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Direct translocation of staphylococci from the skin surface to deep organs

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Abstract

Background—*Staphylococcus aureus* can invade the bloodstream and cause bacteremic infections, but this organism frequently produces serious deep infections without bacteremia or an identifiable portal of entry.

Methods—We used experimental cutaneous *Staphylococcus aureus* infections in mice to determine if the bacteria could reach deep organs without travel through the bloodstream.

Results—After skin surface application the bacteria rapidly distributed to lymph nodes, spleen, kidneys and other organs. In these animals, blood cultures were negative, dissemination was more efficient after surface application than injection near dermal blood vessels, and kidney bacterial localization sites were unlike those of bacteremic infections. Whereas normal mice eventually cleared bacteria from the deep sites, those with prolonged immunosuppression became moribund from these infections; they also had negative blood cultures and kidney localization not consistent with hematogenous dissemination. Bacteria were also found in the intervening abdominal wall outside the spleen and kidney sites, suggesting direct movement of the organisms from the skin surface through connecting tissues.

Conclusions—Although capable of hematogenous dissemination, *S. aureus* can also spread from skin to deep organs by a non-bacteremic process. In this case the bacteria appear to migrate directly from the skin surface to the deep organs below.

Keywords

Staphylococcus aureus; cutaneous infection; translocation; bacteremia

1. Introduction

Staphylococcus aureus frequently causes serious deep infections in the absence of an identifiable portal of entry. This organism is clearly capable of invading the bloodstream and producing bacteremic infections. It has been suggested that staphylococci from nasal colonization sites may infect compromised skin and spread into the bloodstream from there

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[1]. On the other hand, deep staphylococcal infections often occur without documented bacteremia; in this case, it is generally assumed that an unnoticed skin infection may have caused bloodstream invasion that ended before the deeper infection was recognized. In truth, there is very little direct information available about the origins of such deep staphylococcal infections.

Recently we have found in experimental cutaneous infections in mice that staphylococci regularly disseminate from skin to spleen and kidneys within 6–24 hrs [2,3]. Spread to deep organs occurred before significant cutaneous invasion and it seemed possible that a process other than bacteremia might be involved. The following experiments were undertaken to determine if bacteria from the skin traveled to the deep organs through the bloodstream, or if a different route might be involved such as direct passage through intervening tissues.

2. Methods

2.1 Microorganisms

The inoculations were carried out with 10^7 CFU of *Staphylococcus aureus* ATCC strain 25923. The organisms were cultured overnight in tryptic soy broth, and then washed 3 times in sterile water before use. The inoculum of 10^7 CFU was chosen from a review of previous studies of experimental staphylococcal skin infections [4–6]. *S. aureus* strain ATCC #25923 has been shown to be virulent in other animal infection models [7,8] and the virulence factors and enzymes of this staphylococcal strain have been fairly well described [7,9,10].

2.2 Animals

C57BL/6 mice were obtained from Charles Rivers Laboratories (Wilmington, MA). The animals were 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), as previously described [11]; some animals received an extra dose of 100 mg/kg on day 3 to maintain immunosuppression. Our previous work has shown that uninfected animals treated with this regimen recovered a few days after the last cyclophosphamide dose. 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.

2.3 Inoculations

For the *S. aureus* inoculations, the skin over the flanks was shaved with an electric razor, 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 3M, Minneapolis, MN). This technique disrupted the outer stratum corneum, but caused minimal damage to the epidermis or dermis [2]. 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 both flanks (or in some cases only the left flank) and the sites were covered with 1.0 cm² pieces of plastic sheet and then overwrapped with dressings of Transpore tape and Nexcare waterproof tape (3M). The dressings served to prevent spread of the organisms outside the inoculation site and to prevent ingestion of the inoculum by the test animal or its cagemates. In some cases the same inoculum of *S. aureus* was injected intradermally or subcutaneously into the flank sites, or given by intravenous injection into the lateral tail vein. The animals were monitored carefully, and those that became moribund (relatively inactive, showing ruffled fur, or being unable to eat or drink) were killed at that point and processed as discussed below.

