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Role of Bacterial Association with Kupffer Cells in Occurrence of Endogenous Systemic Bacteremia

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Bacteremia in immunocompromised hosts often arises from their endogenous intestinal flora. We produced experimental endogenous bacteremia by administering cyclophosphamide and ampicillin to conventional and specific-pathogen-free mice. The frequencies of bacteremia and mortality in the conventional mice were significantly higher than for the specific-pathogen-free mice. *Pseudomonas aeruginosa* was the major pathogen causing systemic bacteremia in conventional mice and was associated with a high mortality rate. *Morganella morganii* caused systemic bacteremia in both conventional and specific-pathogen-free mice. In contrast, *Escherichia coli*, enterococci, or other species most often caused portal bacteremia only. To determine the mechanism of occurrence of systemic bacteremia, we investigated bacterial blood clearance in mice and association with murine Kupffer cells, using several bacterial strains isolated from mice with bacteremia. Blood clearance rates and the abilities of isolated Kupffer cells to associate with bacteria were significantly greater for the organisms causing portal bacteremia than for those causing systemic bacteremia. There were no significant differences between the blood clearance rates in carrageenan-treated mice and that in normal mice. Moreover, association at 4°C was not different from that at 37°C. The results suggest that blood clearance of bacteria reflects bacterial adherence to Kupffer cells and that the resistance of bacteria to association with Kupffer cells plays an important role in the occurrence of overwhelming systemic bacteremia in this animal model.

Despite the development of numerous potent antimicrobial agents, the morbidity and mortality from severe bacteremia remain high in immunocompromised hosts (12, 15, 34). Most of these infections arise from the patient's own flora, especially from their intestinal flora, in the form of endogenous bacteremia without obvious local clinical signs (7, 28, 35). Interestingly, only some potential pathogens, such as *Pseudomonas aeruginosa*, frequently and usually cause bacteremia, but the other potential pathogens never or only occasionally produce bacteremia (4, 28, 32, 35).

Other investigators have shown that portal bacteremias due to *Escherichia coli* and other bacteria occur in patients with some disease, such as ulcerative colitis (9, 13, 27), and in an animal model of colonic ischemia (6), whereas peripheral vein blood from such patients and animals was sterile.

We developed a simple model of bacteremia in granulocytopenic mice, using conventional mice that have *P. aeruginosa* in their intestines as resident flora and specific-pathogen-free (SPF) mice that do not carry this organism.

Using this model, we determined whether bacteremia was confined to the portal venous system or whether it was systemic in nature. To examine the possibility that the only bacteria which can resist liver clearance produce systemic bacteremia, we also investigated bacterial blood clearance in mice and association with murine Kupffer cells in vitro, using two bacterial strains that caused systemic bacteremia (including *P. aeruginosa*) and four bacterial strains that caused portal bacteremia in granulocytopenic mice.

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MATERIALS AND METHODS

Production of bacteremia. Conventional and SPF male ddY mice weighing 20 to 24 g were used. Both types of mice were fed a sterile diet. The conventional mice were given tap water, and the SPF mice were given distilled water. These mice were administered 150 mg of cyclophosphamide (Endoxan; Shionogi & Co., Ltd., Osaka, Japan) per kg of body weight by intraperitoneal injection on days 0, 2, 5, and 9. Treatment of mice with 200 mg of sodium ampicillin (Viccillin; Meiji Seika Kaisha, Ltd., Tokyo, Japan) per kg by intraperitoneal injection was begun on day 4 and continued every day until day 11. Mice were examined six times daily, and deaths were recorded until day 14. Necropsies were performed within 4 h on mice that died and on mice killed at the end of the experiments. Blood samples were obtained aseptically at necropsy from heart and portal vein by direct puncture. All samples were immediately aerobically cultured on 5% sheep blood agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) at 37°C in humidified air for 24 h. Bacteria isolated from blood were identified by using Vitek Gram-Positive or Gram-Negative Identification cards (Vitek Systems, Inc., Hazelwood, Mo.). Systemic bacteremia was defined as the isolation of bacteria from both heart blood and portal blood. In contrast, portal bacteremia was defined as the isolation of bacteria from portal blood only. Bacterial isolates were stored in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) with 30% glycerol at −80°C.

