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Clearance of *Serratia marcescens* from Blood in Mice: Role of Hydrophobic Versus Mannose-Sensitive Interactions

SHIMON RUMELT,[1] ZVI METZGER,[1] NAAM KARIV,[2] AND MEL ROSENBERG[1,3]*

The Maurice and Gabriela Goldschleger School of Dental Medicine,[1] Glasberg Tower for Medical Research,[2] and Department of Human Microbiology,[3] Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, 69978 Tel-Aviv, Israel

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In the present study, we examined the potential roles of cell surface hydrophobicity and mannose-sensitive (MS) interactions in blood clearance of *Serratia marcescens* in mice. Hydrophobic strain RZ, partially hydrophobic mutant 3162, and nonhydrophobic mutant 3164 were coinoculated into BALB/c male mice, and blood samples were plated out at different time intervals; colonies of the three strains were distinguished by their different morphologies. All three strains were cleared from the blood stream at similar rates, despite their large relative differences in cell surface hydrophobicity. Clearance from blood was subsequently studied by coinoculating two clinical isolates which differ in their abilities to adhere via MS interactions. MS^+ strain 1785 was cleared much more rapidly than MS^- strain 3255; moreover, in the presence of D-mannose, clearance of strain 1785 was inhibited to a rate similar to that of MS^- strain 3255. When D-glucose was substituted for D-mannose, inhibition was not observed. The results suggest that MS, rather than hydrophobic, interactions are primarily responsible for the rapid clearance of *S. marcescens* from blood observed.

Bacterial adhesion has been recognized as an initial step in many instances of host colonization (1). Many adhesion phenomena have been attributed to either hydrophobic interactions or lectinlike (e.g., mannose-sensitive) interactions (1, 2–5, 7–9, 11–15). *Serratia marcescens* is an interesting microorganism in this respect, since strains may independently exhibit either or both adhesion mechanisms (13).

Cell surface hydrophobicity has been cited as an important factor in mediating bacterial adhesion to a variety of surfaces of clinical importance, including tooth surfaces, plastic implants, epithelial cells, and phagocytes (2, 4, 8, 12, 13, 15; for a review, see reference 14). However, little attention has been paid to the possible effects of bacterial hydrophobicity on microbial survival after entry into the bloodstream. Ofek et al. (8) have postulated that *Streptococcus pyogenes* may alter its outer surface hydrophobicity, presenting a hydrophobic outer surface to promote initial adhesion but substituting a hydrophilic hyaluronic acid capsule after invasion to evade clearance mechanisms. The recent finding that clinical *S. marcescens* isolates exhibit pronounced hydrophobicity when grown at 30°C but not at 38°C led to the similar conjecture that modulation of cell surface hydrophobicity may serve as a clearance-avoiding mechanism in *S. marcescens* (13).

Recent studies with *Escherichia coli* (9) and *Salmonella typhimurium* (3, 5) have shown that clearance of these microorganisms from blood is primarily due to mannose-sensitive (MS) interactions. However, neither of these bacteria normally exhibits pronounced cell surface hydrophobicity (14).