2.4 Monitoring of Infections

After 1, 3, 6, or 24 hrs the occlusive dressings were removed and the sites washed 4 times with saline-soaked gauze pads. At various times after inoculation the animals were killed and tissues harvested for either histology or cultures. Blood was taken by cardiac puncture. An incision was made through the skin of the upper thorax, and the skin peeled down to expose a sterile surface overlying the peritoneal cavity and inguinal region. Inguinal subcutaneous tissue containing draining lymph nodes was removed and saved. In some cases sterile saline was injected into the peritoneal cavity and then withdrawn for culture, or a sample of abdominal wall was removed for culture. Upon opening the peritoneal cavity, the spleen and both kidneys were removed, and in some cases, samples of liver and lung were also taken. In one series of experiments only the left flank was inoculated, and left and right kidneys were cultured to determine if the one closest to the inoculation site (i.e., the left kidney) would show more bacteria on cultures. Tissue samples for histology were placed into formalin and then processed for routine histology with paraffin sections stained with a tissue gram stain. The latter were analyzed as discussed below. Samples of lymph nodes, spleen, kidney, abdominal wall, lung, or liver were homogenized in saline to provide a total volume of 1 ml; for lymph node tissue, abdominal wall, liver or lung a specimen was taken that was approximately the same size as the kidney or spleen. The homogenates, as well as whole blood or peritoneal fluid, were cultured on tryptic soy agar in a volume of 0.1 ml. Results were recorded as colony forming units per 0.1 ml. Growth on the plates was confirmed to be *S. aureus* by both colony morphology and gram stains on an as-needed basis.

In order to determine if the bacteria could survive in mouse blood, 0.1 ml samples of blood taken by cardiac puncture from 3 mice were heparinized and then inoculated with approximately 100 CFU of *S. aureus*. After incubation for 2 hrs at 37 degrees C these specimens were cultured on tryptic soy agar and the resulting CFU were compared to those from parallel samples where the organisms were incubated in either saline or distilled water.

2.5 Histology

Histologic sections of kidneys and in some cases the lungs or liver were stained with tissue gram stains and carefully examined for the presence of bacteria. Kidneys were selected for most intensive study because the second one was available for histology and because staphylococcal localization sites have been well described for this organ in previous mouse bloodstream infection studies – showing a characteristic parenchymal abscess pattern [12–14]. Kidney specimens from either control or cyclophosphamide-treated mice were examined; the tissues were taken from 6 hrs to 5 days after inoculation. Similar kidney specimens from mice after intravenous inoculation with *S. aureus* were evaluated in a similar manner. Sites examined for the presence of bacteria included the entire capsule, connective tissue (outside of the capsule) associated with attached adrenals or the hilum, the adrenal glands, the cortex (with at least 30 glomeruli examined), the tubules in the medulla, and whatever blood vessels could be seen.

2.6 Statistics

Data were expressed as mean $\pm$ SE, with statistics carried out in the GraphPad Prism 5.0 for Mac OS X statistical package using ANOVA and Bonferroni's test for multiple comparisons. Generally 4–6 mice per point were studied in 2–4 experiments (each consisting of animals inoculated in a similar manner on a single day). Statistical significance was taken as $P < .05$.

3. Results

As shown in Table 1 the tissues sampled at the earliest time point (1 hour) showed very few *S. aureus* CFU, except for the abdominal wall samples of which 5 of 6 were positive; at this time most of the spleen, kidney, and lymph node samples were negative. By 3–6 hrs, more of the spleen, kidney, and lymph node specimens had become positive, with the abdominal wall samples showing the most bacteria. Another finding shown in Table 1 is that whereas over half of the spleen and kidney cultures were positive at 6 hrs, all the corresponding blood cultures (of 20) were negative; blood cultures were also negative at the 1 and 3 hour time points. In contrast, when the inoculum was given intravenously, all of the 6 hour blood cultures were positive (8 of 8). We also did an in vitro study to determine if *S. aureus* could survive in mouse blood for the approximately 2 hour period required for tissue processing. After a 2 hour incubation in 0.1 ml of heparinized mouse blood or equivalent volumes of saline or distilled water, an initial inoculum of 178 ± 76 CFU of *S. aureus* yielded 487 ± 103 CFU in blood versus 49 ± 12 in saline and 26 ± 11 in distilled water (means of data from 3 experiments). Therefore, the organisms do appear to persist quite well in mouse blood during the time needed to process the samples.

