INFECTION AND IMMUNITY, July 1992, p. 2636–2640
0019-9567/92/072636-05$02.00/0

Vol. 60, No. 7

Murine Model of Cutaneous Infection with Gram-Positive Cocci

CHRIS BUNCE,[1] LISA WHEELER,[1] GEORGE REED,[2] JAMES MUSSER,[3] AND NEIL BARG[1]*

Division of Infectious Disease, Department of Medicine,[1] and Department of Preventive Medicine,[2] Vanderbilt University Medical School, Nashville, Tennessee 37232, and Section of Molecular Pathobiology, Department of Pathology, Baylor College of Medicine, Houston, Texas 77030[3]

Received 18 February 1992/Accepted 20 April 1992

Staphylococcus aureus **has remained an important cause of nosocomial wound infections, but standardized or reproducible systems for analyzing cutaneous infections caused by *S. aureus* do not exist. A variety of foreign materials, variable inocula, and skin traumas have been used to promote infection. To minimize these variables and ensure reproducibility, we chose a model using subcutaneous injections of a fixed quantity of dextran microbeads (Cytodex) as the foreign material added to standardized broth suspensions of *S. aureus*. Suspensions (0.2 ml) injected into an outbred strain of immunocompetent hairless mice generated reproducible, measurable lesions. With *S. aureus* Smith Diffuse, fluctuant, erythematous lesions with a peak diameter of 15 mm were observed; these lesions yielded purulent material containing gram-positive cocci and neutrophils and yielded growth of *S. aureus* on culture. Lesion size was proportional to the bacterial inoculum size. Histologic examination of excised lesions revealed typical abscesses. A second strain of *S. aureus* (SLC3) produced dermonecrosis instead of abscesses at an inoculum size of 10^7 CFU. Control injections with a sterile Cytodex suspension regularly produced nondraining, nonerythematous nodules with maximum diameters of ≤5 mm. *Streptococcus pyogenes* produced late-onset necrotic lesions and abscesses. Using a foreign substance, this model generates easily observed and reproducible cutaneous infection with *S. aureus* and streptococci that can potentially discriminate between inter- and intrastrain differences. Such a model could be used to test the pathogenicity of isogeneic strains of these bacterial species and to evaluate the efficacy of antimicrobial agents.**

Staphylococcus aureus is a common cause of all types of nosocomial infections and is the leading cause of nosocomial wound infections (21). Although animal models have been used to examine several virulence factors of *S. aureus*, none have specifically addressed the pathogenesis of wound infections (1, 3, 10, 13, 17, 18, 22). Animals have been infected so as to produce peritonitis, mastitis, or renal abscesses. These infections were less relevant to human nosocomial staphylococcal infections, and the inoculum sizes (10^8 to 10^{10} CFU) used were far greater than those required to produce infection in humans.

Other models of staphylococcal infections used to evaluate therapy have required traumatic injury to the integument (12) or the introduction of foreign material to generate an abscess at an inoculum size of $<10^8$ CFU. These models have certain disadvantages. Foreign substances such as plastic materials (23), soft agar (7), and cotton (19) often differ in composition and particle size or, in the case of metal coils (20) and capsules (8), require surgical implantation. Traumatic injury or implantation, required by some models, may induce variable damage to the skin which may obscure the exact effect of the infecting organism.

For these reasons, we sought to develop a model that was simple and reproducible and allowed repeated observation of an infected lesion. We also wanted a model that more closely mimicked human cutaneous (wound) infection than did published models. Ford et al. developed a model that (6) did not require large inocula or traumatic injury and that used a uniform and commercially available material, dextran microcarrier beads, as a foreign substance. To be useful, the model had to be capable of discriminating among different strains of *S. aureus*. We report the development of a model of cutaneous infection based on the methods of Ford et al.