In the present study, we examined the potential roles of cell surface hydrophobicity and MS interactions in mediating clearance of *S. marcescens* from blood in mice. The data suggest that MS interactions, not bacterial hydrophobicity, are mainly responsible for the rapid clearance from blood observed.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. marcescens* RZ was originally obtained from R. Zack, Tel-Aviv University. Mutants 3162, exhibiting intermediate cell surface hydrophobicity, and 3164, almost completely devoid of cell surface hydrophobicity, were derived from strain RZ, as reported previously (12). Pigmented clinical isolate 1785 was obtained from the Rambam Hospital, Haifa, Israel; nonpigmented isolate 3255 was obtained from the Chaim Sheba Medical Center, Tel-Hashomer, Israel (13). Relevant properties of the strains used are presented in Table 1. The bacteria were maintained on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, Mich.) at 4°C and transferred every month. Starter cultures were prepared by inoculating bacteria to tubes containing 4 ml of BHI broth (Difco) which were incubated for 18 h with shaking at 30°C. Bacteria were then inoculated into flasks containing 50 ml of BHI broth with 0.25 ml of liquid culture and incubated for 18 h at 30°C in a G76 Gyrotory shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 300 rpm. After growth, bacteria were harvested by centrifugation at 10,000 × *g*, washed twice, and suspended in a phosphate-buffered saline (PBS; 8 g of NaCl, 0.2 g of KCl, 1.15 g of Na_2HPO_4, 0.2 g of KH_2PO_4, distilled water to 1 liter [pH 7.2]) to an optical density of ca. 1.0 at 400 nm, as measured with a Kontron 710 spectrophotometer (Uvikon, Zurich, Switzerland). Suspensions were tested for cell surface hydrophobicity on the bases of (i) adhesion to 0.2 ml of hexadecane, as previously described (12, 13) but with PBS as the suspending buffer, and (ii) adhesion to untreated polystyrene (13); MS adhesion (6, 7) was determined by mannose-inhibitable aggregation of *Saccharomyces cerevisiae* cells as described previously (9, 13).

Survival of bacteria in fresh mouse blood in vitro. To 0.5 ml of fresh mouse blood was added 0.05 ml of a bacterial suspension containing ca. 10^6 bacteria per ml and heparin (20 U). The mixtures were tumbled at 37°C for 60 min. The suspensions were then diluted 1:10 in distilled water and subsequently in PBS and plated on BHI agar. Test tubes

TABLE 1. Properties of the *S. marcescens* strains used

Strain	Distinguishing colony morphology	Cell surface hydrophobicity[a]	MS adhesion[b]
RZ	Pigmented	High	+
3162	Nonpigmented	Intermediate	+
3164	Pigmented, translucent[c]	Low	+
1785	Pigmented	High	+
3255	Nonpigmented	High	−

[a] As determined by percent adhesion to 0.2 ml of hexadecane (13); high, >75%; intermediate, 25 to 75%; low, <25%.
[b] As determined by mannose-inhibitable agglutination of *S. cerevisiae* cells (6, 13).
[c] With respect to wild-type RZ colonies.

containing heparinized PBS instead of blood served as controls.

Clearance of bacteria from blood. Male BALB/c mice (age, 6 to 7 weeks; three mice in each group) were injected by the tail vein with 0.5 ml containing bacterial mixtures (ca. 10^8 CFU/ml total) and 50 U of heparin (to test the effects of sugars, the mixture was supplemented with 90 mg of D-mannose or D-glucose, as indicated; such concentrations were found to be below the lethal level). At various times after injection, 20-μl samples of blood were obtained from the retroorbital plexus with disposable micropipettes. The samples were diluted 1:10 into distilled water and subsequently in PBS and plated on BHI agar. Differentiation between the different strains was based on colony morphology and pigmentation (Table 1).

Statistical analysis. Statistical analysis was carried out with the paired *t* test.

RESULTS

The survival of the various bacterial strains in fresh mouse blood is shown in Table 2. In no instance was killing observed during a 60-min period.

Figure 1 represents a typical experiment showing the blood clearance of three isogenic strains that differ in their hydrophobic properties: wild-type strain RZ, which exhibits pronounced cell surface hydrophobicity; mutant 3162, which exhibits intermediate affinity for hydrophobic surfaces; and nonhydrophobic mutant 3164 (13). All three strains were cleared from the blood stream at similar rates, despite the extreme differences in their respective surface hydrophobicities.