In that it seemed possible for the bacteria to be travelling directly from the flank inoculation site to the kidney underneath we inoculated only the left flank, and then compared CFU in the left and right kidneys. In each of 6 mice studied in this fashion in 3 experiments, the left kidney was found to have more CFU on culture (left kidney with range of 1 to 79 CFU versus right kidney with range of 0 to 1 CFU). This finding supports the hypothesis that the bacteria did migrate directly from the skin surface to the kidney below, rather than being carried by the bloodstream in which case the numbers in both kidneys should have been similar.

As an alternative way to evaluate bacterial spread from the skin surface, we used different inoculation methods to associate the bacteria more closely with the cutaneous blood vessels. Since the latter are found in the dermis and subcutaneous tissue (and not in the epidermis), we compared deep organ culture results after skin surface (epicutaneous), intradermal, and subcutaneous inoculation. If blood vessel invasion caused dissemination to the deep organs, then organism numbers in these organs should be highest with inoculation by injection into the tissues nearest the blood vessels. As seen in Figure 1, spleen and kidney CFU were actually highest when the bacteria were applied epicutaneously; therefore, blood vessel invasion and hematogenous spread seems less likely.

We next used an analysis of bacterial localization sites in the kidneys to evaluate staphylococcal travel in these infections. As shown in Figure 2, after epicutaneous inoculation we found the bacteria in an unusual site - namely in connective tissue outside the renal capsule, often near the renal hilum and/or between the adrenal glands and the renal capsule (Fig. 2A, Table 2). In immunosuppressed mice moribund from the infections at day 4–5, the bacteria were also found in this location but in much greater numbers (Fig. 2B). In contrast, renal abscesses (which were located in renal parenchyma within the capsule) were identified only in animals given the *S. aureus* inoculum intravenously (Fig. 2C, Table 2). These abscesses were more prominent at 4–5 days and represented much larger foci than were seen in the outer connective tissue sites in a few of these animals. None of the animals with intravenous inoculation (total of 23) had outer connective tissue foci in the absence of parenchymal abscesses (data not shown). The immunosuppressed mice inoculated epicutaneously also had bacteria invading from the liver surface (Fig. 2D). Bacteria were found mostly at times after 6 hrs (Table 2), presumably because proliferation after initial dissemination made them more visible then. The organisms in the outer connective tissue

site were within the tissue and often separated from the surface by other structures, as shown in Fig. 3A&B.

Previously we found the staphylococci in spleen and kidney to disappear over time even after a short course of cyclophosphamide; therefore, for the present study we prolonged the immunosuppression to determine the fate of the disseminated bacteria. As shown in Table 3, the spleen and kidney bacterial numbers in the mice with prolonged immunosuppression to markedly increased over time and the animals became moribund from the infection by day 4–5. Blood cultures were done in 4 of these animals and all were negative (data not shown). As noted above (Fig. 2B and Table 3), the kidney localization site (outer connective tissue) was the same as in non-immunosuppressed animals, but the bacterial numbers appeared much larger. Thus, the dissemination process described in the present study can produce fatal infections in appropriately immunosuppressed hosts.

4. Discussion

The findings discussed above suggest that staphylococci from the skin surface inoculation sites in these experimental infections did not get to the deep organs by either contamination during processing or by dissemination through the bloodstream. If contamination during processing caused the positive kidney cultures, then the bacteria should have been found on the outside of the specimens. However, they were actually found within the connective tissue and often separated from the surface by other structures, as discussed above. It could also be argued that bacteria in the outer connective tissue were more visible microscopically than those within the internal renal parenchyma; however, small numbers of staphylococci are easily visible in tissue of similar density such as the epidermis [2] and the liver. In addition, large and easily visible collections of bacteria were seen in the immunosuppressed mice, but only in the outer connective tissue sites and not within the capsule. Many previous studies on staphylococcal dissemination after intravenous inoculation have found that the resulting infections in the kidneys show a characteristic pattern of parenchymal abscesses within the renal capsule [12–14]. Therefore, the outer connective tissue localization site (outside the renal capsule) of the bacteria after cutaneous inoculation indicates that after skin infection the organisms did not travel to the kidneys through the bloodstream.