MATERIALS AND METHODS

Bacterial strains. Smith Diffuse, a highly encapsulated strain of *S. aureus*, shown to be virulent in a rat model of peritonitis (25), was used as the initial test strain. SLC3, a nosocomial clinical strain isolated from a patient with a sternal wound infection, was used in later comparisons. Strains similar to SLC3 have been isolated from patients with nosocomial infections at six locations within the United States (14). A colonizing strain of *Staphylococcus haemolyticus* obtained during a surveillance study was provided by D. Low, Toronto, Ontario, Canada. Since *Streptococcus pyogenes* also causes cutaneous infections, this species was evaluated. The following strains of *S. pyogenes* were tested: MGAS 158, a producer of streptococcal pyrogenic exotoxin A (SPEA); MGAS 279, a producer of SPEB; MGAS 326, a producer of SPEC; and MGAS 252, a clinical isolate which does not produce SPEA, SPEB, or SPEC. These strains were recovered from human invasive disease episodes (15). All strains were subcultured from brain heart infusion (Difco, Detroit, Mich.) agar plates to 10 ml of brain heart infusion broth and incubated at 37°C for 2.5 to 3 h until an optical density at 600 nm of 1.0 was reached. Serial dilutions were prepared and plated to determine the actual inoculum sizes used.

Microcarrier beads. Dextran beads (Cytodex; Sigma Chemicals, St. Louis, Mo.) were used as the foreign material. Beads were first prepared as a stock solution by suspending 1 g in 50 ml of phosphate-buffered saline and autoclaving.

Preparation of the inoculum. Aliquots of the Cytodex

* Corresponding author.

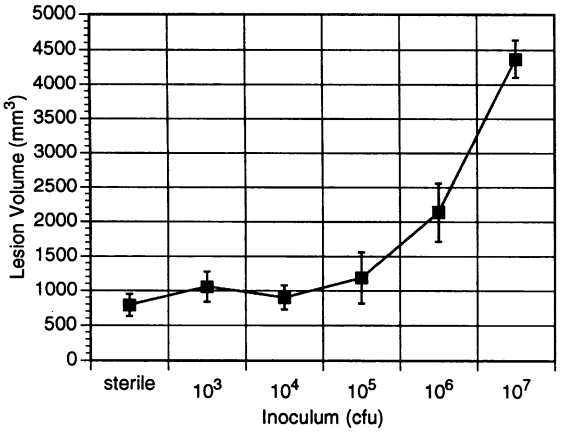

FIG. 1. Mean lesion volume for mice injected with *S. aureus* Smith Diffuse at inoculum sizes of 10^3 to 10^7 CFU plus 0.038 ml of Cytodex (n = 6). The animals were observed for 14 days. The mean lesion volume increased in proportion to the size of the inoculum of *S. aureus*. Error bars, standard error of the mean.

suspension, ranging from 0.01 to 0.1 ml, were added to 0.5 ml of brain heart infusion broth with or without bacteria. Additional broth was added for a final volume of 1 ml. From this suspension, 0.2 ml was injected subcutaneously in the right flank of each animal with a 1-ml tuberculin syringe. All syringes were coded, and mice were tagged according to the syringe codes.

Animals. Four-week-old (15- to 20-g), outbred, immunocompetent, hairless male mice, strain Crl:SKH1(hrhr)Br (Charles River, Wilmington, Mass.), were divided into groups of five to eight animals and injected with the same inoculum. Mice were examined and weighed serially by a blinded observer.

Bacteremia. To determine whether the mice developed high-level bacteremia and metastatic infection, two animals from each group were sacrificed at the end of the observation period. Both kidneys were removed by a sterile technique and placed in a sterile glass homogenizer containing 1 ml of brain heart infusion broth. Serial dilutions were made and plated as described above to determine bacterial counts.

Measurements. Animals were weighed immediately prior to inoculation. Thereafter, animals were observed at 24-h intervals after inoculation for the first 5 days and then at 48- or 72-h intervals for a total of 14 to 21 days. Lesions were measured with a caliper. Abscess size was calculated by using the formula for a spherical ellipsoid [$V = (\pi/6) \cdot L \cdot W^2$], where L is length and W is width. Areas were calculated for dermonecrosis by using the formula $A = \pi(L \cdot W)/2$. Lesion sizes were recorded by day of observation and graphed. For each animal, the area under the lesion size curve (lesion volume) was determined and used as a measure of disease.

