JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1990, p. 20–26
0095-1137/90/010020-07$02.00/0

Vol. 28, No. 1

Immunophysical Characterization of Human Isolates of *Serratia marcescens*

R. M. HAMADEH,[1,2]* R. E. MANDRELL,[1] AND J. McLEOD GRIFFISS[1,2]

Centre for Immunochemistry, Veterans Administration Medical Center, San Francisco, California 94121,[1] and Departments of Laboratory Medicine and Medicine, University of California, San Francisco, California 94143[2]

Received 16 June 1989/Accepted 14 September 1989

The immunophysical characteristics of 29 *Serratia marcescens* strains isolated from hospitalized patients in three different cities were studied. Their outer membrane antigens were compared by solid-phase radioimmunoassay inhibition, and their proteinase K-treated, whole-cell lysates were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis. The strains had a limited number of unique outer membrane lipopolysaccharide (LPS) and capsular polysaccharide (K) antigens. By solid-phase radioimmunoassay inhibition, these strains could be divided into four distinct LPS and five K antigenic groups. By SDS-PAGE, the LPS groups could be further divided into three distinct SDS-PAGE core polysaccharide profiles and five distinct O-side-chain polysaccharide profiles. Immunoblot analysis with rabbit antiserum confirmed the limited heterogeneity of these isolates. Of the strains tested, no PAGE profile was unique to blood or nonblood isolates or to organisms collected from a given hospital. Variability of O and core PAGE profiles was not a function of organism growth cycle. Five representative *Serratia* strains were tested by SDS-PAGE and immunoblot analysis and in a bactericidal assay with normal human serum. We found that (i) the normal human serum had antibodies to the LPS of each of the strains, (ii) the anti-LPS antibody measured by immunoblot did not correlate with the level of bactericidal activity in the normal human serum, (iii) three of four sepsis isolates were serum sensitive, (iv) two *Serratia* strains serum sensitive in log-phase growth became serum resistant in late stationary-phase growth and under limiting nutrient conditions, and (v) no LPS PAGE profile distinguished serum-sensitive from serum-resistant strains.

Serratia marcescens has emerged as a significant human pathogen over the past two decades (4, 28, 29). Most efforts have been directed at identifying the various serotypes of *Serratia* spp. Thus far, 24 different O antigens and 26 flagellar or H antigens have been described (12, 24). Many *Serratia* spp. also have capsular or K antigens (1, 5), but these remain untyped. The opsonization requirements for *S. marcescens* have been examined in some detail (19, 23). Little is known, however, about the outer membrane antigens of *Serratia* spp. and how they relate to its pathogenicity. Studies examining this question have looked mostly at the antigenic basis for the serum resistance of *Escherichia coli*. In *E. coli* strains, increased concentrations of lipopolysaccharides (LPS or O antigen) (17) or K antigen (7), long O side chains (6), or different protein envelope composition (22) has been associated with serum resistance. In addition, Taylor et al. (21) suggested that the limiting nutrient environment, which occurs in vivo, may promote the cell surface changes in *E. coli* which are responsible for the development of serum resistance.

Disease-causing strains of *Serratia* spp. have not been similarly studied. To determine whether strain differences were involved in disease caused by *Serratia* spp., we examined the degree of homogeneity in outer membrane LPS and K antigens of *S. marcescens* strains causing sepsis and those isolated from sites other than blood. We used sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblot analysis, and solid-phase radioimmunoassay (SPRIA) inhibition to characterize and compare these outer membrane antigens immunophysically. In addition, we studied the serum reactivity of a sample group of *S. marcescens* strains and compared the immunophysical characteristics of serum-sensitive and serum-resistant strains.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. A total of 29 *S. marcescens* strains were studied, and these were isolated from hospitalized patients at the Beth Israel Hospital in Boston, Mass. (five blood isolates), the Veterans Administration Medical Center in San Francisco, Calif. (five blood isolates and seven nonhematogenous isolates), and the Erie County Medical Center in Buffalo, N.Y. (three blood and nine nonhematogenous isolates; kindly provided by M. A. Apicella). Nonhematogenous sources included urine, sputum, skin, and peritoneal sites. Blood isolate 21 (first isolate from Beth Israel Hospital) was arbitrarily chosen as a reference strain against which all other strains were compared.