It has recently been reported that after injection into the ears of mice, spores of *Bacillus anthracis* localized to draining lymph nodes within 2–3 minutes and to liver and spleen within 1 hour (although blood cultures were negative) [15]. This rapid dissemination to deep organs parallels our findings with *S. aureus*. Therefore, such rapid dissemination of bacteria from the skin could be a general phenomenon for bacterial infections, and may involve initial travel through local lymphatics. We considered the latter possibility in our system. We found that at 3 hrs after inoculation the inguinal lymph nodes did have positive cultures, suggesting early bacterial entry into interstitial fluid and lymph. Spread through some kind of lymphangitis process is certainly possible. The skin does have a rich lymphatic system that drains away lymph originating from epidermal interstitial fluid; it involves a complex vasculature that eventually merges with larger lymphatics such as the thoracic duct [16,17]. The latter terminates in the venous system, indicating that bacteria in major lymph vessels should end up in the bloodstream. Drainage of staphylococci to the renal lymph nodes is another possibility [18]. However, these nodes were not included in our culture specimens. Although the bacteria in our experimental infections do not appear to pass through the larger lymphatics and into the bloodstream, it is possible that they may be carried by smaller lymph vessels or channels through the skin and subcutaneous tissue to the underlying organs.

If the bloodstream and/or major lymph vessels do not carry the bacteria to deep organs in these infections, then some other process must be involved. Finding bacteria in abdominal wall cultures suggests direct migration between inoculated skin and deep organs underneath. The mechanisms involved would have to account for small numbers of bacteria crossing distinct tissue planes without damaging the intervening tissues. In fact, the previously described phenomenon of bacterial translocation would appear to have this characteristic. Bacterial translocation is closely defined as the transit of bacteria from the gut lumen to extra-intestinal tissues [19–23], and likely involves escape the intestine by one of 3 mechanisms: (a) passage between cells of damaged mucosa (extracellular route); (b) passage through intact epithelial cells (intracellular route); and (c) ingestion and carriage by intestinal macrophages during their immune surveillance function (phagocytic cell route). Each of the 3 may be involved at times. In our earlier studies, free bacteria were often found in dermis underneath intact keratinocyte layers [2], suggesting a similar translocation process in the skin.

In our system, the staphylococci in spleen and kidney disappeared over time even after a short course of cyclophosphamide; however, for the present study, prolonging the immunosuppression caused the spleen and kidney bacterial numbers to markedly increase and the animals to become moribund from the infection by day 4–5. As in non-immunosuppressed animals, blood cultures were negative and the bacteria were also found in the outer connective tissue site. Thus, the dissemination process described in the present study can produce fatal infections in appropriately immunosuppressed hosts.

Epicutaneous inoculation (as opposed to deeper injections) may cause entry into interstitial fluid over a large surface area, thereby augmenting travel to deep organs. Such travel means crossing intervening tissue planes, as with bacteria translocating from the gut. The kidney occupies a retroperitoneal location and the outer connective tissue site may be more directly connected to the inoculated skin than is the rest of the organ. Carriage by host immune surveillance cells (i.e., macrophages or epidermal Langerhans cells) might be possible, but the rapid times involved and unusual distribution in the kidney make this consideration less likely. Staphylococci themselves are non-motile, but locomotive force could be applied to interstitial fluids containing them by respiration or other animal movements [25].

In summary, it would appear that after inoculation to the skin surface *S. aureus* can rapidly travel through intervening tissues to reach deep organs situated beneath the infection site. The direct travel of bacteria through tissues rather than through the bloodstream is likely more relevant for shorter distances. However, this type of bacterial movement could pertain particularly to invasion from skin to directly underlying sites in adjacent organs, bones, joints, or implanted foreign materials.

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References

1. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. *New Engl J Med*. 2001; 344:11–16. [PubMed: 11136954]
2. Hahn BL, Onunkwo CC, Watts CJ, Sohnle PG. Systemic dissemination and cutaneous damage in a mouse model of staphylococcal skin infections. *Microb Pathogen*. 2009; 47:16–23. [PubMed: 19397991]