Clearance from blood of two clinical isolates, 1785 and 3255, which have similar hydrophobic properties but differ in MS adhesion, was subsequently studied in the absence (Fig. 2A) or presence (Fig. 2B) of D-mannose. In the absence of D-mannose, strain 1785 (MS^+) was cleared much more rapidly than strain 3255 (MS^-). For example, at 15 min after injection, the clearance of strain 1785 was 30-fold higher than that of strain 3255. However, when mannose was present in the coinoculation mixture, the clearance of MS^+ strain 1785 was greatly inhibited and similar to that of MS^- strain 3255 (Fig. 2B). As expected, clearance of the latter strain was not significantly affected by addition of the monosaccharide.

To determine whether the observed inhibition by D-mannose was a general sugar effect, additions of D-mannose and D-glucose were compared (Table 3). D-Glucose did not appear to inhibit rapid clearance of MS^+ strain 1785 from blood as did D-mannose. For example, 10 min after inoculation, only 2.7% of the MS^+ cells could be recovered when glucose was present, as opposed to 32% when mannose was present. As expected, MS^- strain 3255 was cleared at the same rate whether mannose or glucose was present.

TABLE 2. In vitro survival of *S. marcescens* strains in fresh mouse blood versus PBS

Strain	Mean (±SD) CFU (10^3)/ml after incubation[a] in:	
	PBS	Fresh mouse blood
RZ	16.6 ± 1.7	25.4 ± 2.4
3162	41.3 ± 4.8	50.9 ± 4.2
3164	74.4 ± 12.6	139 ± 24
1785	67.0 ± 1.4	80.0 ± 41
3255	33.1 ± 5.3	106 ± 38

[a] After 1 h of incubation at 37°C.

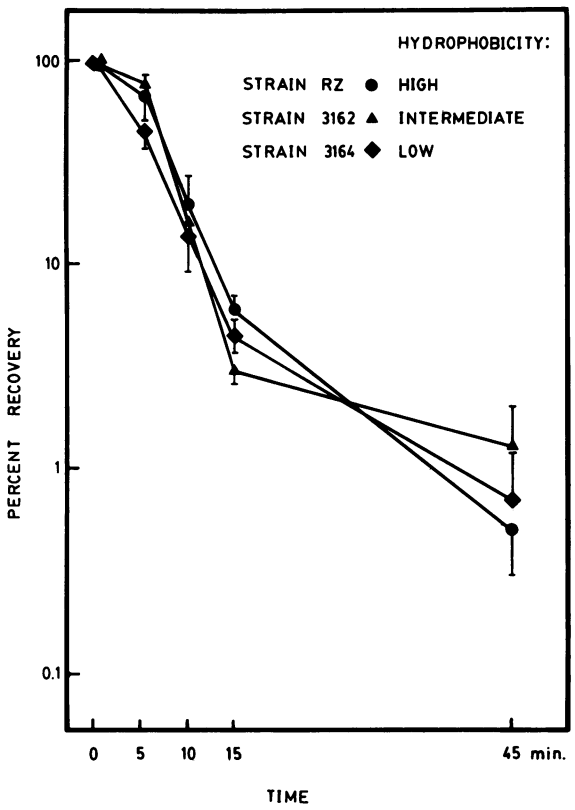

FIG. 1. Effect of cell surface hydrophobicity on clearance from blood of *S. marcescens*. Strains RZ, 3162, and 3164 were grown, washed, and suspended in PBS as described in Materials and Methods. Equal volumes of bacterial suspensions were combined to obtain the inoculation mixture. At various times after inoculation, blood samples were taken, diluted first into tap water and then into PBS, and plated. The results are expressed as the mean recovery percentages (± the standard deviation) of strains RZ, 3162, and 3164 as a function of time. The time zero values were calculated by estimating the total blood volume as 8% (vol/wt) of the average weight (20 g) of the mice used (5).

