stratum corneum or crusts had 3.3%, epidermal keratinocytes had 0%, and dermis had 0%; comparable values found with viable bacteria were 77.8%, 42.2%, and 24.4%. Hair follicle outlets demonstrated organisms in 3.3% with the heat-killed inoculum as compared to 60.5% with viable bacteria; there were no deep hair follicle infections seen at this time point with the heat-killed inoculum (compared to 8.8% with viable bacteria). Therefore, it appears that viable bacteria are required for entry into the skin.

2.3. Cutaneous damage from the infections

Damage to the inoculated skin from the experimental infections was significant (Fig. 5). The major types of damage seen were loss of the epidermis (Fig. 5a), infiltration of neutrophils into the epidermal keratinocyte layers (Fig. 5b), and complete necrosis of the dermis (Fig. 5c). In general, the same types of cutaneous damage developed after inoculation with *S. aureus* onto normal or cyclophosphamide-treated C57BL/6 mice, and with Balb/c mice, although the amounts of damage varied for the different experimental conditions (Fig. 6a–e). Dermal necrosis resulted in conversion of the dermis and overlying epidermis into a shrunken and discolored layer overlying the remaining fat cells of the subcutaneous tissue underneath. This process did not occur until 48 h after inoculation, but it was frequent in the infected skin; for example, with the #25923 strain of *S. aureus* in C57BL/6 mice (Fig. 6a) it affected approximately 67% of fields at 48 h after inoculation. Since dermal necrosis also occurred in cyclophosphamide-treated animals (Fig. 6b), it was apparently due to effects of the bacteria and not to those of the host inflammatory response. Studies using saline-inoculated mice demonstrated that tape-stripping alone produced only minimal amounts of cutaneous damage as compared to the experimental infections (Fig. 6e).

2.4. Characteristics of dermal bacterial foci

Each site in the inoculated skin from either cyclophosphamide-treated or untreated C57BL/6 mice at 6 h after inoculation was examined for dermal bacterial foci (presence of bacteria located below the dermal-epidermal junction), and then the characteristics of each focus with

respect to accompanying epidermal damage was assessed (Table 1). This study was undertaken to determine if the bacteria gained access to the dermis and the blood vessels located there because the epidermis had either been lost (absent epidermis) or significantly damaged by the infection process. However, most of the dermal foci were found either beneath intact epidermis or associated with hair follicle outlet infections, indicating that epidermal damage was not required for invasion into the dermis.

3. Discussion

In this model system it appears that *S. aureus* can rapidly (within 6 h) penetrate through intact keratinocyte layers of the epidermis and into the dermis below; at some point within this 6 h the invading organisms also distribute to distant organs. Although severe damage to the dermis and dermal blood vessels does develop in these infections, the visible effects would seem to occur too late (between 24 and 48 h) after inoculation to really be involved in the dissemination process. More subtle cutaneous changes might perhaps be involved, as well as a number of other routes into the bloodstream, as discussed below. The dermal necrosis process is apparently due to the bacteria themselves and not the host inflammatory cells in that it also occurs in cyclophosphamide-treated animals. Infections of hair follicles, especially at the infundibular outlets, do occur in this model system and could be involved in the dissemination process by affording the bacteria more rapid ingress to the deeper dermal blood vessels. However, deep hair follicle infections (below 100 mm from the skin surface) were relatively uncommon in the *S. aureus*-inoculated skin at early time points and did not seem to disrupt the follicle integrity or cause significant inflammatory responses. It should be noted that the two strains of *S. aureus* used in these studies have previously been found to be virulent in a variety of animal models and to produce a number of important virulence factors; the organisms are described more fully in the Methods section below.