Statistical methods. Lesion volumes were compared among groups by using analysis of variance (ANOVA). Post hoc tests were done by using Fisher's protected least significant difference. Kruskal-Wallis (nonparametric) tests were also carried out to make sure that the results did not depend on assumptions of normality. All tests were consistent. P values reported are for the ANOVA tests. Significant differences between pairs of groups are all $P < 0.05$ by Fisher's protected least significant difference. Weights over time were tested with repeated measures of ANOVA. Fisher's exact test was used to compare counts of abscesses and dermonecrotic lesions.

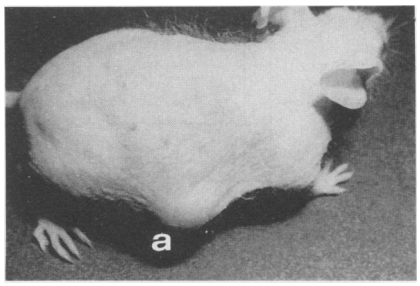

FIG. 2. Flank abscess (a) generated after inoculation of Smith Diffuse (10^6 CFU) plus Cytodex (0.038 ml), 12 days after injection.

RESULTS

Generation of lesions. For a Cytodex dose of 0.038 ml, abscess volumes increased in proportion to the *S. aureus* Smith Diffuse inoculum size. The infected lesions were significantly larger than those of sterile controls at an inoculum size of $\geq 10^6$ CFU (P = 0.0001 [ANOVA]) (Fig. 1). Infected lesions (Cytodex plus *S. aureus*) became erythematous and fluctuant by day 3 to 4. These lesions progressively enlarged to 7 to 15 mm in diameter over 7 to 9 days and then drained externally through the overlying epidermis. A total of 75 to 80% of the lesions were healing by 10 days. Abscesses excised at 6 and 12 days (Fig. 2) postinoculation yielded purulent material, which was shown by Gram stain to contain gram-positive cocci and neutrophils. *S. aureus* ($\geq 10^8$ CFU) was recovered from day 12 abscesses. Histologic examination of a day 12 abscess revealed edema of the subepidermal tissues, encapsulation of the contents, infiltration of the abscess cavity with neutrophils, and numerous gram-positive cocci visible with tissue Gram stain. Cytodex microcarrier beads were distributed throughout the abscess (Fig. 3). Control lesions were firm, nonerythematous, nondraining, subcutaneous nodules which reached

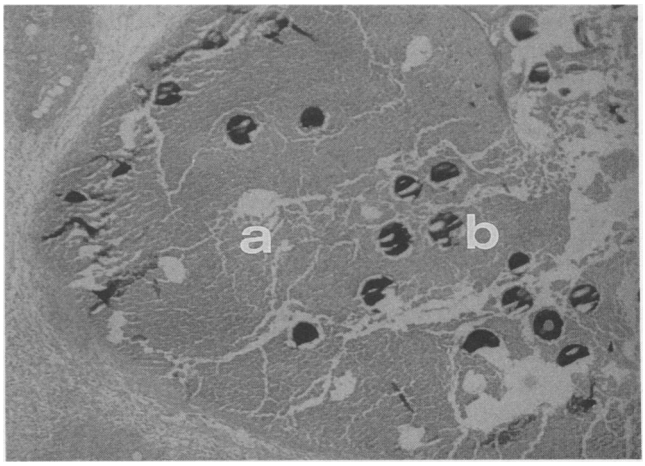

FIG. 3. Microscopic appearance of a typical abscess excised 10 days after infection and fixed. An inflammatory infiltrate and necrotic debris (a) are visible within the abscess cavity. With Brown-Brenn stain, numerous cocci are visible. Large, dark, circular structures (b) within the abscess are sectioned Cytodex beads.