Bacterial strains and growth media. Six representative bacterial strains isolated from bacteremic mice were used for subsequent experiments. *Pseudomonas aeruginosa* D4 was isolated from a conventional mouse that died of systemic bacteremia on day 8. *Morganella morganii* L48 was isolated from a SPF mouse with systemic bacteremia. *Escherichia coli* L424, *Enterobacter cloacae* L522, and *Enterococcus*

faecalis L425 were isolated from SPF mice with portal bacteremia. *Klebsiella pneumoniae* H29 was isolated from a conventional mouse with portal bacteremia. The last five bacterial strains were isolated from mice killed on day 14.

Enterococcus faecalis L425 was grown on sheep blood agar, and the others were grown on Trypticase soy agar at 37°C for 18 h. Bacteria originating from a single colony were inoculated in Mueller-Hinton broth which was incubated at 37°C in air for 18 h. Bacteria were harvested from the broth by centrifugation at 10,000 × *g*, washed three times with sterile saline or medium 199 (M-199; Nissui), and adjusted to suitable concentration for each experiment in saline or M-199 by optical density measurements with a spectrophotometer (UVIDEC-40; Jasco, Tokyo, Japan).

Blood clearance of bacteria in mice. SPF male ddY mice weighing 20 to 24 g were injected in the tail vein with 0.2 ml of bacterial suspension in saline containing 10^8 CFU/ml. At 5, 10, 15, and 30 min after injection, 20-μl samples of blood were obtained from the retroorbital plexus with disposable heparinized capillary tubes. At the end of experiments, mice were killed by ether inhalation, after which the livers were removed and homogenized. The blood and liver homogenate samples were diluted in saline and plated on sheep blood agar or Trypticase soy agar. The time zero values were calculated by estimating the total blood volume as 8% (vol/wt) of each body weight of the mice used (20). Whenever desired, 100 mg of D-mannose (Nacalai Tesque, Inc., Kyoto, Japan) was added to the bacterial suspension or injected intravenously at 10 min before bacterial challenge. In some experiments, mice were administered 150 mg of cyclophosphamide per kg or 200 mg of carrageenan type II (Sigma Chemical Co., St. Louis, Mo.) per kg intraperitoneally at 6, 4, and 1 days or at 24 h before bacterial challenge.

Susceptibility of bacteria to serum factors. Survival of bacteria was tested with pooled fresh normal murine serum. Specific antibody against any of the bacterial strains examined was not detected in the serum used by double immunodiffusion (22) or bacterial agglutination method (19). Bacteria were suspended in saline to a concentration of 10^6 CFU/ml, and 0.1 ml of this suspension was added to 0.4 ml of serum and incubated at 37°C for 60 min with gentle rocking. At 30 and 60 min after incubation, samples were withdrawn, and the numbers of viable cells were determined by dilution plating. Serum resistance was defined by maintenance of 100% viability after 60-min incubation.

