# Natural History of Chronic *Staphylococcus epidermidis* Foreign Body Infection in a Mouse Model

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**The development and characterization of a mouse model of chronic *Staphylococcus epidermidis* foreign body infection was done with two clinical isolates that differed in degree of extracellular slime production. Segments of Silastic catheters bearing preformed *S. epidermidis* biofilms were implanted intraperitoneally, and mice were assessed after 3 and 6 months. Both test strains of *S. epidermidis* persisted at the implant site through the 6-month follow-up in 80% of the mice, regardless of the degree of slime production. There was no evidence of overt animal morbidity, and microbiologic assessment of other peritoneal sites did not reveal dissemination of bacteria from the infected focus. In comparison with control mice, animals harboring chronic foreign body infection presented marked peripheral neutrophilia and mild anemia.**

The increased prevalence of infection associated with medical implants has been frequently reported [1]. Animal studies have demonstrated impaired phagocytic and bactericidal functions of polymorphonuclear leukocytes at the site of tissue cage implants, which might be attributed to persistent complement activation and exhaustive stimulation for phagocytosis [2–4]. Furthermore, tissue damage due to surgical implantation and host inflammatory responses to the implant may lead to vascular thrombosis and ischemia, thereby limiting the influx of immune constituents to the implant site.

*Staphylococcus epidermidis* is notoriously associated with foreign body infections [5]. These microorganisms adhere to surfaces of foreign implants where they may form confluent, multilayer bacterial biofilms [6]. Within the biofilm colony, microorganisms are protected from eradication by host defense mechanisms and antibiotic therapy [7, 8]. A key characteristic of *S. epidermidis* foreign body infection may be the ability of the infecting strain to produce an extracellular slime substance that provides a "cementing" matrix around the biofilm colony. In vitro assessments have demonstrated an inhibitory influence of staphylococcal slime on polymorphonuclear leukocyte chemotaxis, opsonophagocytic and bactericidal activities [9, 10], and T and B cell blastogenesis [10, 11].

We have developed a mouse model of chronic slime-producing *S. epidermidis* foreign body infection. The peritoneal cavity was selected as the implant site because of the prevalence of *S. epidermidis* peritonitis and *S. epidermidis* colonization of the peritoneal catheter in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) [5].

## Materials and Methods

*Preparation of catheter-associated S. epidermidis biofilms.* The two test strains of *S. epidermidis* (29260 and 2503) were clinical isolates recovered from peritoneal dialysis effluents of CAPD patients with peritonitis. Compared with reference slime-producing strains of *S. epidermidis* (supplied by G. D. Christensen, VA Hospital, Columbia, MO), strain 29260 was a moderate slime producer (++) and 2503, a prolific slime producer (+++).

Custom-made segments of Silastic, Oreopoulos-Zellerman CAPD catheters (Accurate Surgical Instruments, Toronto) had inner and outer diameters of 3.0 and 5.0 mm, respectively, and the 12-mm length contained six regularly spaced perforations. Both open ends of the catheter segment were beveled smooth. Catheter segments were placed in 5 ml of trypticase soy broth (TSB) that had been inoculated with $10^4$ cfu of *S. epidermidis* 29260 or 2503 in the log phase of growth and were incubated at 37°C for 72 h. During the incubation time, the TSB was decanted and replaced with sterile medium every 24 h. Preliminary studies indicated that this procedure ensured the development of confluent catheter-associated biofilms. Control catheters were prepared as above with the exclusion of bacterial inoculation. Catheter preparations were gently rinsed in sterile saline immediately before implantation.

*Implantation of biofilm-catheter preparations.* Five-week-old female C57BL/6 mice were obtained from Charles River Breeding Laboratories (Kingston, NY). Mice were anesthetized with ether, and through a left flank incision, catheter preparations were placed intraperitoneally and secured to the lateral abdominal wall with a single suture. The incision was closed with a running suture, and surgical clips were applied to the skin.