3. Onunkwo CC, Hahn BL, Sohnle PG. OClearance of experimental cutaneous *Staphylococcus aureus* infections in mice. Arch Dermatol Res. 2010; 302:375–82. [PubMed: 20130894]
4. Abe Y, Akiyama H, Arata J. Furuncle-like lesions in mouse experimental skin infections with *Staphylococcus aureus*. J Dermatol. 1993; 20:198–202. [PubMed: 8315108]
5. Kugelberg E, Norstrom T, Petersen TK, Duvoid T, Andersson DI, Hughes D. Establishment of a superficial skin infection model in mice by using *Staphylococcus aureus* and *Streptococcus pyogenes*. Antimicrob Agents Chemother. 2005; 49:3435–41. [PubMed: 16048958]
6. Molne L, Tarkowski A. An experimental model of cutaneous infection induced by superantigen-producing *Staphylococcus aureus*. J Invest Dermatol. 2000; 114:1120–25. [PubMed: 10844554]
7. Callegan MC, Hobden JA, Hill JM, Insler MS, O'Callaghan RJ. Topical antibiotic therapy for the treatment of experimental *Staphylococcus aureus* keratitis. Invest Ophthalmol Vis Sci. 1992; 33:2017–23. [PubMed: 1399405]
8. Cirioni O, Giacometti A, Ghiselli R, Kamysz W, Orlando F, Mocchegiani F, et al. Temporin A alone and in combination with imipenem reduces lethality in a mouse model of staphylococcal sepsis. J Infect Dis. 2005; 192:1613–20. [PubMed: 16206076]
9. Blaiotta G, Ercolini D, Pennacchia C, Fusco V, Casaburi A, Pepe O, et al. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus spp.* strains isolated from meat and dairy products. Evidence for new variants of seG and seI in *S. aureus* AB-8802. J Applied Microbiol. 2004; 97:719–30. [PubMed: 15357721]
10. Monecke S, Berger-Bachi B, Coombs G, Holmes A, Kay I, Kearns A, et al. Comparative genomics and DNA array-based genotyping of pandemic *Staphylococcus aureus* strains encoding Pantone-Valentine leukocidin. Clin Microbiol Infect. 2007; 13:236–49. [PubMed: 17391377]
11. Sohnle PG, Hahn BL. Inhibition of pseudohyphal growth as a neutrophil-mediated host defense mechanism against experimental deep *Candida albicans* infections in mice. J Lab Clin Med. 1993; 121:235–43. [PubMed: 8433039]
12. Richardson AR, Libby SJ, Fang FC. A nitric oxide-inducible lactate dehydrogenase enables *Staphylococcus aureus* to resist innate immunity. Science. 2008; 319:1672–76. [PubMed: 18356528]
13. Cheng AG, McAdow M, Kim HK, Bae T, Missiakas DM, Schneewind O. Contribution of coagulases towards *Staphylococcus aureus* disease and protective immunity. Plos Pathogens. 2010; 6:e1001036.10.1371/journal.ppat.1001036 [PubMed: 20700445]
14. Albus A, Arbeit RD, Lee JC. Virulence of *Staphylococcus aureus* mutants altered in type 5 capsule production. Infect Immun. 1991; 59:1008–14. [PubMed: 1847696]
15. Corre JP, Piris-Gimenes A, Moya-Nilges M, Jouvion G, Fouet A, Glomski JJ, et al. In vivo germination of *Bacillus anthracis* spores during murine cutaneous infection. J Infect Dis. 2013; 207:450–7. [PubMed: 23148288]
16. Skobe M, Detmar M. Structure, function, and molecular control of the skin lymphatic system. J Invest Dermatol Symposium Proc. 2000; 5:4–19.
17. Oliver G, Detmar M. The rediscovery of the lymphatic system: old and new insights into the development and biological function of the lymphatic vasculature. Genes & Dev. 2002; 16:773–83. [PubMed: 11937485]
18. Harrell ML, Iritani BM, Ruddell A. Lymph node mapping in the mouse. J Immunol Methods. 2008; 332:170–4. [PubMed: 18164026]
19. Edmiston CE, Condon RE. Bacterial translocation. Surg Gynecol Obstet. 1991; 173:73–83. [PubMed: 1866678]
20. O'Boyle CJ, MacFie J, Mitchell CJ, Johnstone D, Sagar PM, Sedman PC. Microbiology of bacterial translocation in humans. Gut. 1996; 42:29–35. [PubMed: 9505882]
21. Berg RD. Bacterial translocation from the gastrointestinal tract. Trends Microbiol. 1995; 3:149–154. [PubMed: 7613757]
22. Wells CL, Maddaus MA, Simmons RL. Proposed mechanisms for the translocation of intestinal bacteria. Rev Infect Dis. 1988; 10:958–79. [PubMed: 3055194]
23. Mora EM, Cardona MA, Simmons RL. Enteric bacteria and ingested inert particles translocate to intraperitoneal prosthetic materials. Arch Surg. 1991; 126:157–63. [PubMed: 1825166]