Preparation of Kupffer cells. Kupffer cells were harvested as previously described (14), with minor modifications. Briefly, SPF male ddY mice weighing 24 to 28 g were anesthetized with pentobarbital. The livers were perfused through the portal vein in situ, first with Ca^{2+}-free Hanks balanced salt solution (Sigma) for 5 min and then with M-199 containing 0.05% collagenase type I (290 U/mg; lot 128F-0295; Sigma), 0.001% DNase (3,008 U/mg; lot 58P719; Worthington Biochemical Co., Freehold, N.J.), 5% fetal calf serum, 5 mM $CaCl_2$, and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) for 10 min, at 4°C at a rate of 2 ml/min. The livers were then removed and forced through 50-gauge stainless steel mesh (Cellector; Bellco Glass, Inc., Vineland, N.J.). The cells in the resulting filtrate were incubated in the solution containing the enzymes as described above for 60 min at 37°C with constant stirring. The suspension was filtered through twin nylon mesh (pore size, 20 μm) and then centrifuged at 300 × *g* for 10 min. Nonparenchymal cells were separated from hepatocytes by differential sedimentation over 30.0% (wt/vol) metrizamide (Sigma) cushions. Centrifugation was performed at 3,000 × *g* for 15 min. After two washes, nonparenchymal cells were resuspended in M-199 with 20% fetal calf serum and 10 mM HEPES at a concentration of 3.0×10^6 cells per ml. A 0.3-ml portion of cell suspension was placed in each tissue culture chamber slides (Lab-Tek; Miles Laboratories, Inc., Naperville, Ill.). Chamber slides were incubated at 37°C in 5% CO_2 for 1 h. After nonadherent cells were removed by gentle washing, adherent cells were incubated with the medium as described above. More than 99% of adherent cells were esterase positive. All experiments were performed by using Kupffer cells after 24 h of culture.

Visual assessment of bacterial association with Kupffer cells. Chamber slides containing Kupffer cells were washed three times with warm M-199, and 0.2 ml of bacterial suspension in M-199 containing 10^8 CFU/ml with or without 20% murine serum was added to each chamber. The chamber slides were incubated at 37 or 4°C for 60 min with gentle rocking and washed 5 times with M-199. The chambers were removed from the slides, and the slides were air dried, fixed with methanol, and stained with acridine orange (Vacutainer; Becton Dickinson and Co., Rutherford, N.J.). The slides were examined at ×400 magnification with oil immersion under a fluorescence microscope (BH2-RFL-T2; Olympus Optical Co., Ltd., Tokyo, Japan). A total of 200 Kupffer cells were examined in each experiment, and the percentage of bacteria-associated Kupffer cells and the number of bacteria per Kupffer cell were recorded.

Effect of antiserum on association of *P. aeruginosa* D4 and *M. morganii* L48. Antiserum was raised in rabbits by three intramuscular injections (days 1, 8, and 15) of Formalin-killed whole bacterial cells emulsified in Freund incomplete adjuvant. The serum was collected on day 21 and stored at −80°C. Bacteria and Kupffer cells were incubated with 20% normal rabbit serum or antiserum against each strain at 37°C for 60 min. Examination was performed as described above.

Screening for mannose sensitivity and bacterial surface mannose. To examine mannose sensitivity of bacterial strains, agglutination was performed by mixing 25 μl of Mannosylated Taxonobeads (E-Y Laboratories, Inc., San Mateo, Calif.) with 25 μl of bacterial suspension containing 10^{10} bacteria per ml in the absence or presence of 0.25 M D-mannose on a slide. To examine bacterial surface mannose, agglutination was performed with 25 μl of concanavalin A (Vector Laboratories, Inc., Burlingame, Calif.) at a concentration of 1 mg/ml instead of Taxonobeads. After gentle shaking, agglutination was observed visually within 15 min at room temperature. Mannose-inhibitable agglutination was considered positive for either mannose sensitivity (Mannosylated Taxonobeads) or bacterial surface mannose (concanavalin A).

Assessment of hydrophobicity. Cell surface hydrophobicity was determined by adherence to liquid hydrocarbon (25). Briefly, bacteria were suspended in saline to an optical density of 1.0 to 1.2 at 400 nm (1-cm cell width) with a spectrophotometer, and 0.6 ml of *n*-hexadecane (Nacalai) was added to 3.6 ml of an aqueous bacterial suspension, and mixtures were agitated uniformly for 2 min. After allowing 15 min for the hydrocarbon phase to separate completely, the bottom aqueous phase was carefully removed and transferred to cuvettes, and the optical density at 400 nm was read. Results were expressed as the percentage of decrease in the absorbance of the the lower aqueous phase compared with the absorbance of the initial cell suspension.