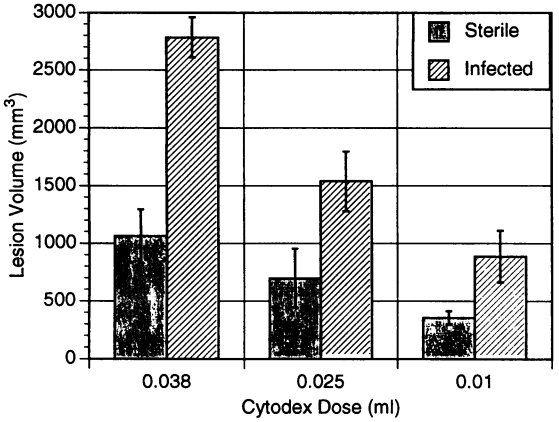

FIG. 4. Mean lesion volume for sterile and infected lesions (*S. aureus* Smith Diffuse, 10^6 CFU), obtained with three doses of Cytodex, in mice observed for 14 days ($n = 6$). At the Cytodex doses shown, infected lesions were significantly larger than sterile lesions. The lesion size was proportionate to the Cytodex dose. Error bars, standard error of the mean.

maximum size (2 to 4 mm in diameter) 3 to 5 days after injection. No subepidermal tissue edema was observed on histologic examination of a day 8 lesion. The contents, consisting of Cytodex beads, neutrophils, and lymphocytes, were surrounded by several layers of fibroblasts, in contrast to infected lesions. No organisms were seen. These lesions were sterile.

Lesion volume also correlated with Cytodex dose. For doses of 0.025 to 0.1 ml of Cytodex stock, a marked increase in lesion volume was noted ($P < 0.0001$ [ANOVA]). At Cytodex doses of 0.01, 0.025, and 0.038 ml, lesions produced by infected inocula (Smith Diffuse, 10^6 CFU) were significantly larger than lesions produced by Cytodex alone. However, infected lesions were not significantly larger than sterile lesions at the higher Cytodex doses (≥0.075 ml). Of Cytodex doses for which infected lesions were significantly larger than sterile lesions, the 0.038-ml dose produced the most easily measured difference in lesion volume between infected (Smith Diffuse, 10^6 CFU) lesions and sterile lesions (Fig. 4). A Cytodex dose of 0.038 ml was chosen for subsequent experiments. The size of infected lesions, therefore, depended on the size of the bacterial inoculum and the quantity of Cytodex.

Baseline weights were obtained prior to inoculation and again when lesions were measured. Repeated-measures ANOVA indicated a significant trend over time ($P = 0.0001$) which corresponded to the increase in weights over time for all mice. The analysis also indicated a significant group-time interaction ($P = 0.0001$), which implied that all groups did not increase over time in the same fashion. This interaction was not significant after day 2, indicating that the differences in weight change among groups occurred in the first day. The largest difference occurred between days 0 and 1, when mean weights in groups infected with 10^7 CFU dropped significantly ($P < 0.001$). Subsequent analysis of weight change focused on this period.

Comparison of *S. aureus* Smith Diffuse and SLC3. To determine whether the model could show potential differences between strains, we compared lesions generated by Smith Diffuse with lesions generated by the nosocomial strain SLC3. At inoculum sizes of 10^3 and 10^5 CFU, little difference was seen in mean lesion volume for abscesses

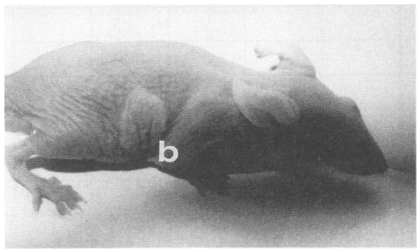

FIG. 5. Dermonecrosis generated by *S. aureus* SLC3 48 h after infection. Such lesions (b) were apparent 24 h after injection. These lesions slowly healed, forming eschars, over 14 to 21 days.

generated by either strain. For Smith Diffuse, the largest abscesses were observed in the group infected with 10^7 CFU. However, at the same inoculum size, no abscesses were observed in the group infected with SLC3. Instead, a lesion consistent with dermonecrosis was noted 24 h after inoculation (Fig. 5). Such lesions were flat and ulcerated and had an underlying purulent exudate which yielded neutrophils and gram-positive cocci on Gram stain and coagulase-positive cocci on culture. Histologic examination at 24 h showed marked tissue edema, linear inflammatory cell infiltrates, and linear collections of bacteria in the subcutaneous tissue. Vessels within the lesion were patent and filled with inflammatory infiltrate. Only one of six necrotic lesions were healed by day 14. Therefore, a necrotic lesion was considered to be more severe than an abscess.