Our staphylococcal skin infection model is based upon a number of similar ones that have been described previously. In general, the previously described experimental infections have used either intracutaneous injections of the organisms or epicutaneous application onto damaged skin with coverage by occlusive dressings in a manner similar to that used here [4,8,11]. Resulting skin changes were consistent with what we found and include hair follicle infections [4], abscess formation [9–11], epidermal necrosis [4,8], and cutaneous ulcer formation [10]. These previous model systems were developed primarily to assess local cutaneous changes from the infections rather than bacterial dissemination from the skin inoculation site. Blood cultures were performed in a model system based on intracutaneous injections of *S. aureus*, and were found to be negative in normal animals, but positive in some cyclophosphamide-treated mice (1 of 6 at 6 h and 5 of 6 at 48 h) [11]. With our experimental infections we wanted to assess the ability of the inoculated organisms to penetrate a relatively intact epidermis and disseminate systemically. Therefore, we used a mild tape-stripping method to induce stratum corneum damage while leaving the underlying keratinocyte layers relatively intact; in this model system the organisms would have to traverse the keratinocytes in order to gain access either to the bloodstream or the dermis below. Also, we felt that organ cultures might be a more sensitive method than blood cultures to determine bacterial dissemination in that residence of organisms in the bloodstream would likely be transient. These studies did demonstrate a remarkable ability of *S. aureus* organisms inoculated onto the skin surface to distribute to distant organs within 6 h.

A number of routes for bacteria to pass through the epidermal keratinocytes are possible. Small defects from shaving or tape-stripping might allow direct entry into the dermis. We did not find such defects in the 6-hr samples of saline-inoculated skin on microscopic exam, although they still could have been present in very small numbers. On the other hand, organisms were found in the dermis at 6 h in our experimental infections, and these sites were generally located

beneath an intact keratinocyte layer. Otherwise, there is evidence that *S. aureus* can either enter keratinocytes, or bypass them by damaging their intercellular connections. This organism can be internalized by either immortalized human keratinocytes [13–15] or primary keratinocytes [14–16]. Internalization appears to require the presence of fibronectin-binding proteins on the bacteria in most cases [14]. *S. aureus* ATCC 25923 does have the gene for fibronectin-binding protein A (Gen-Bank accession number EU195388), as discussed in the Methods section. Ingestion of *S. aureus* by keratinocytes may result in increased expression of β -defensins and cathelicidins by the cells and killing of the ingested bacteria [15,16]. Alternatively, interaction between keratinocytes and *S. aureus* may result in necrotic or apoptotic keratinocyte death unrelated to staphylococcal hemolysins [13], although staphylococcal α -toxin (hemolysin) is capable of killing human keratinocytes in vitro by permeabilizing the plasma membrane for monovalent ions [17]. Direct killing of epidermal cells by staphylococcal enzymes could produce cavities in the epidermis that would allow entry of the bacteria to deeper sites in the skin.

Passage between epidermal keratinocytes is another route that staphylococci might take to gain entrance to the dermis and the blood vessels located there. *S. aureus* produces an impressive array of enzymes and exotoxins that can either directly damage the skin or subvert the host's immune defenses against the invading organisms [12,18,19]. Staphylococcal exfoliative toxins produce subcorneal acantholytic cleavage that causes the bullous lesions characteristic of staphylococcal scalded skin syndrome and bullous impetigo. These enzymes have been shown to damage the intercellular junctions between keratinocytes by cleaving desmoglein 1, a desmosomal cadherin that is expressed in the upper epidermis [20]. Enzymes from *Bacteroides fragilis* and *Helicobacter pylori* act similarly on intercellular junctions of endothelial cells, although some of the proteins affected may be different [21–23]. As discussed below in the Methods section, the Newman strain of *S. aureus* is positive for exfoliative toxin, whereas ATCC #25923 is negative. We did not see acantholysis analogous to that of bullous impetigo in our experimental infections, but in mice this process generally requires use of exfoliative toxin in neonatal animals. Even so, more subtle damage to keratinocyte intercellular junctions by exfoliative toxins or other proteolytic enzymes could have allowed the organisms to transverse the epidermis more readily.