DISCUSSION

Bacterial hydrophobicity has emerged in recent years as an important factor in a wide array of adhesion and adhesion-related phenomena (14), including hemagglutination (4)

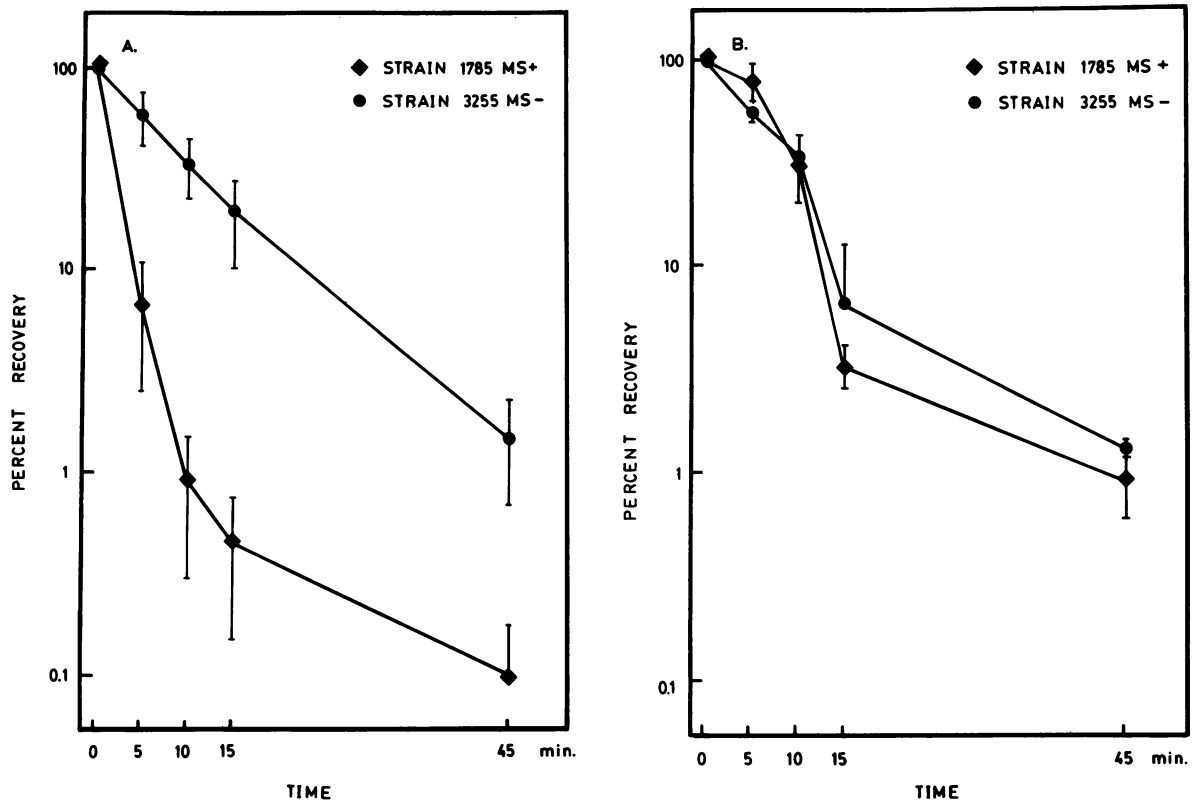

FIG. 2. Clearance of MS^+ versus MS^- *S. marcescens* from blood in the absence or presence of D-mannose. MS^+ strain 1785 and MS^- 3255 were grown and prepared as described in Materials and Methods. Equal volumes of the two bacterial suspensions were combined to obtain the inoculation mixture. At various time intervals after inoculation, blood samples were taken, diluted into PBS, and plated. The results are expressed as the mean recovery percentages (± the standard deviation) of strains 1785 and 3255 as a function of time. (A) Results obtained without mannose. The differences between the recoveries of strains 1785 and 3255 were statistically significant ($P < 0.05$) at 5 and 10 min after injection. (B) Results obtained with D-mannose in the inoculation mixture. Both experiments used the same bacterial suspensions and were carried out simultaneously. The time zero values were calculated by estimating the total blood volume as 8% (vol/wt) of the average weight (20 g) of the mice used (5).