Statistics. Frequencies of bacteremia and mortality were analyzed by the χ^2 test. Student's *t* test was used to compare means, and a level of 5% was taken to be significant.

TABLE 1. Organisms isolated from conventional mice with bacteremia

Organism	No. of causative organisms	
	Systemic bacteremia	Portal bacteremia
Pseudomonas aeruginosa	15	1
Morganella morganii	9	1
Escherichia coli	0	3
Klebsiella pneumoniae	0	1
Proteus vulgaris	0	2

RESULTS

Frequency of bacteremia and causative organisms. Bacteremia occurred in 25 (83.3%) of 30 conventional mice, from which 32 organisms were isolated (Table 1). Portal bacteremia was observed in 7 (28.0%) of the 25 mice, and 8 (25.0%) of the 32 organisms were isolated from portal blood only. Of the 25 mice, 6 (24.0%) had polymicrobic bacteremia: systemic bacteremia due to *P. aeruginosa* and *M. morganii* (3 mice), portal bacteremia due to *E. coli* and *Proteus vulgaris* (1 mouse), systemic bacteremia due to *P. aeruginosa* and *M. morganii* and portal bacteremia due to *P. vulgaris* (1 mouse), and systemic bacteremia due to *M. morganii* and portal bacteremia due to *P. aeruginosa* (1 mouse). The mortality from bacteremia was 64% (16 of the 25 mice died). *Pseudomonas aeruginosa* was isolated from blood from the hearts of all dead mice, except for one mouse which died of *M. morganii* systemic bacteremia. These mice died between day 7 and day 13.

In contrast, bacteremia occurred in 27 (20.3%) of 133 SPF mice, from which 30 organisms were isolated (Table 2). Portal bacteremia was observed in 8 (29.6%) of the 27 mice, and 10 (33.3%) of the 30 organisms were isolated from portal blood only. Of the 27 mice, 3 (11.1%) had polymicrobic bacteremia: systemic bacteremia due to *P. aeruginosa* and *M. morganii* (one mouse), portal bacteremia due to *M. morganii* and *Enterococcus faecium* (1 mouse), and portal bacteremia due to *E. coli* and *E. faecalis* (1 mouse). One mouse died of systemic bacteremia caused by *P. aeruginosa* on day 8, and the mortality rate was 3.7%. *Pseudomonas aeruginosa* that caused bacteremia in the SPF mice was considered to be acquired during the experiments.

Both the frequency of occurrence of bacteremia and the mortality were significantly higher in conventional mice ($P < 0.01$). The frequency of occurrence of portal bacteremia based on the number of mice examined was also significantly higher in the conventional mice (23.3% versus 6.0%; $P < 0.01$). The most frequent organism causing bacteremia was *P. aeruginosa* in conventional mice and *M. morganii* in SPF mice. Almost all of the bacteremias caused by *P. aeruginosa* and *M. morganii* were systemic in nature. In contrast, most of the bacteremias due to the other bacteria isolated were portal bacteremias.

TABLE 2. Organisms isolated from SPF mice with bacteremia

Organism	No. of causative organisms	
	Systemic bacteremia	Portal bacteremia
Pseudomonas aeruginosa	2	1
Morganella morganii	17	1
Escherichia coli	0	2
Enterobacter cloacae	1	3
Enterococcus faecalis	0	1
Enterococcus faecium	0	2