24. Hess DJ, Garni RM, Henry-Stanley MJ, Wells CL. *Escherichia coli* modulates extraintestinal spread of *Staphylococcus aureus*. Shock. 2005; 23:376–81. [PubMed: 16205324]
25. Ikomi F, Hund J, Hanna G, Schmidt-Schonbein GW. Interstitial fluid, plasma protein, colloid, and leucocyte uptake into initial lymphatics. J Appl Physiol. 1996; 81:2060–7. [PubMed: 8941530]

Highlights

- *Staphylococcus aureus* rapidly spread to deep organs in mice after skin inoculation
- Blood cultures were negative in these experimental infections
- Kidney histology was different after skin versus bloodstream infection
- The skin infections caused death in mice with prolonged leucopenia
- Thus, *S. aureus* can travel from skin to deep organs without bloodstream invasion

Fig. 1.

Dissemination of bacteria to spleen and kidney after epicutaneous inoculation or intradermal or subcutaneous injection of *Staphylococcus aureus*. Values represent log₁₀ CFU (mean ± SE) cultured from 0.1 ml specimens of spleen (SP) or kidney (KD) after epicutaneous inoculation (EPI) or intradermal (ID) or subcutaneous (SC) injection of 10⁷ *S. aureus* onto or into the flank skin of mice 24 hrs previously. Values for SC injection compared to EPI inoculation were significantly less ($P < .05$ and $.01$ respectively for SP and KD by ANOVA and Bonferroni's tests).

Fig. 2.

Microscopic location of bacteria in kidney and liver after epicutaneous or intravenous inoculation. For epicutaneous inoculation, the organisms were applied to the flank skin under occlusive dressings. Intravenous injections of the inoculum were given into the lateral tail vein. Immunosuppression was induced by intraperitoneal injections of cyclophosphamide (CY), with some animals receiving an extra dose at day 3 to maintain immunosuppression. Paraffin sections were stained with a tissue gram stain. **(A)** Photomicrograph at 24 hrs of bacteria in outer connective tissue outside the renal capsule in a non-immunosuppressed mouse after epicutaneous inoculation (1000 $\times$; bacteria shown by the arrow, renal surface by the arrowhead; bar = 10 microns). **(B)** Photomicrograph at 4 days of bacteria in outer connective tissue outside the renal capsule in a mouse with prolonged immunosuppression after epicutaneous inoculation (1000 $\times$; bacteria shown by the arrow, renal surface by the arrowhead; bar = 10 microns). **(C)** Photomicrograph at 4 days of a large abscess with prominent microorganisms in renal parenchyma inside the renal capsule after intravenous inoculation of *S. aureus* (400 $\times$; bar = 25 microns). **(D)** Photomicrograph at 4 days of bacteria at the liver surface after epicutaneous inoculation in a mouse with prolonged immunosuppression (1000 $\times$; bacteria shown by the arrow; bar = 10 microns).

Fig. 3.

Low power photomicrographs of bacterial localization sites in kidney. **(A)** Photomicrograph at 24 hrs of location where bacteria were found in outer connective tissue (marked by an X) at the renal hilum in a non-immunosuppressed mouse after epicutaneous inoculation (100 $\times$; bar = 100 microns). **(B)** Photomicrograph at 24 hrs of location where bacteria were found in outer connective tissue outside the renal capsule (marked by an X) in a non-immunosuppressed mouse after epicutaneous inoculation (100 $\times$; bar = 100 microns).

Table 1

Distribution of *Staphylococcus aureus* bacteria to various tissues after epicutaneous (or intravenous) inoculation.

