A Model of Infected Burn Wounds Using Escherichia coli O18:K1:H7 for the Study of Gram-Negative Bacteremia and Sepsis

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A difficulty that has emerged in the development and preclinical evaluation of adjuvant therapies for gram-negative sepsis is the lack of easily studied animal models that closely mimic human infection. An objective of this study was to adapt a previously described model of infection in burned mice to rats with a defined bacterial strain of Escherichia coli. Challenge with two colonies of live E. coli O18:K1:H7 bacteria into an 8% full-thickness burn of the dorsal skin surface of rats produced predictable bacteremia at 24 to 48 h and 80 to 100% mortality at 3 to 4 days. E. coli O18:K1:H7 was approximately 10-million-fold more virulent than several other gram-negative bacterial strains. The model should be a useful tool in studying the pathogenicity of burn wound infections and in evaluating the efficacy of novel adjuvant therapies for gram-negative sepsis.

Secondary infections with gram-negative bacteria cause considerable morbidity and occasional mortality in burn patients. Sometimes the infection rapidly leads to overwhelming bacteremia with shock and subsequent death.

Although numerous candidate therapies have been proposed in the last decade for the adjuvant treatment of gram-negative sepsis, it has become clear that many of the animal models that are currently available for studying gram-negative sepsis may inadequately mimic human disease. Problems with current models include the following: purified lipopolysaccharide is sometimes utilized, large amounts of bacterial inoculum are needed that result in endotoxin intoxication rather than infection, the bacteria are injected or infused as a bolus, and the organism utilized either is not virulent or is not a human pathogen. These issues have been recently summarized (1).

There are few models in which a small amount of bacterial inoculum of a clinically relevant gram-negative human pathogen is utilized that subsequently multiplies in vivo, leading to predictable bacteremia and eventual mortality. Simpler models using purified lipopolysaccharide or large amounts of bacterial inoculum may not reflect the complex interplay between host defense and pathogenicity of the organism.

Several burn models have previously been reported (6, 8–11). In many models, Pseudomonas species and/or a relatively large amount of bacterial inoculum has been utilized. In order to develop a more relevant model for the evaluation of novel therapies and to study the roles of capsule and certain outer membrane proteins common to Enterobacteriaceae in protective immunity (2), we sought to adapt one of these models that had utilized mice infected with a strain of Pseudomonas aeruginosa (8) for use in rats infected with a highly defined strain of Escherichia coli. This report describes these experiments.

MATERIALS AND METHODS

E. coli strains O16:K1:H6, O18:K1:H7 (and the K- mutant of this strain), and O25:K5:1H1 and P. aeruginosa strain 12.4.4 were the kind gifts of Alan Cross (University of Maryland Cancer Center, Baltimore, Md.). E. coli O4:K54:H5 was the kind gift of Thomas Russo (State University of New York at Buffalo, Buffalo, N.Y.) (7). The outer membrane protein A (ompA)-negative mutant (ompA-) of E. coli O18:K1:H7 was the kind gift of Kwang Sik Kim, (Los Angeles Children's Hospital, Los Angeles, Calif.) and has been previously described (5). P. aeruginosa ATCC 19660 was obtained from the American Type Culture Collection, Rockville, Md.

A single colony of bacteria was inoculated into 10 ml of Trypticase soy broth (Difco Labs, Detroit, Mich.) and incubated overnight at 37°C. A 20-μl aliquot was added to 10 ml of Trypticase soy broth, incubated on a shaker at 37°C, and grown to mid-log phase. The bacteria were centrifuged at 2,800 × g for 10 min, and the cell pellet was washed three times with 0.9% saline solution and resuspended in 5 ml of 0.9% saline solution. Bacterial concentrations were determined by generating standard curves of bacterial concentration versus absorbance at 550 nm. The final concentration was adjusted as required by serial dilutions with 0.9% saline solution. Exact numbers of CFU injected were determined by plating serial 10-fold dilutions of the bacteria on Trypticase soy agar plates, incubating them overnight at 37°C, and counting the colonies the next day.