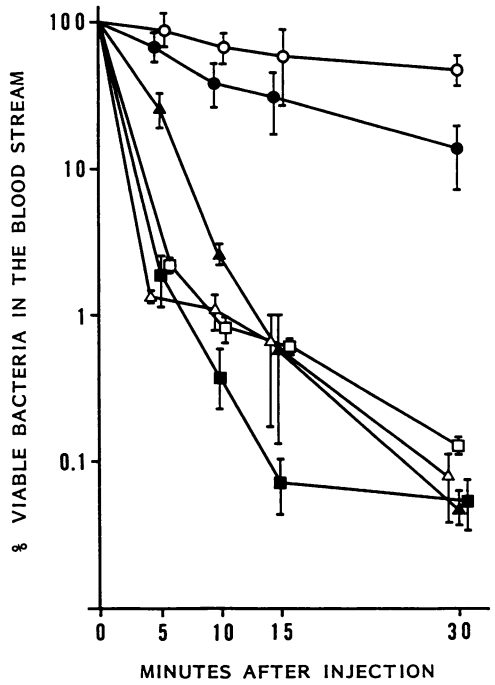

FIG. 1. Blood clearance of bacterial strains in mice. The results are expressed as the mean recovery percentages ± standard deviations of five experiments. The clearance of *M. morganii* L48 and *P. aeruginosa* D4 was significantly slower than those of the other bacterial strains at all time points ($P < 0.01$). Symbols: ●, *P. aeruginosa* D4; ○, *M. morganii* L48; ■, *E. coli* L424; □, *E. cloacae* L522; ▲, *K. pneumoniae* H29; △, *E. faecalis* L425.

Blood clearance and susceptibility to serum factors. Blood clearance rates of the bacterial strains are shown in Fig. 1. Normal mice rapidly cleared all bacterial strains isolated from portal blood, i.e., *E. coli* L424, *E. cloacae* L522, *K. pneumoniae* H29, and *E. faecalis* L425. At the end of the experiments, the recovery of viable *E. coli* L424, *E. cloacae* L522, *K. pneumoniae* H29, and *E. faecalis* L425 in the livers was 80.25% ± 27.29%, 77.03% ± 7.75%, 98.58% ± 17.10%, and 70.13% ± 9.32%, respectively (mean ± standard deviation of five experiments). In contrast, the blood clearance of bacterial strains isolated from heart blood, *P. aeruginosa* D4 and *M. morganii* L48, was significantly slower than the clearance of the others at all time points ($P < 0.01$).

Figure 2 shows that the clearance of *E. coli* L424 in granulocytopenic mice treated with cyclophosphamide or carrageenan-treated macrophage-depleted mice was not significantly different from the clearance in normal mice. The recovery of viable *E. coli* L424 in the livers of carrageenan-treated mice was calculated to be 89.48% ± 11.56%, which was not significantly different from that of normal mice. Moreover, there were no significant differences in the clearance rates of *E. cloacae* L522, *K. pneumoniae* H29, and *E. faecalis* L425 between normal mice, cyclophosphamide-treated mice, and carrageenan-treated mice (data not shown).

Table 3 shows the survival rates of bacterial strains in murine sera. No killing was observed for any of the bacterial strains.

Bacterial association with Kupffer cells. Association of *E.*

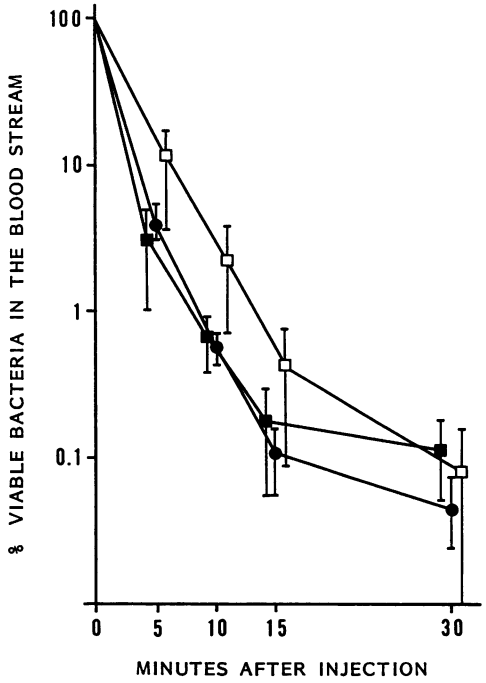

FIG. 2. Blood clearance of *E. coli* L424 in granulocytopenic mice and macrophage-depleted mice. The results are expressed as the mean recovery percentages ± standard deviations of five experiments. There were no significant differences among the clearance rates of *E. coli* L424 in the three groups. Symbols: ●, control mice; ■, granulocytopenic mice treated with cyclophosphamide; □, macrophage-depleted mice treated with carrageenan.