No weight loss was observed in groups infected with either strain or in controls at an inoculum size of 10^3 CFU. However, at both the 10^5- and 10^7-CFU inoculum sizes, animals infected with SLC3 lost significantly more weight in the first 24 h than animals infected with Smith Diffuse ($P < 0.0001$ [ANOVA]) (Fig. 6).

Comparison of *S. aureus* SLC3 with *S. haemolyticus*. *S. haemolyticus* (10^7 CFU) also did not appear as virulent as SLC3. None of the animals (zero of six) infected with *S.*

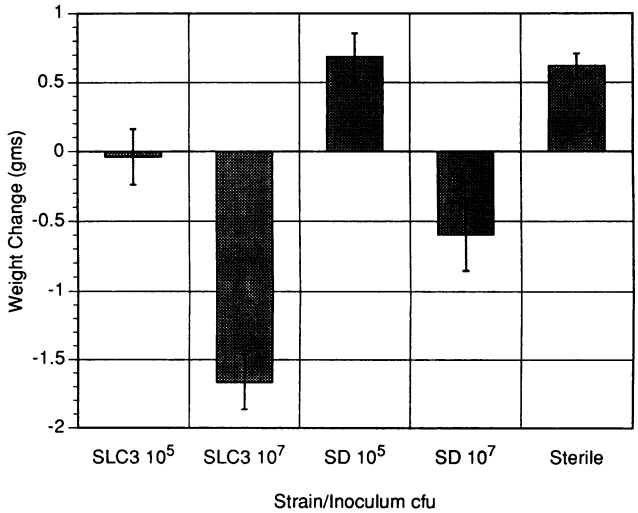

FIG. 6. Mean weight change 24 h after inoculation for mice infected with *S. aureus* Smith Diffuse (SD) and SLC3 at inoculum sizes of 10^5 and 10^7 CFU ($n = 6$). At both inoculum sizes, mice infected with SLC3 lost significantly more weight than mice infected with Smith Diffuse and uninfected mice (sterile controls). Error bars, standard error of the mean.

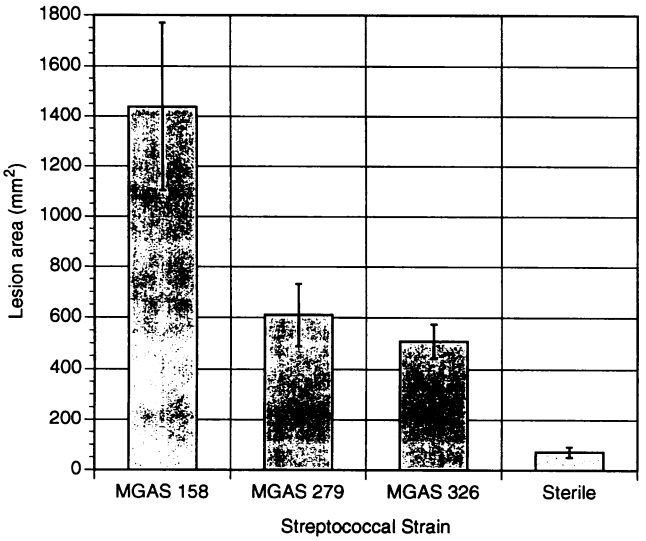

FIG. 7. Mean lesion area over 8 days for mice with necrotizing lesions that were infected with *S. pyogenes* (10^5 CFU) MGAS 158 (elaborating SPEA), MGAS 279 (elaborating SPEB), or MGAS 326 (elaborating SPEC) (n = 8). MGAS 158 generated the largest lesions. Animals given toxin-negative strains and sterile controls did not develop necrotizing lesions. The mean lesion size of sterile controls is shown for comparison. Error bars, standard error of the mean.

haemolyticus developed dermonecrosis; instead, they developed small abscesses (4 to 6 mm in diameter). All (six of six) animals infected with SLC3 developed dermonecrosis. The mean weight change over 24 h in animals infected with *S. haemolyticus* was +0.34 g, versus −1.66 g for animals infected with SLC3 and +1.42 g for those injected with Cytodex alone ($P < 0.0001$ [ANOVA]).