Culture conditions. Bacteria were grown in Mueller-Hinton (MH) broth at 37°C with shaking. We used organisms from stationary-phase (16-h) cultures for whole-cell SPRIA inhibition experiments and for preparation of whole-cell lysates. For time course experiments, we withdrew samples from the cultures at various times. In several SPRIAs, we used organisms grown overnight on solid MH medium. Organisms grown in modified Frantz medium (2) supplemented with 1% maltose instead of glucose were used for isolation of LPS and K polysaccharide.

LPS extraction. LPS was extracted from *S. marcescens* isolate 21 by the hot phenol-water method described by Westphal and Jann (27). The protein content of the LPS preparation was <5% by the method of Lowry et al. (13). The nucleic acid content was <5% as quantified by sample $A_{280/260}$.

K-antigen purification. Capsular polysaccharide was prepared from *S. marcescens* 21 by following the methods

outlined by Zollinger and Mandrell for meningococci (33). Protein and nucleic acid contamination was determined by methods outlined by Lowry et al. (13) and Wong et al. (30), respectively. The polysaccharide preparation was analyzed by SDS-PAGE to detect any contaminating LPS or proteins (25).

Antiserum production. Rabbit antisera against *S. marcescens* 21 (anti-21) were raised in New Zealand White rabbits (3 to 4 kg) by intravenous inoculation at 3- to 4-day intervals with 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0 ml of a freshly prepared suspension of live bacteria (5×10^8 to 1×10^9 bacteria per ml in saline). Blood was collected by cardiac puncture 5 to 7 days after the final inoculation, and sera were stored at −20°C.

SPRIA inhibition. The SPRIA method has been outlined in detail by Zollinger and Mandrell (32). The isolate 21 LPS and K antigens were diluted in Dulbecco phosphate-buffered saline to 10 to 50 μg/ml and used to coat the wells of polyvinyl microdilution plates. Nonspecific binding sites were blocked by incubation with a "filler" solution consisting of 0.5% casein, 0.5% bovine serum albumin, and 0.1% sodium azide in Dulbecco phosphate-buffered saline. Inhibition mixtures were prepared composed of dilutions of agar-grown whole *Serratia* organisms at the inhibitors and a dilution of anti-21 that, uninhibited, would result in 30% of maximal binding to the target 21 antigen. Inhibitor, rabbit antibody, and ^{125}I-labeled goat anti-rabbit immunoglobulin steps were done as described previously (32). The percentage of inhibition was determined for each inhibitor: [1 − (cpm of inhibited well/cpm of uninhibited control well)] × 100. *S. marcescens* strains were grouped based on their ability to inhibit the binding of the isolate 21 antiserum to its homologous LPS or K antigens. Results shown are the means of at least four experiments.

Preparation of proteinase K whole-cell lysates from organisms at different phases of growth. As a rapid method to study the morphology of the LPS of these strains, we prepared proteinase-treated lysates (8) for all 29 *Serratia* strains grown in MH broth and sampled at 2, 6.5 (mid-log phase), 24 (stationary phase), and 48 (late stationary phase) h. For single-growth-time comparisons, only stationary-phase (16-h) cultures were sampled. Viability of the samples was monitored on MH agar by comparing the CFU for each time point.

SDS-PAGE of LPS. Proteinase K-treated lysates were separated by SDS-PAGE by the method outlined by Laemmli (11). Samples were applied to discontinuous slab gels (3% acrylamide spacer gel, 14% acrylamide resolving gel) and electrophoresed at a 10-mA current for 4 to 5 h. Lipooligosaccharides from *Neisseria meningitidis* 8032, whose M_rs of individual components have been determined by comparison with *Salmonella* rough mutant LPS (10), were used as low-M_r markers. The SDS-PAGE-separated LPS components were visualized by the silver stain method described by Tsai and Frash (26). The O and core LPS component profiles of each of the strains were compared, and LPS PAGE profiles (PP) were assigned to strains that shared similar PP.

Immunoblotting with anti-21. Samples separated by SDS-PAGE were transferred to nitrocellulose by using a modification of the Western blot (immunoblot) transfer method of Burnette (3). The transfer was done overnight at 30 V. The transblot was incubated for 30 min in a 1% casein filler (1% casein, 0.1% sodium azide in phosphate-buffered saline) to block nonspecific binding sites and then for 1 h in a 1:500 dilution of the anti-21 antiserum diluted in 1% casein filler. The antibody-treated nitrocellulose paper then was washed and incubated in ^{125}I-labeled goat anti-rabbit immunoglobulin diluted in casein filler. The transblots were then washed, air dried, and used for autoradiography with X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.).