coli L424, *E. cloacae* L522, *K. pneumoniae* H29, and *E. faecalis* L425 was significantly greater than those of *P. aeruginosa* D4 and *M. morganii* L48 at 37°C in the absence of murine sera ($P < 0.01$) (Table 4). Because this experiment did not show whether the bacteria were ingested or adhered to the surface of Kupffer cells, the association at 37°C was compared with that at 4°C, a temperature at which ingestion is sluggish. There was no significant decrease in the association of bacterial strains performed. Murine sera significantly enhanced the association of *E. faecalis* L425 ($P < 0.01$). However, the association of the others was not significantly enhanced by murine sera. Table 5 shows that antisera significantly enhanced the association of *P. aeruginosa* D4 and *M. morganii* L48 ($P < 0.01$). Kupffer cells poorly associated with these strains under all other conditions.

TABLE 3. Percentage of survival of bacterial strains in murine serum

Bacterial strain	% Survival[a] after:	
	30 min	60 min
P. aeruginosa D4	131.8 ± 12.9	164.7 ± 27.6
M. morganii L48	151.0 ± 26.5	165.7 ± 20.3
E. coli L424	107.8 ± 4.7	140.5 ± 5.6
E. cloacae L522	115.6 ± 7.7	124.4 ± 9.5
K. pneumoniae H29	120.4 ± 9.6	122.5 ± 11.3
E. faecalis L425	114.7 ± 11.3	130.8 ± 16.7

[a] Results are expressed as means ± standard deviations of five independent assays.

Agglutination and inhibition of blood clearance by sugars. Table 6 shows that *E. coli* L424 and *E. cloacae* L522 were positive in their mannose sensitivity. All other bacterial strains did not agglutinate Mannosylated Taxonobeads and were considered negative with respect to their mannose sensitivity. On the other hand, *P. aeruginosa* D4, *K. pneumoniae* H29, and *E. faecalis* L425 were positive in the agglutination with concanavalin A, and their agglutination was mannose inhibitable. Clearance of *E. coli* L424 and *E. cloacae* L522, however, was not inhibited by D-mannose mixed with bacteria (data not shown). Moreover, the clearance of *K. pneumoniae* H29 and *E. faecalis* L425 was not inhibited by D-mannose injected before bacterial challenge (data not shown).

Hydrophobicity. The hydrophobicity of *E. faecalis* L425 and *M. morganii* L48 was significantly higher than those of the other bacteria ($P < 0.01$) (Table 6). More than 89% of *E. faecalis* L425 and over 50% of *M. morganii* L48 were removed from the aqueous phase by hexadecane. The other bacteria showed little or no affinity towards hexadecane.

DISCUSSION

Some investigators have produced endogenous bacteremia by feeding animals *E. coli*, *K. pneumoniae* (37), or *P. aeruginosa* (10). Others have produced bacteremia by instillation of *P. aeruginosa* into the conjunctival sacs of rabbits (36). In contrast, we produced endogenous bacteremia in mice by administering cyclophosphamide and ampicillin without artificial inoculation of bacteria. We consider this model to closely resemble the situation observed in neutropenic humans. Our data suggest that *P. aeruginosa* colonizing the gastrointestinal tract contributed to the high incidence of systemic bacteremia with great mortality.