*Collection and processing of specimens.* At the selected assessment times of 3 and 6 months after catheter implantation,

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mice were anesthetized with ether and exsanguinated by cardiac puncture. Routine hematology was done using a Coulter counter (model 2B1; Coulter Electronics, Hialeah, FL, and differential white blood cell counts were done on Wright-stained blood smear preparations.

Peritoneal washing was done by retracting the abdominal skin and injecting and slowly withdrawing 10 ml of MEM (Eagle's modified, containing 10% heat-inactivated fetal calf serum and 20 m*M* HEPES buffer) from the peritoneal cavity. Total cell counts were done in Neubauer counting chambers, and differential cell counts were determined from cytocentrifuge preparations stained with Diff-Quick (American Scientific Products, McGaw Park, IL).

Specimens for microbiologic culture included peritoneal washing, parietal peritoneum, catheter-associated granulation tissue, spleen (6-month end point), and catheters. Catheter segments were removed by fine dissection, and the associated granulation tissue was peeled away. Tissue specimens were homogenized in HEPES-buffered normal saline. Specimens were quantitatively cultured onto 5% horse blood Columbia agar for bacterial content and identity.

Viable catheter-associated bacterial biofilms were assayed by scraping and culture of scrapings onto agar and by using the 2,3,5-triphenyltetrazolium chloride (TTC) assay [12]. TTC is reduced to insoluble red formazan by electron transfer associated with active oxidative bacterial metabolism and is precipitated intracellularly, thereby causing bacterial biofilms to stain red. Recovered catheter segments were incubated in 5 ml of TSB containing one drop of a 1% TTC solution (wt/vol sterile distilled water). Subculture of broth from TTC-positive catheter segments identified the biofilm-originating organism.

All cultured specimens were incubated at 37°C for 24 h. Positive identification of recovered *S. epidermidis* organisms was ascertained by colony morphology, Gram's stain, and antibiogram.

*Expression of results and statistical analysis.* Results are expressed as mean ± SD. Statistical analysis was done using techniques for analysis of variance and the Tukey-Kramer test for multiple comparisons. A significance level of .05 was used for all statistical tests.

## Results

The surgical implantation of the biofilm-catheter preparations was well tolerated. Of 48 mice, 3 animals were not included in the 3- or 6-month assessments due to complications after catheter implantation in 1 control mouse (sterile implant) and 2 biofilm-catheter–implanted mice.

*Bacterial recoveries.* Up to 6 months after biofilm-catheter implantation, *S. epidermidis* (both strains) persisted at the catheter site in a large majority of animals, and all recovered microorganisms were positively identified as the originally inoculated bacteria (table 1). Specimens recovered from control mice (i.e., mice into which sterile catheter segments had been implanted) were consistently culture-negative. Catheter scrapings revealed culture-positive results for 10 of 16 *S. epidermidis* 29260 catheter specimens (3/7 at 3 months, 7/9 at 6 months) and for 11 of 14 *S. epidermidis* 2503 catheter specimens (8/9 at 3 months, 3/5 at 6 months). The direct assessment of catheter-associated biofilms by the TTC assay demonstrated viable biofilms of *S. epidermidis* 29260 in 13 of 16 specimens (6/7 at 3 months, 7/9 at 6 months) and of *S. epidermidis* 2503 in 11 of 14 specimens (8/9 at 3 months, 3/5 at 6 months). The intensity of formazan deposition on catheter segments appeared to be comparable between test strain groups.

The catheter implant was invariably surrounded by a granulation tissue sheath in all control and test mice. *S. epidermidis* was recovered from the catheter-associated granulation tissue in a large majority of biofilm-catheter–implanted mice. Culture results for granulation tissue most often concurred with the TTC assessment of catheters (*S. epidermidis* 29260, 10/13 concurred; *S. epidermidis* 2503, 11/11 concurred). Six months after implantation, quantitative recoveries from granulation tissue specimens were comparable between the two *S. epidermidis* test strains. *S. epidermidis* was infrequently recovered from peritoneal structures other than the catheter site.