Since staphylococci inoculated onto the skin surface could rapidly make their way into the dermis, it seems likely that they might enter the bloodstream through many blood vessels located there. However, passage into the lymphatic system might be another route for systemic dissemination. In inhalational anthrax, the spores of *Bacillus anthracis* are thought to germinate within alveolar macrophages, which then carry them to regional lymph nodes [24] where they produce a destructive lymphadenitis and eventually enter the systemic circulation. Epidermal Langerhans cells are a type of dendritic antigen-presenting cell located in the epidermis and capable of ingesting *S. aureus* [25]. Epidermal Langerhans cells have been shown to migrate to regional lymph nodes [26] and could possibly carry ingested microorganisms there. In any event, distribution through the lymphatic system represents an alternative to direct bloodstream invasion as a route to distant organs for staphylococci on the skin surface.

In summary, *S. aureus* is capable of causing significant cutaneous damage after being inoculated onto the skin surface, and also has the ability to disseminate systemically from the skin within 6 h of such inoculation. However, because the major dermal damage does not develop until dissemination has already taken place, it would seem that the two processes are not directly related.

4. Experimental/materials and methods

4.1. Organisms

The inoculations were carried out with 10^7 CFU of *S. aureus* ATCC strain 25923, with some parallel studies using the Newman strain of *S. aureus*. The organisms were cultured overnight in tryptic soy broth, then washed 3 times in sterile water before use. The inoculum of 10^7 CFU was chosen from a review of previous studies [4,7,9]. *S. aureus* strain ATCC #25923 has been shown to be virulent in other animal models of staphylococcal infection [27,28]. In addition to fibronectin-binding protein A, other virulence factors and enzymes of this staphylococcal strain include the Panton–Valentine leukocidin (PVL), coagulase, catalase, DNase, heat shock protein 60, thermostable nuclease, α -toxin, and staphylococcal enterotoxins G, I, M, N, and O, but not β -lactamase, exfoliative toxins, or toxic shock syndrome toxin 1 (TSST) [27,29,30]. The Newman strain has been sequenced and found positive for enterotoxin A, exfoliative toxin A, and a variety of other exotoxins and enzymes [31].

4.2. Animals

C57BL/6 and Balb/c mice were obtained from Charles Rivers Laboratories (Wilmington, MA). C57BL/6 mice were used for most of the studies, but some experiments were repeated with Balb/c mice because they have previously been found to differ from C57BL/6 mice in sensitivity to certain *S. aureus* infections [32]. It should also be noted that mouse leukocytes appear to be more resistant to staphylococcal exotoxins such as PVL than are human leukocytes [33], suggesting that inflammatory responses to the infections may differ between species.

The animals were both male and female from 8–14 weeks of age and were described by the supplier as being free of specific pathogens (including *S. aureus*). Some mice were treated with cyclophosphamide (150 mg/kg intraperitoneally 3 days before and 100 mg/kg 1 day before the inoculations) to render them leukopenic, as previously described [34]. The mice for these studies were housed in a separate BSL 2 enhanced section of the Veterinary Medical Unit at the Milwaukee VA Medical Center. The experimental procedures were approved by the appropriate committees at the Milwaukee Veterans Affairs Medical Center and the Medical College of Wisconsin.

4.3. Epicutaneous inoculations

The skin was shaved with an electric razor, and then disinfected with iodine, washed with alcohol followed by saline, and then dried with gauze. The skin surface was prepared by gentle tape-stripping 7× with Transpore tape (approximately 27 mm in width, from 3 M, Minneapolis, MN). As shown in Fig. 6e, this technique was found to cause only minimal damage to the epidermis or dermis. An inoculum of 10^7 *S. aureus* CFU in 0.025 ml of saline was added to 4 mm filter paper discs placed on prepared skin of the animal's left flank, with saline on the opposite side. Both sites were covered with 1.0 cm² pieces of plastic sheet and overwrapped with dressings of Transpore tape and Nexcare waterproof tape (3 M). Photographs of the tape-stripping and inoculation procedures are shown in Fig. 7.