Infection with group A streptococci. In contrast to the early fluctuant lesions seen with *S. aureus* Smith Diffuse, *Streptococcus pyogenes* (10^5 CFU) produced induration and erythema. Lesions enlarged over 3 to 4 days, and then central necrosis was observed. The necrosis enlarged for another 3 to 4 days, and eventually eschar formed. In mice not developing necrotic lesions, abscesses were observed by day 6. All (eight of eight) mice infected with SPEA producers developed necrotic lesions. Of animals infected with SPEB producers, six of eight developed necrotic lesions, while five of eight animals infected with SPEC producers developed necrotic lesions. In comparisons among only the mice with necrotic lesions, those infected with SPEA producers had significantly larger lesions than those infected with SPEB and SPEC producers ($P < 0.0001$ [ANOVA]), but no significant difference between animals infected with SPEB producers and those infected with SPEC producers was seen (Fig. 7). Two mice infected with SPEB producers did not develop necrotic lesions (two abscesses), and three mice infected with SPEC producers did not develop necrotic lesions (three abscesses). All mice (eight of eight) infected with the toxin-negative strain developed abscesses. Mean weight loss at 24 h in animals infected with toxin-producing strains was greater than 1 g (SPEA, −1.96 g; SPEB, −1.9 g; and SPEC, −1.15 g). Compared with animals infected with the toxin-negative strain (mean weight loss, −0.3 g), animals infected with SPEA and SPEB producers lost significantly more weight ($P < 0.0001$ [ANOVA]). All infected groups lost significantly more weight ($P < 0.0001$ [ANOVA]) than did Cytodex controls (mean, +1.05 g). An *S. aureus* inoculum size 2 logs higher (10^7 CFU) was required to cause weight loss of >1 g.

DISCUSSION

Of the 34 different exoproteins known to be elaborated by *S. aureus*, only a few have been evaluated in animal models and identified as possible virulence factors (9). Multiple exoproteins are probably involved in the generation of human staphylococcal wound infection. Because previously reported animal models for staphylococcal infection have not closely simulated human cutaneous staphylococcal infection, these models are more like animal test systems as defined by Onderdonk et al. (16). These animal test systems are designed to evaluate a single virulence factor or antibiotic therapy. For example, intraperitoneal injection of mice has been used to determine differences in virulence related to the presence of capsule (25). Alpha-toxin has been evaluated in a model of mouse mastitis and a model using subcutaneous injection with large inocula (17). We wanted to develop a potentially relevant animal model for nosocomial cutaneous staphylococcal infections. The requirements of such an animal model are simulation of human infection, reproducible experimental disease, and a similar degree of infection for all animals in a group (16). We also sought to minimize the effect of the recognized variables (foreign material and skin trauma) in such a model and to choose techniques that would be easy to perform.

We wished to eventually examine the pathogenesis of cutaneous staphylococcal infections which are augmented by foreign materials (5). Bacterial infections in animals may also be augmented by the addition of foreign substances (6). Therefore, our first goal was to establish cutaneous infection in an animal by using a foreign substance. The hairless mouse seemed ideal, since infections could be directly observed. The infections generated in this animal, abscesses or a necrotizing infection of the skin, were analogous to staphylococcal cutaneous infections in humans (24). These lesions were reproducible and appeared to be bacterial strain dependent and inoculum dependent. With this model, strain SLC3 appeared more virulent than strain Smith Diffuse and a coagulase-negative staphylococcus, on the basis of lesion type, lesion volume, and early weight loss. Furthermore, a different pattern of disease was observed in animals infected with *S. pyogenes*, indicating that the model can distinguish between staphylococcal and streptococcal cutaneous infection.