**Table 1.** Microbiologic assessment of peritoneal structures of mice after intraperitoneal placement of sterile and *Staphylococcus epidermidis* biofilm-catheter preparations.

| | | | *S. epidermidis* biofilm-catheter | | | |
|---|---|---|---|---|---|---|
| | Sterile catheter | | Strain 29260 | | Strain 2503 | |
| | 3 mo. ($n$ = 7) | 6 mo. ($n$ = 8) | 3 mo. ($n$ = 7) | 6 mo. ($n$ = 9) | 3 mo. ($n$ = 9) | 6 mo. ($n$ = 5) |
| Catheter scraping | 0 | 0 | 3 | 7 | 8 | 3 |
| Catheter TTC assay | 0 | 0 | 6 | 7 | 8 | 3 |
| Granulation tissue | 0 | 0 | 4 | 6 | 8 | 3 |
| Peritoneal washing | 0 | 0 | 0 | 0 | 1 | 1* |
| Parietal peritoneum | 0 | 0 | 0 | 0 | 0 | 1* |
| Spleen | ND | 0 | ND | 4 | ND | 1* |

NOTE. Results are number of animals harboring culture-positive specimens. TCC, 2,3,5-triphenyltetrazolium chloride; ND, not done.
* Same animal.

**Table 2.** Host inflammatory responses in mice at selected times after intraperitoneal placement of sterile and *Staphylococcus epidermidis* biofilm-catheter preparations.

| | Sterile catheter ($n$ = 15) | *S. epidermidis* biofilm-catheter | |
|---|---|---|---|
| | | Strain 29260 ($n$ = 13) | Strain 2503 ($n$ = 11) |
| Peritoneal washing | | | |
| Leukocytes, $\times 10^6$/ml | 0.5 ± 0.3 | 0.7 ± 0.4 | 0.8 ± 0.7 |
| Polymorphs, % | 6.2 ± 14.4 | 7.0 ± 15.4 | 5.8 ± 10.8 |
| Lymphocytes, % | 28.6 ± 10.4 | 35.7 ± 20.5 | 35.3 ± 19.5 |
| Macrophages, % | 63.9 ± 12.4 | 57.0 ± 21.1 | 58.3 ± 18.0 |
| Peripheral blood | | | |
| Leukocytes, $\times 10^6$/ml | 2.9 ± 1.7 | 3.5 ± 1.7 | 5.5 ± 1.9*† |
| Polymorphs, % | 8.7 ± 5.4 | 24.7 ± 15.1* | 42.1 ± 17.9*† |
| Lymphocytes, % | 90.1 ± 6.2 | 73.6 ± 15.8 | 55.2 ± 19.0*† |
| Monocytes, % | 1.2 ± 1.6 | 1.7 ± 1.6 | 2.7 ± 1.6 |
| Hemoglobin, g/dl | 14.0 ± 0.5 | 12.4 ± 1.5* | 12.0 ± 1.1* |
| MCV, fl | 44.2 ± 0.9 | 43.5 ± 1.1 | 43.1 ± 0.6* |
| MCHC, g/dl | 36.8 ± 0.9 | 37.2 ± 1.1 | 38.6 ± 2.6* |

NOTE. Pooled results (mean ± SD) of assessments 3 and 6 months after catheter implantation. In biofilm-catheter groups, only results of culture-positive mice are shown: *S. epidermidis* 29260, 13/16 mice; *S. epidermidis* 2503, 11/14 mice.

* $P \leq .05$ between control and test mice.

† $P \leq .05$ between *S. epidermidis* strains 29260 and 2503.

*Inflammatory responses.* Total and differential counts of peritoneal leukocytes, complete blood counts, and differential counts of circulating leukocytes of mice were used to assess local and systemic inflammatory responses (table 2). There was no statistically significant difference between parameters assessed at 3 and 6 months; therefore, data for the respective *S. epidermidis* strains were pooled.