Immunoblotting with normal human sera (NHS). Samples were electroblotted as described in the preceding section. The transblot was then placed in a 1:50 dilution of serum from a laboratory volunteer. After a 2-h incubation, the nitrocellulose paper was washed and incubated for 1 h with alkaline phosphatase-conjugated goat anti-human (immunoglobulins G and M) secondary antibody. The secondary antibody was removed, the paper was washed, naphthol AS-MX phosphate and Fast Red TR salt were added (Sigma Chemical Co., St. Louis, Mo.) as described before (9), and the development of pink color was assessed visually.

Bactericidal assay. The bactericidal activity of NHS for five *S. marcescens* strains was determined by methods described previously (31). Briefly, organisms grown overnight on MH agar were transferred to a flask containing MH broth and incubated at 37°C in a shaking water bath. When an optical density of 0.6 was reached, the organisms were pelleted and suspended to an optical density of 0.6 in 0.005 M Veronal-buffered saline containing 1 mM $MgCl_2$–0.15 mM $CaCl_2$, pH 7.35 (bactericidal buffer) (16). The suspension of organisms was then diluted to 1:15,000 in bactericidal buffer. Reaction mixtures were prepared by combining 0.025 ml of bacteria, 0.025 ml of undiluted NHS (stored at −70°C to preserve intrinsic complement activity), and 0.075 ml of bactericidal buffer. The mixtures were incubated at 37°C in a shaking water bath for 1 h. A 20-μl aliquot from each reaction mixture was plated on MH agar plates. After an 18-h incubation at 37°C, the number of colonies produced by surviving organisms was counted. Bactericidal activity was determined by comparing the number of survivors in samples with NHS (intact complement activity) with those in samples with heat-inactivated (56°C; 30 min) NHS.

RESULTS

SPRIA inhibition experiments. LPS and K antigens of each of the *Serratia* isolates were compared for ability to inhibit the binding of purified LPS (Fig. 1) and polysaccharide (Fig. 2) antigens of *Serratia* 21 to homologous anti-21 antiserum. On both figures, line A represents maximal inhibition of the binding of *Serratia* 21 LPS or polysaccharide antigen to anti-21 and, thus, maximal homology with *S. marcescens* 21. Conversely, the inhibition of anti-21 by the organisms represented by line D (Fig. 1) or E (Fig. 2) indicates minimal homology with strain 21. These results suggested that *S. marcescens* strains of human origin have a limited number of unique outer membrane O and K antigens. The organisms in this study can be divided into five groups with distinct K antigens and four with distinct O antigens. Blood isolates did not belong exclusively to any of these groups.

PP of proteinase K-treated, whole-cell lysates and immunoblotting with anti-21. Proteinase K-treated, whole-cell lysates of the *S. marcescens* strains were separated by SDS-PAGE and immunoblotted with anti-21 antiserum to examine the degree of heterogeneity among these disease-causing organisms. We wanted to see whether antigenic profiles among the sepsis and nonsepsis isolates correlated with their site of isolation. Table 1 shows the 29 *S. marcescens* strains grouped by the site of isolation and by the PP of complete LPS (O-PP) and core LPS (core-PP). At least three distinct core-PP could be distinguished among the strains

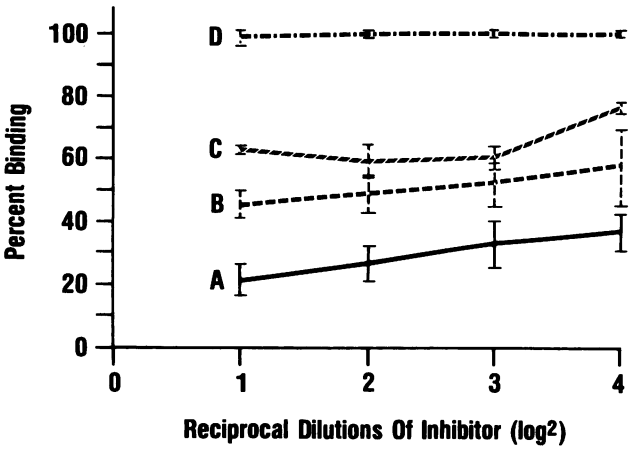

FIG. 1. Inhibition of anti-21 binding to strain 21 LPS. Values are expressed as percent residual binding (± standard deviation) of anti-21 to 21 LPS after incubation of anti-21 with the inhibitor bacteria. Line A, Inhibitors 21, 41, 149, 193, and C2; line B, inhibitors 7, 8, 9, 10, 109, and C23; line C, inhibitors C1 and C4; line D, inhibitors C3 and C9.