4.4. Monitoring of infections

After 1–24 h the occlusive dressings were removed and the sites washed 4 times with saline-soaked gauze pads. At various times from 1–96 h after inoculation the animals were killed and skin removed from the inoculated sites for histology. Paraffin sections were prepared and stained with tissue gram stains, with analysis as discussed below. In some cases when the mice were killed, skin scrapings or homogenates of spleen or kidneys in 1 ml of saline were cultured on tryptic soy agar, with results recorded as CFU per ml. For skin scrapings the entire inoculation site was sampled by abrasion with a scalpel blade until a glistening surface could

be seen; the material removed was vortexed in 1 ml of saline and then cultured for CFU determinations.

Sections of either *S. aureus* or saline-inoculated skin were examined in a blinded fashion for bacteria and cutaneous changes at a 400× magnification under light microscopy. For the purposes of this study, the epidermis was defined as the epidermal keratinocyte layers above the dermal–epidermal junction, but excluding the stratum corneum or crusts. In ten random fields in each section the site of bacteria location was determined as being in the stratum corneum or crusts, the epidermis, the dermis, or combination thereof. Each hair follicle infundibular outlet across the sections was also examined for the presence of bacteria, with the data being recorded as percent of outlets infected and percent of outlets with inflammatory cell infiltrates present. In addition, the hair follicles located in the dermis were also examined for the presence of bacteria located greater than 100 mm below the skin surface; the data were recorded as the percent of hair follicles with deep infections. It should be noted that mouse skin structure is generally similar to that of humans except for a thinner epidermis and grouping of hair follicles together in areas of ones that are actively growing (anagen) or those that are resting (telogen) [35].

Cutaneous damage was also assessed for each linear high power (400×) field across the section's entire epidermis as absent epidermis, neutrophil infiltration into the keratinocyte layers, or complete dermal necrosis. The latter represented conversion of the entire dermis into a discolored amorphous crust. Examples of cutaneous damage are shown in Fig. 5.

Characteristics of dermal bacterial foci were assessed from sections of inoculated skin taken at 6 h in order to determine if epidermal damage was required for invasion of the organisms into the dermis. Dermal bacterial foci were defined for the purposes of this study as foci of bacteria located below the dermal–epidermal junction of the inoculated skin. The foci were evaluated to determine if they were associated with a hair follicle or were located beneath epidermis with an intact keratinocyte layer; otherwise, epidermal damage associated with the dermal foci was assessed as absent epidermis (with the epidermal keratinocyte layers missing entirely) or as those with epidermis disrupted with or without host inflammatory cells being present. Such data were generated from cyclophosphamide-treated or untreated C57BL/6 mice inoculated with the ATCC #25923 strain of *S. aureus*, and were expressed as the percentages of each type of dermal focus found.

4.5. Statistics

Data were expressed as mean ± SE, with statistics carried out in the GraphPad Prism 4.0c statistical package using ANOVA and Dunnett's test for multiple comparisons. Generally 4–12 mice per point were studied in 3–5 experiments (each consisting of animals inoculated in a similar manner on a single day). Statistical significance was taken as $P < .05$.

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Fig. 1.

S. aureus colony forming units (CFU) found in skin (checked bars), spleen (solid bars), and kidney (open bars) at various times (in hrs on the X-axis) after epicutaneous inoculation. A. *S. aureus* ATCC strain #25923 in C57BL/6 mice; B. *S. aureus* ATCC strain #25923 in cyclophosphamide-treated C57BL/6 mice; C. *S. aureus* strain Newman in C57BL/6 mice; D. *S. aureus* strain ATCC #25923 in Balb/c mice. Note in panel A that no CFU were found in kidney and spleen at 1 h after inoculation, indicating lack of contamination during the organ harvesting procedure; also, the difference in skin CFU between 1 and 48 h was significant ($P < .001$ by ANOVA and Tukey's tests), indicating growth at the skin surface. Data are from 4–13 animals per point, tested in 2–5 experiments.

Fig. 2.

Location of bacteria in different skin layers, expressed as percent of microscopic fields with organisms at each site on the Y-axis, for locations in the stratum corneum or crusts (checked bars), epidermal keratinocytes (solid bars), or dermis (open bars); times after epicutaneous inoculation are given in hrs on the X-axis. A. *S. aureus* ATCC strain #25923 in C57BL/6 mice; B. *S. aureus* ATCC strain #25923 in cyclophosphamide-treated C57BL/6 mice; C. *S. aureus* strain Newman in C57BL/6 mice; D. *S. aureus* strain ATCC #25923 in Balb/c mice. Data are from 4–11 animals per point, studied in 3–5 experiments.