The model represents an adaption of a previously reported murine model (8). Permission for animal studies was obtained from the Subcommittee on Animal Studies of Massachusetts General Hospital, Boston, Mass., and development of the burn and infection protocol was closely supervised by a veterinarian from the Office for Laboratory Animal Research of Massachusetts General Hospital.

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nitrocellulose paper and immunostaining with a rabbit immunoglobulin G specific for the O polysaccharide antigen of E. coli O18. The animals were observed twice a day for 10 days.

To measure colony counts in tissues, rats were humanely sacrificed at intervals up to 72 h following burn and infection. The burn area and a remote, previously denuded area were immediately washed with 95% ethanol and allowed to dry in a sterile area. The skin was removed, by scissoring, under sterile conditions, and one triangle of tissue was cut from each of the extreme corners of two diagonally opposed quadrants. The underlying eschar and musculature were each removed in a similar fashion. The tissue specimens were weighed and homogenized in 3 ml of 0.9% saline solution by 5 min of grinding in a sterile Ten Broeck tissue grinder (Wheaton Science Products, Millville, N.J.). Samples were then diluted and cultured on agar plates. Quantitative colony counts were performed for each serial dilution, and the concentration of bacteria was calculated per gram of wet tissue.

Statistics of survival data were determined by the log rank test with GraphPad Prism, version 2.01 (GraphPad Software, Inc., San Diego, Calif.).

RESULTS

We initially assumed that a relatively large amount of bacterial inoculum might be necessary for the induction of bacteremia. However, because of high mortality, we progressively reduced the number of E. coli O18:K1:H7 injected into the 4-in² burn. We found that the injection of only 2 CFU resulted in predictable bacteremia and death (Fig. 1, top panel). There was 100% survival in the absence of a bacterial inoculum. To confirm that a one-to-one correspondence existed between the number of viable bacteria and the number of colonies formed on the agar plates, E. coli O18:K1:H7 bacteria were grown as described above and serially diluted. One 100-μl aliquot of each 10-fold dilution was plated onto agar plates as above, and another was inoculated into 10 ml of broth; all aliquots were then held for 7 days. At dilutions in which there were no CFU on the plates, the broth remained sterile.

Statistics of survival data were determined by the log rank test with GraphPad Prism, version 2.01 (GraphPad Software, Inc., San Diego, Calif.).

The relationship of burn size to survival in the model is shown in Fig. 1, middle panel. Survival was significantly higher in rats with a 2-in² burn than in rats with larger burns (P \leq 0.002). To assess whether the configuration of the burn was important for survival, we compared two burns of 2 in² each (one of which was infected) with a single infected burn of 4 in². Animals with the same total burn size (4 in²) composed of two burns (one of which was infected) had a significantly higher
rate of survival than animals with a single infected burn ($P < 0.0001$) (Fig. 1, bottom panel).

The small amount of bacterial inoculation permitted us to determine the kinetics of bacterial multiplication in the tissues and blood. These data, shown in Fig. 2, suggest that local bacterial replication begins immediately in the burn wound and is detectable in blood after 18 h.

The striking pathogenicity of *E. coli* O18:K1:H7 prompted us to evaluate several other gram-negative bacteria with the same model. These data are shown in Fig. 3. For all other *E. coli* strains, two previously studied strains of *P. aeruginosa*, and *E. coli* O18 organisms that were deficient in either the K1 capsule or OmpA had higher survival rates under identical conditions. The contributions of the capsule and OmpA to pathogenicity have been previously described for models of meningitis (3, 5).

At this time, we do not have an explanation for the striking pathogenicity of *E. coli* O18:K1:H7. While some caution is needed because the virulence of *E. coli* O18:K1:H7 is so much higher than the other organisms studied, an advantage of the model for studies of efficacy and pathogenicity compared to most previously described models of gram-negative infection is that it is a model in which gram-negative bacteria replicate in vivo prior to bacteremia.

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