tested (Fig. 3 and 4). Examples of these are strains 21 (Fig. 3B, core-PP1), 41 (Fig. 3B, core-PP2), and C23 (Fig. 3B, core-PP3). By reference to a neisserial lipooligosaccharide having components of known M_r, core-PP1 had three LPS components of differing M_r: a component of 5,200, components of 4,600 to 5,200, and components of 4,100 to 4,600. Core-PP1 was the most frequently encountered pattern among the strains. Core-PP2 had two major components, one of M_r >5,600 and one of M_r between 5,200 and 5,600. Core-PP3 had two closely migrating components of M_rs between 3,600 and 4,600.

The profiles representing the O-side-chain repeat units of LPS (O-PP) had five patterns based on the distance between the O repeating units and the overall pattern of the O side chains. These patterns are best shown by strains 21 (Fig. 3A, O-PP1), 41 (Fig. 3A, O-PP2), C22 (Fig. 3A, O-PP3), and C9

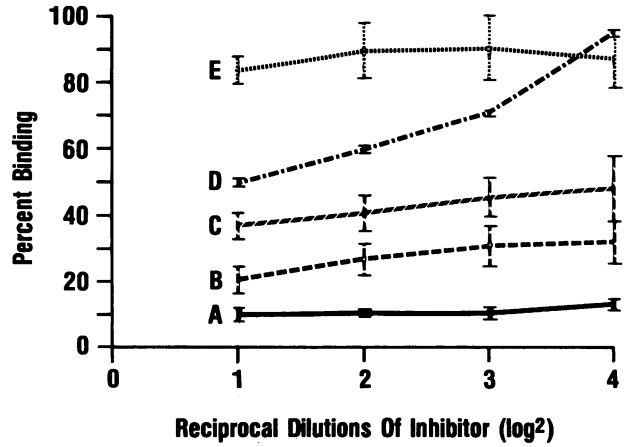

FIG. 2. Inhibition of anti-21 binding to strain 21 K antigens. Values are expressed as percent residual binding (± standard deviation) of anti-21 to 21 K after incubation of anti-21 with the inhibitor bacteria. Line A, Inhibitors 10, 9, and C4; line B, inhibitors 21, 41, 109, 149, and C2; line C, inhibitors 7, 8, and 193; line D, inhibitor C23; line E, inhibitors C1, C3, and C9.

TABLE 1. *S. marcescens* strains grouped by site of isolation and LPS O-PP and core-PP

Strain(s)	Site of isolation	LPS O-PP and core-PP
7, 21, 193	Blood	O-PP1, core-PP1
8, 9, 10, 22, 27, 109	Blood	O-PP2, core-PP1
41	Blood	O-PP2, core-PP2
28, 149	Blood	O-PP3, core-PP1
26[a]	Blood	O-PP5, core-PP3
C13	Sputum	O-PP1, core-PP1
C18, C19, C21	Sputum	O-PP2, core-PP1
C22	Sputum	O-PP3, core-PP1
C20, C16, C17	Urine	O-PP2, core-PP1
C14	Urine	O-PP2, core-PP2
C23[a]	Urine	O-PP5, core-PP3
C4, C15	Wound	O-PP2, core-PP1
C3	Wound	O-PP3, core-PP2
C1	Wound	O-PP4, core-PP1
C2	Wound	O-PP5, core-PP1
C9	Peritoneal fluid	O-PP4, core-PP1

[a] Isolates from same patient.

(Fig. 4A, O-PP4). It is interesting to note that core-PP1 was found in at least one strain of each of the O-PP types and that strains C23, 26, and C2 had LPS of the rough (low O copy number) type (Fig. 3A and 4A, O-PP5). Strains C23 and 26, urine and blood isolates from the same patient, had identical core-PP and O-PP. Sepsis was caused by both strains with high-O-copy-number LPS and a strain having rough LPS (strain 26). Of the strains tested in our study, no PP was unique to blood isolates, nonblood isolates, or organisms collected from a given hospital.