All animals within a group inoculated with *S. aureus* did not exhibit the same degree of infection. However, human cutaneous infections with staphylococci also vary from mild to very severe. In this model, the variability may be caused in part by the use of outbred mice. The use of an inbred, hairless strain might reduce variability in lesion size. We think that repeated observation of lesions over time could offset the lesion variability within a group, when compared with a single set of lesion measurements. Other models typically measure disease during a single observation (4). We also observed that lesions did not all become obvious at the same time in a group infected with the same strain (data not shown). However, serial observations enabled us to detect late-onset lesions. A delayed appearance of obvious infection also simulates staphylococcal infection in humans. Human staphylococcal sternal wound infections may show marked variability in time of onset (2). The lack of a distinct end point, such as death, is another disadvantage of this

model, but lesion size and weight change are measurable. Also, a nonlethal model may be more desirable. Examination of lesions over time provides an objective measurement of disease within a group of animals, as do weight changes in the first 24 h after inoculation. Weight changes have been useful in other murine models as a measure of microbial virulence (11).

Other advantages of this model include the minimization of foreign body variability and no need for immunosuppression, surgical implantation, and traumatic injury. Cytodex is uniform and easily available and can be precisely measured. Confounding procedures such as depilation, sacrifice, and lesion excision are not necessary to infect or to directly observe the resultant lesion. Infection is produced with an easily administered subcutaneous injection.

In summary, we have shown that a model of subcutaneous gram-positive infection of hairless mice using dextran microcarrier beads as a foreign substance can discriminate among infections caused by different strains of staphylococci and streptococci. Differences in the disease intensity caused by certain strains of staphylococci can be based on the measurement of lesion size over time, lesion type, and weight loss. This hairless-mouse model of subcutaneous infection should be suitable both for comparing isogeneic strains of gram-positive cocci to examine factors necessary for the pathogenesis of cutaneous infections augmented by a foreign substance and for evaluating antibacterial agents.