Both *P. aeruginosa* and *M. morganii* were likely to cause systemic bacteremia, but only *P. aeruginosa* bacteremia caused a high mortality rate. These results suggest that there are major pathogenic differences between *P. aeruginosa* and *M. morganii*. However, it is still not fully understood how bacteria invade blood vessels and enter the blood stream. It is possible that polymorphonuclear cells, especially neutrophils, play an important role as the main effector in protection of the host against bacteremia and that granulocytopenia encourages the development of these infections (5, 8, 10). It is also possible that disruption of the intestinal mucosal barrier is induced by bacterial exoenzymes, antimicrobial agents, and therapy for treatment of underlying disease. It is also unclear why the frequency of portal bacteremia in the conventional mice was higher than for the SPF mice. There may be differences in the composition and number of the intestinal flora involved in the protection of mice against overgrowth with potential pathogens because diet drastically affects the intestinal flora (16). In addition, a recent study showed that fecal counts of antibiotic-resistant bacteria able to cause systemic infections were significantly higher in subjects receiving a normal diet than in those taking a sterile diet (11). It is also speculated that exoenzymes produced by *P. aeruginosa* may also help other bacteria to enter the blood stream in conventional mice.

Our data suggest that blood clearance rate is one of the important factors that determine whether latent portal bacteremia in the initial stage progresses to systemic infection or not. Some investigators have demonstrated that liver trapping reflects blood clearance (20) and used perfused liver to study the phagocytosis and killing activity of hepatic macrophages (3). In contrast, we examined the association of

TABLE 4. Bacterial association with murine Kupffer cells

Bacterial strain	37°C without serum		4°C without serum		37°C with serum[a]	
	% Kupffer cells[b]	No. of bacteria[c]	% Kupffer cells	No. of bacteria	% Kupffer cells	No. of bacteria
P. aeruginosa D4	27.7 ± 13.4[d]	0.3 ± 0.2[d]	NT[e]	NT	30.0 ± 13.9[d]	0.4 ± 0.2[d]
M. morganii L48	13.3 ± 7.9[d]	0.2 ± 0.1[d]	NT	NT	19.0 ± 8.8[d]	0.3 ± 0.1[d]
E. coli L424	95.5 ± 1.7	4.2 ± 0.3	93.9 ± 2.5	4.0 ± 0.3	96.9 ± 2.2	5.0 ± 0.9
E. cloacae L522	94.1 ± 2.2	3.8 ± 0.1	93.3 ± 3.2	3.8 ± 0.3	95.9 ± 2.4	4.3 ± 0.6
K. pneumoniae H29	93.7 ± 2.4	3.9 ± 0.2	92.3 ± 1.8	3.7 ± 0.5	95.4 ± 5.1	5.2 ± 1.1
E. faecalis L425	69.8 ± 4.3	3.9 ± 0.5	NT	NT	96.3 ± 1.8[f]	4.2 ± 0.2[f]

[a] Bacteria and Kupffer cells were incubated with 20% murine serum.
[b] Percentage of bacteria-associated Kupffer cells. Results are expressed as means ± standard deviations of five independent assays.
[c] Number of bacteria per Kupffer cell. Results are expressed as means ± standard deviations of five independent assays.
[d] Significantly less than the values of *E. coli* L424, E. cloacae L522, *K. pneumoniae* H29, and E. faecalis L425 ($P < 0.01$).
[e] NT, Not tested.
[f] Significantly greater than the value at 37°C without serum ($P < 0.01$).

several bacterial strains with isolated murine Kupffer cells. We harvested Kupffer cells by a method which leaves Fc and complement receptors on their surfaces intact (14). In our preliminary time course studies, the association reached a maximum at 16 to 24 h after removing nonadherent cells, and then the association was static (data not shown). We think that the receptors on the surfaces of Kupffer cells had already reappeared by 24 h of culture. Our results can be best explained in conjunction with the studies showing that liver or hepatic macrophages are responsible for rapid blood clearance of bacteria.