Fig. 3.

Location of bacteria after epicutaneous inoculation of C57BL/6 mice with *S. aureus* ATCC strain #25923 24 h previously (photomicrographs of tissue gram-stained sections at 1000× original magnification under oil immersion): A. Section of normal uninoculated C57BL/6 mouse skin with the bracket marking the epidermal keratinocyte layers; the stratum corneum lies above these layers and the dermis lies below; B. Bacteria located in epidermal keratinocytes (arrow); C. Bacteria located in the dermis (arrow); D. Bacteria associated with a dermal blood vessel in a cyclophosphamide-treated mouse.

Fig. 4. Infected hair follicle outlets (checked bars), infected hair follicle outlets with inflammatory cell infiltration (solid bars), and deep (greater than 100 μ m from the skin surface) hair follicle infections (open bars) at 6 or 24 h after inoculation with *S. aureus* ATCC strain #25923 in C57BL/6 mice.

Fig. 5. Types of cutaneous damage after epicutaneous inoculation of C57BL/6 mice with *S. aureus* 24 h previously (photomicrographs of tissue gram-stained sections at 1000×): A. Loss of epidermis with organisms present in the superficial dermis; B. Infiltration of neutrophils into the epidermis, with associated bacteria (arrow); C. Dermal necrosis (arrows).

Fig. 6.

Quantitation of cutaneous damage after experimental cutaneous *S. aureus* infections, including loss of epidermis (checked bars), neutrophil infiltration into the epidermal keratinocyte layers (solid bars), and dermal necrosis (open bars) on the Y-axis at various times after epicutaneous inoculation (in hrs on the X-axis). A. *S. aureus* ATCC strain #25923 in C57BL/6 mice; B. *S. aureus* ATCC strain #25923 in cyclophosphamide-treated C57BL/6 mice; C. *S. aureus* strain Newman in C57BL/6 mice; D. *S. aureus* strain ATCC #25923 in Balb/c mice; E. Inoculation with saline alone. Note the minimal damage that occurred after inoculation with saline alone; also, at 24 h loss of the epidermis was greater and neutrophil infiltration into the epidermis was less in cyclophosphamide-treated than normal mice ($P < .01$ and $.05$ respectively by ANOVA and Tukey's tests). Data represent mean $\pm$ SE of percent of fields (of 10 counted per section) showing cutaneous damage; results are from 4–11 animals studied in 3–5 experiments per point.

Fig. 7. Stages of the epicutaneous inoculation procedure. A. Appearance of skin after shaving and tape-stripping; B. Arrangement of filter disc and plastic covering after inoculation of *S. aureus* onto the disc.

Table 1

Characteristics of dermal bacterial foci after epicutaneous *Staphylococcus aureus* inoculation onto tape-stripped skin of mice.

Type of Dermal Focus	CY ^a -Treated Mice	Untreated Mice
HF-Associated ^b	40.0 ± 24.5	27.7 ± 7.2
Epidermis Intact	43.3 ± 20.9	44.2 ± 8.3
Epidermis Absent	11.0 ± 11.0	4.4 ± 3.0
Epidermis Damaged with IC ^c	0.0 ± 0.0	11.4 ± 4.7
Epidermis Damaged w/o IC	0.5 ± 0.5	2.4 ± 1.7

^a CY = Mice treated with cyclophosphamide to render them leukopenic.

^b HF-Associated = Dermal foci associated with a hair follicle.

^c Epidermis damaged with IC = Epidermal keratinocyte layers disrupted with or w/o host inflammatory cells (IC) being present. Data represent mean ± SE of percent of dermal bacterial foci found of the various types; dermal foci were counted as sites where bacteria were found below the dermal-epidermal junction of infected skin, with data from 4 CY and 10 normal animals studied in 3–5 experiments.