REFERENCES

1. **Albus, A., R. D. Arbeit, and J. C. Lee.** 1991. Virulence of *Staphylococcus aureus* mutants altered in type 5 capsule production. Infect. Immun. **59:**1008–1014.
2. **Bor, D., R. Rose, J. Modlin, R. Weintraub, and G. Friedland.** 1983. Mediastinitis after cardiovascular surgery. Rev. Infect. Dis. **5:**885–897.
3. **Bramley, A. J., A. H. Patel, M. O'Reilly, R. Foster, and T. J. Foster.** 1989. Roles of alpha-toxin and beta-toxin in virulence of *Staphylococcus aureus* for the mouse mammary gland. Infect. Immun. **57:**2489–2494.
4. **Dye, E. S., and F. A. Kapral.** 1981. Characterization of a bactericidal lipid developing within staphylococcal abscesses. Infect. Immun. **32:**98–104.
5. **Elek, S. D., and P. E. Cohen.** 1957. The virulence of *Staphylococcus pyogenes* for man. A study of the problems of wound infection. Br. J. Exp. Pathol. **38:**573–586.
6. **Ford, C. W., J. C. Hamel, D. Stapert, and R. J. Yancey.** 1988. Establishment of an experimental model of a *Staphylococcus aureus* abscess in mice by use of dextran and gelatin microcarriers. J. Med. Microbiol. **28:**259–266.
7. **Fujimoto, T., M. Sato, K. Katami, et al.** 1986. Chemotherapeutic efficacy of ofloxacin on renal and subcutaneous infection models with *Staphylococcus aureus* in mice. Chemotherapy (New York) **32:**291–298.
8. **Gerding, D. N., B. Bean, L. R. Peterson, J. Moody, and K. Bettin.** 1987. Cephalothin clearance of *Staphylococcus aureus* from two experimental infection sites in the presence and absence of local phagocytic cells. J. Antimicrob. Chemother. **20:**685–695.
9. **Iandolo, J.** 1989. Genetic analysis of extracellular toxins of *Staphylococcus aureus*. Annu. Rev. Microbiol. **43:**375–402.
10. **Jonsson, P., M. Lindberg, I. Haraldsson, and T. Wadström.** 1985. Virulence of *Staphylococcus aureus* in a mouse mastitis model: studies of alpha hemolysin, coagulase, and protein A as possible virulence determinants with protoplast fusion and gene cloning. Infect. Immun. **49:**765–769.
11. **Kinsman, O. S., and J. P. Arbuthnott.** 1980. Experimental staphylococcal infections in newborn mice: inhibition of weight gain as an index of virulence. J. Med. Microbiol. **13:**281–290.
12. **Kraft, W. G., P. T. Johnson, B. C. David, and D. R. Morgan.** 1986. Cutaneous infection in normal and immunocompromised mice. Infect. Immun. **52:**707–713.
13. **Lee, J. C., M. J. Betley, C. A. Hopkins, N. E. Perez, and G. B. Pier.** 1987. Virulence studies in mice of transposon-induced mutants of *Staphylococcus aureus* differing in capsule size. J. Infect. Dis. **156:**741–750.
14. **McMurray, L. W., D. S. Kernodle, and N. L. Barg.** 1990. Characterization of a widespread strain of methicillin-susceptible *Staphylococcus aureus* associated with nosocomial infections. J. Infect. Dis. **162:**759–762.
15. **Musser, J. M., A. R. Hauser, M. H. Kim, P. M. Schlievert, K. Nelson, and R. K. Selander.** 1991. *Streptococcus pyogenes* causing toxic-shock-like syndrome and other invasive diseases: clonal diversity and pyrogenic exotoxin expression. Proc. Natl. Acad. Sci. USA **88:**2668–2672.
16. **Onderdonk, A. B., R. L. Cisneros, R. Finberg, J. H. Crabb, and D. L. Kasper.** 1990. Animal model system for studying virulence of and host response to *Bacteroides fragilis*. Rev. Infect. Dis. **12:**S169–S177.
17. **O'Reilly, M., J. C. S. de Azavedo, S. Kennedy, and T. J. Foster.** 1986. Inactivation of the alpha-haemolysin gene of *Staphylococcus aureus* 8325-4 by site-directed mutagenesis and studies on the expression of its haemolysins. Microb. Pathog. **1:**125–138.
18. **Patel, A. H., P. Nowlan, E. D. Weavers, and T. Foster.** 1987. Virulence of protein A-deficient and alpha-toxin-deficient mutants of *Staphylococcus aureus* isolated by allele replacement. Infect. Immun. **55:**3103–3110.
19. **Renneberg, J., and M. Walder.** 1989. The role of β-lactamase in mixed infections in mice in relation to treatment with ampicillin. J. Infect. Dis. **160:**337–341.
20. **Rossi, R., P. Castellani, G. Younes, and C. Della Bruna.** 1989. Activity of FCE 22891 compared with cefuroxime axetil and cefixime in pulmonary and subcutaneous infections in mice. J. Antimicrob. Chemother. **23**(Suppl. C)**:**149–155.
21. **Schaberg, D., D. Culver, and R. Gaynes.** 1991. Major trends in the microbial etiology of nosocomial infection. Am. J. Med. **91:**72S–75S.
22. **Seki, K., M. Ogasawara, J. Sakurada, M. Murai, and S. Masuda.** 1989. Altered virulence of a pleiotropic *Staphylococcus aureus* mutant with a low producibility of coagulase and other factors in mice. Microbiol. Immunol. **33:**981–990.
23. **Sherertz, R. J., D. M. Forman, and D. D. Solomon.** 1989. Efficacy of dicloxacillin-coated polyurethane catheters in preventing subcutaneous *Staphylococcus aureus* infection in mice. Antimicrob. Agents Chemother. **33:**1174–1178.
24. **Swartz, M. N.** 1990. Subcutaneous tissue infections and abscesses, p. 808–811. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases. Churchill Livingstone, New York.
25. **Yoshida, K., and M. Takahashi.** 1973. Colonial morphologies of the Smith Diffuse strain of *Staphylococcus aureus* related to mouse virulence. Jpn. J. Microbiol. **17:**321–322.