<u>Tissue</u>	<u>Cultures positive for <i>S. aureus</i> at different sites (#pos/#tested)</u>		
	<u>1 Hr</u>	<u>3 Hrs</u>	<u>6 Hrs</u>
Lymph Node	0/6	2/5	11/14
Abdominal Wall	5/6	5/7	4/5
Peritoneal Cavity	NT	0/5	2/15
Spleen	3/10	4/12	13/22
Kidney	1/14	4/12	12/22
Blood	0/6	0/12	0/20
Blood IV	NT	NT	8/8
Liver	NT	NT	2/6
Lung	NT	NT	1/6
<u><i>S. aureus</i> CFU isolated from different sites (Mean $\pm$ SE)</u>			
Lymph Node	0.0 $\pm$ 0.0	5.0 $\pm$ 4.8	11.2 $\pm$ 4.0
Abdominal Wall	10.2 $\pm$ 5.4	278.0 $\pm$ 275.0	110.0 $\pm$ 61.7
Spleen	0.6 $\pm$ 0.3	0.6 $\pm$ 0.3	110.0 $\pm$ 62.3
Kidney	0.6 $\pm$ 0.6	4.3 $\pm$ 3.6	29.2 $\pm$ 15.3
Blood	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Blood IV	NT	NT	4.6 $\pm$ 1.4
Liver	NT	NT	1.2 $\pm$ 0.3
Lung	NT	NT	0.3 $\pm$ 0.3
Peritoneal Cavity	NT	0.0 $\pm$ 0.0	0.3 $\pm$ 0.2
<u>Range of <i>S. aureus</i> CFU isolated from different sites</u>			
Lymph Node	0 – 0	0 – 24	0 – 42
Abdominal Wall	0 – 35	0 – 1928	0 – 340
Peritoneal Cavity	NT	0 – 0	0 – 3
Spleen	0 – 3	0 – 3	0 – 1,290
Kidney	0 – 9	0 – 44	0 – 280
Blood	0 – 0	0 – 0	0 – 0
Blood IV	NT	NT	1 – 11
Liver	NT	NT	0 – 5
Lung	NT	NT	0 – 2

CFU = Colony-forming units; NT = Not tested.

Blood IV represents results of blood cultures after IV inoculation; all other data represent cultures after epicutaneous inoculation. Data are expressed as number of specimens positive over number tested or mean $\pm$ SE of *S. aureus* CFU cultured from 0.1 ml of tissue specimens or range of CFU isolated.

Table 2

Microscopic bacterial locations in the kidneys after either epicutaneous or intravenous inoculation.

Microscopic location of bacteria in kidney tissue sections				
	<u>Outer CT</u>	<u>Capsule</u>	<u>Cortex</u>	<u>Medulla</u>
Normal Mice - Epi				
6 Hrs	1/12	0/12	0/12	0/12
1D	4/7	0/7	0/7	0/7
2D	3/6	1/6	0/6	0/6
4D	3/5	0/5	0/5	0/5
CY- Mice - Epi				
1D	4/9	0/9	0/9	0/9
4-5D (+CY D3)	5/5	3/5	0/5	0/5
Normal Mice – IV				
6 Hrs	0/7	0/7	0/7	0/7
1-2D	1/10	0/10	2/10	0/10
4-5D	2/5	1/5	3/5	1/5

Data represent numbers of mice with sections showing bacteria at each location over total of mice examined

Outer CT = Connective tissue outside of renal capsule; Epi = Epicutaneous inoculation; D = Day(s); CY = Cyclophosphamide; +CY D3 = Extra dose of CY at D3; IV = Inoculum given intravenously instead of epicutaneously

Table 3

Results of spleen and kidney cultures and survival of non-immunosuppressed and cyclophosphamide (CY)-treated mice after inoculation of *Staphylococcus aureus* onto flank skin under occlusive dressings

Number of <i>S. aureus</i> CFU cultured and mouse survival after epicutaneous inoculation (Mean $\pm$ SE of <i>S. aureus</i> CFU with #pos/#cultured in parentheses and #surviving/#tested in right-hand column)			
	<u><i>S. aureus</i> Spleen</u>	<u><i>S. aureus</i> Kidney</u>	<u>Survival</u>
Normal Mice			
2 D	6,950 $\pm$ 4,360 (6/6)	1,900 $\pm$ 1,040 (6/6)	6/6
4 D	108 $\pm$ 80 (4/5)	64 $\pm$ 19 (5/5)	5/5
6 D	2 $\pm$ 1 (3/6)	4 $\pm$ 2 (5/6)	6/6
CY-Treated Mice			
2 D	2,350 $\pm$ 1,300 (4/4)	2,400 $\pm$ 1,480 (4/4)	4/4
4 D	476 $\pm$ 292 (4/4)	408 $\pm$ 200 (4/4)	4/4
4-5 D (+CY D3)	18,700 $\pm$ 9,470 (6/6)	27,100 $\pm$ 10,100 (6/6)	0/6

CFU = Colony-forming units; D = Day(s); CY = Cyclophosphamide; +CY D3 = Extra dose of CY at Day 3.

Culture data represent *S. aureus* CFU isolated from 0.1 ml of tissue specimen