Compared with those of control mice (sterile catheters), peritoneal washing specimens of culture-positive test mice did not reveal evidence of inflammation in terms of total leukocyte and differential counts. However, mice harboring culture-positive catheters presented a marked circulating polymorph response that was most evident in animals harboring *S. epidermidis* 2503 biofilm-catheters. Polymorphs and total leukocyte counts were significantly elevated compared with control mice and animals receiving *S. epidermidis* 29260. Mice that received *S. epidermidis* 29260 presented a significant polymorph response, but leukocyte counts were comparable to those of control mice. Chronically infected mice in both *S. epidermidis* groups developed mild anemia.

## Discussion

We have developed a reproducible mouse model of stable foreign body infection in the peritoneal cavity without overt evidence of animal morbidity. Implanted catheter segments bore preformed *S. epidermidis* biofilms, and the two test strains used for the model varied in degree of slime production. At 3 and 6 months after peritoneal implantation of biofilm-catheter preparations, both test strains were persistently and almost exclusively recovered from the catheter site without evidence of dissemination. In response to the ongoing colonization of the peritoneal catheter implant, there was a marked systemic neutrophilia, but there was no evidence of peritoneal inflammation. Chronically infected mice developed mild anemia.

To maximize the reproducibility of the mouse model, we chose to implant established biofilm-catheter preparations. Following this protocol, 80% of test mice developed chronic foreign body infection. All microbiologic recoveries from test mice were positively identified as the originally inoculated staphylococcus, and specimens from control mice that received sterile catheters were invariably culture-negative.

Scanning electron microscopic studies of in vitro catheter colonization by slime-producing strains of *S. epidermidis* have documented the progressive formation of a confluent multilayer biofilm encased by a slime matrix [6]. It is proposed that microorganisms in a biofilm colony do not present the same immunologic stimulus to the host as they would in a free-floating phase [13]. Periodically, microcolonies break away from the biofilm and disperse to distant sites, at which time host inflammatory responses may be triggered. Our results did not reveal significant dissemination of staphylococci from the catheter site to other peritoneal structures. After 6 months, spleen specimens of a small number of mice harbored *S. epidermidis*, but this presumably represented bacterial transport via the peritoneal lymphatics and effective clearing of bacteria from the systemic circulation.

Mice with ongoing *S. epidermidis* colonization of the peritoneal catheter site presented systemic neutrophilia that was significantly more evident in animals with *S. epidermidis* 2503 infections. Whether or not host responses could be

modified by the slime-producing characteristics of the test inocula remains unknown; however, prolific slime production by *S. epidermidis* 2503 may block primary adhesin receptor sites, thereby favoring dispersement from the biofilm colony. In the dispersed state, bacteria may more effectively trigger inflammatory processes. Nevertheless, the systemic neutrophil response failed to eliminate the infecting microorganisms from the implant site.

The anemia of chronic disease is associated with any chronic infection or inflammation and is induced by decreased red blood cell production. Normal erythropoiesis is slowly interrupted as iron from senescent erythrocytes is retained by hepatic reticulum cells, thus making it unavailable for reuse and, importantly, for uptake by infecting microorganisms [14, 15]. In the current series, mice with chronic, catheter-associated *S. epidermidis* 29260 or 2503 infection had mild anemia. These findings parallel clinical observations of chronic infection, inflammation, or both.

Due to the attachment of *S. epidermidis* to smooth surfaces, assessments based on standard microbiologic culture may lead to falsely negative culture results. The assay of bacterial biofilms used in this series, using TTC as a metabolic indicator, is technically simple to conduct with minimal setup time. Even when catheter scrapings yielded no bacterial growth, TTC assessments demonstrated *S. epidermidis* biofilms that were positively identified by subculture of the fluid media from TTC-positive catheter segments.

The currently described mouse model presents several of the key characteristics of foreign body infection that have been observed clinically. This model will be used for future studies of the pathogenesis and treatment of foreign body infection.

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