Of considerable interest was the observation that the blood clearance in carrageenan-treated mice was not different from that in normal mice and that the bacterial association did not decrease at 4°C in vitro. The blood clearance in carrageenan-treated mice may be due to bacterial adherence to the surfaces of Kupffer cells which have lost their ability to ingest and kill bacteria. Pruzzo et al. (24) have demonstrated that *K. pneumoniae* carrying the mannose-inhibitable adhesin efficiently binds to polymorphonuclear leukocytes incubated at 4°C but not at 37°C. It is possible that there is a difference between the mechanism of association of the bacteria that we examined at 37°C and at 4°C. Our data suggest that blood clearance and association of the bacterial strains examined may reflect adherence phase of phagocytosis rather than ingestion phase. However, our data do not exclude the possibility that hepatic clearance may also be due to bacterial adherence to other sinusoidal cells, such as endothelial cells, because Friedman and Moon (17) have shown that hepatic trapping of *Salmonella typhimurium* involves adherence to both endothelial and Kupffer cells.

Serum opsonins frequently serve an adherence and ingestion function (18). Nonetheless, some bacterial species, including *E. coli*, *K. pneumoniae*, and *P. aeruginosa* are susceptible to nonopsonic phagocytosis (30). Opsonic phagocytosis and nonopsonic phagocytosis seem to act as complementary processes in protection against invading microorganisms. Nonopsonic phagocytosis, such as lectinophagocytosis (21) and hydrophobic interaction (1, 33), may play an important role in host defense against infection, especially in the initial phase of infection, at sites where levels of antibody and complement are naturally low, in a complement-deficient host, or in the case of infection with bacteria that do not activate the alternative pathway of complement (21, 31). Nonopsonic clearance is also important in host defense against bacteremia (31). In this study, only the association of *E. faecalis* L425 was enhanced by normal murine sera. It is possible that fibronectin in serum mediated the association of this strain by binding to teichoic acid or lipoteichoic acid which are major structural components of gram-positive cocci walls (2, 29). It is also possible that the association of *E. faecalis* L425 depends on cell surface hydrophobicity in the absence of serum, although hydrophobicity did not seem to be related to the association of other bacterial strains examined.

Recent studies with *E. coli* (23), *S. typhimurium* (20) and *Serratia marcescens* (26) have shown that blood clearance or liver clearance of these microorganisms is mannose inhibitable. Our data have shown that all of the bacterial strains cleared rapidly from blood had mannose-type lectins or mannose on their surfaces enabling lectinophagocytosis, but we observed no inhibition of their blood clearance by mannose. These data suggest that the rapid blood clearance of some isolates cannot be explained by a single factor and that additional factors may be involved.

In conclusion, we have shown that there are significant differences in blood clearance and association with Kupffer

TABLE 5. Effect of antiserum on bacterial association with Kupffer cells

Bacterial strain	Normal serum[a]		Antiserum[b]	
	% Kupffer cells	No. of bacteria	% Kupffer cells	No. of bacteria
P. aeruginosa D4	31.0 ± 7.7	0.4 ± 0.1	92.2 ± 3.3[c]	6.4 ± 1.5[c]
M. morganii L48	11.4 ± 3.4	0.1 ± 0.0	91.3 ± 5.4[c]	6.8 ± 1.1[c]

[a] Bacteria and Kupffer cells were incubated with 20% normal rabbit serum.
[b] Bacteria and Kupffer cells were incubated with 20% antiserum.
[c] Significantly greater than the value with normal serum ($P < 0.01$).

TABLE 6. Mannose sensitivity, cell surface mannose, and hydrophobicity

Bacterial strain	Mannose sensitivity	Cell surface mannose	Hydrophobicity (%)[a]
P. aeruginosa D4	−	+	7.65 ± 2.82
M. morganii L48	−	−	51.13 ± 9.29[b]
E. coli L424	+	−	2.59 ± 0.31
K. pneumoniae H29	−	+	8.13 ± 1.49
E. faecalis L425	−	+	89.66 ± 2.99[b]

[a] Results are expressed as means ± standard deviations of five independent assays.
[b] Significantly higher than the values of the other bacterial strains.

cells between the bacterial strains causing systemic bacteremia and the strains causing portal bacteremia. Although it is still not clear how the former bacterial strains resist association with Kupffer cells, resistance to association may play an important role in the occurrence of overwhelming systemic bacteremia.

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