and phagocytosis (11, 15). Stereospecific, lectinlike interactions have also been shown to mediate associations of many bacterial species with host cells (1–3, 5, 7, 9), including phagocytosis (2) and clearance from blood (3, 5, 9). It was thus of interest to see whether either or both of these mechanisms mediate clearance from blood in *S. marcescens*, a microorganism which has been increasingly implicated in nosocomial infections (10, 16) and which can simultaneously exhibit both MS^+ and hydrophobic surface properties (13).

To assess the potential role of cell surface hydrophobicity, the clearances from blood of hydrophobic wild-type strain RZ, mutant 3162, which is partially deficient in hydrophobic surface properties, and nonhydrophobic mutant 3164 were compared. The different colony morphologies of the wild-type and mutant cells (Table 1) enabled us to coinoculate them into test animals. Despite the large differences in cell surface hydrophobicity, they did not differ in their respective clearance rates. The possibility that pigmentation is involved in clearance from blood can be ruled out, since nonpigmented strain 3162 was cleared in a manner similar to that of the two pigmented strains, RZ and 3164.

To study the potential role of MS adhesins, two clinical isolates with similar hydrophobic properties but differing in MS properties were compared. One pigmented and one nonpigmented isolate were chosen so that they could be coinoculated and subsequently distinguished. MS^+ strain 1785 was cleared much more rapidly than MS^- strain 3255. To further establish a role for MS interactions in the rapid clearance of strain 1785, the two strains were also coinoculated in the presence of D-mannose. Whereas clearance of strain 3255 (MS^-) was not appreciably affected, mannose greatly inhibited the clearance of strain 1785 (MS^+). Addition of D-glucose did not similarly inhibit the rapid clearance of MS^+ strain 1785, a further indication of the specificity of the mannose-mediated inhibition.

TABLE 3. Inhibition of rapid clearance from blood by D-mannose but not by D-glucose[a]

Time (min) after injection	Mean (±SD) % recovery of[b]:			
	Strain 1785 (MS^+)		Strain 3255 (MS^-)	
	D-Glucose	D-Mannose	D-Glucose	D-Mannose
5	19 ± 5	50 ± 2	66 ± 13	61 ± 10
10	2.7 ± 2	32 ± 10	29 ± 16	33 ± 7
45	0.37 ± 0.16	4.5 ± 0.8	3.6 ± 1.2	4.3 ± 0.8

[a] The clearance of a mixture of strains 1785 (MS^+) and 3255 (MS^-) from blood was studied, as described in the legend to Fig. 2, in the presence of either D-mannose or D-glucose (180 mg/ml).

[b] Results are expressed as mean percent recovery to the initial inoculum. The differences between the recoveries of strains 1785 and 3255 in the presence of glucose were statistically significant ($P < 0.05$) at 5 and at 45 min after injection.

These data suggest that MS interactions play a role in the rapid clearance from blood observed. This finding concurs with previous reports of the importance of MS interactions in clearance from blood of other gram-negative bacteria (3, 5, 9).

In a previous study, cell surface hydrophobicity was observed in all 14 clinical isolates of *S. marcescens* tested (13). Nevertheless, hydrophobicity does not appear to play a significant role in the clearance of *S. marcescens* from blood in mice under the conditions studied. These data do not rule out a role for cell surface hydrophobicity in host colonization by *S. marcescens*, e.g., via the gastrointestinal tract. Studies in this direction are currently in progress.

S. marcescens is a common environmental microorganism which has recently been cited in a wide variety of nosocomial infections (10, 16). Treatment is often difficult because of the resistance of clinical strains to many antibiotics (10). Clarification of the roles of various adhesins and adhesion mechanisms in host colonization by *S. marcescens* may thus be of potential therapeutic benefit.

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