Although no correlation was shown between the silver stain profiles of the LPS and the site of infection, each of the LPS was tested with anti-21 to see whether antigenic activity correlated with the site of infection. The autoradiographs of blots with anti-21 are shown in Fig. 3C and 4C. The antiserum recognized several low-molecular-weight, regularly spaced bands that were shared by all strains, regardless of their core and O-PP. These shared, relatively low-M_r molecules did not reduce silver and were not visible after staining with Coomassie brilliant blue. In a few samples, the anti-21 also bound to LPS molecules migrating in the middle of the gel and to some migrating at the top of the gel (Fig. 3C and 4C, arrows). Two of 13 blood (15.4%) and 4 of 16 nonblood (25%) isolates bound anti-21 to LPS molecules of medium M_r, while 9 of 12 blood (75%) and 12 of 16 nonblood (75%) isolates bound anti-21 to the higher-O-copy LPS molecules. Although there were no marked differences in the anti-21 binding to the LPS of blood and nonblood isolates, there were considerable quantitative differences in the binding of anti-21 to the various strains and in the O-copy-number LPS to which it bound.

Comparison of LPS of four blood isolates and one wound isolate of *Serratia* spp. (i) O-PP and core-PP at different times in their growth cycles. To determine whether some of the variability in the M_r of the O repeat units of the LPS was dependent on environmental conditions, growth curves on five representative *S. marcescens* strains were done and the variability of the PP was tested over time. Five *S. marcescens* strains, including blood isolates 21, 7, 41, and 193 and wound isolate C2, were sampled at 2, 6.5, 24, and 48 h of growth and tested for O-PP, core-PP, and susceptibility to NHS. Figures 5 and 6 show that the previously designated O-PP and core-PP for each strain did not change to any of the other identified O- and core-PP. The observed heterogeneity

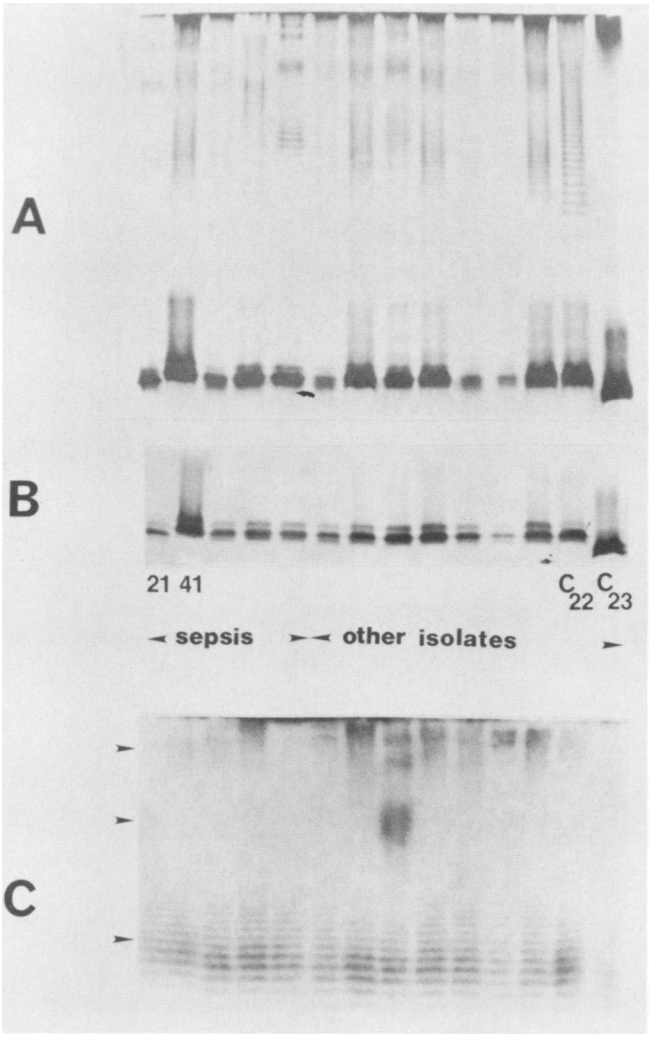

FIG. 3. SDS-PAGE and immunoblot of *S. marcescens* human isolates. (A) Silver-stained gel showing O polysaccharide repeat units. Four O polysaccharide PAGE profiles are noted in panel A: O-PP1, strain 21; O-PP2, strain 41; O-PP3, strain C22; and O-PP5, strain C23. (B) Silver-stained gel of the core oligosaccharide region of the whole LPS. Three core oligosaccharide PAGE profiles are noted in this panel: core-PP1, strain 21; core-PP2, strain 41; and core-PP3, strain C23. (C) Immunoblot of the gel with anti-21. Three distinct antigens bind anti-21. The lower arrow points to several regularly spaced lower-M_r bands that are shared by all of the strains and that did not reduce silver. The middle and upper arrows show the location of middle- and higher-M_r shared antigens, respectively.

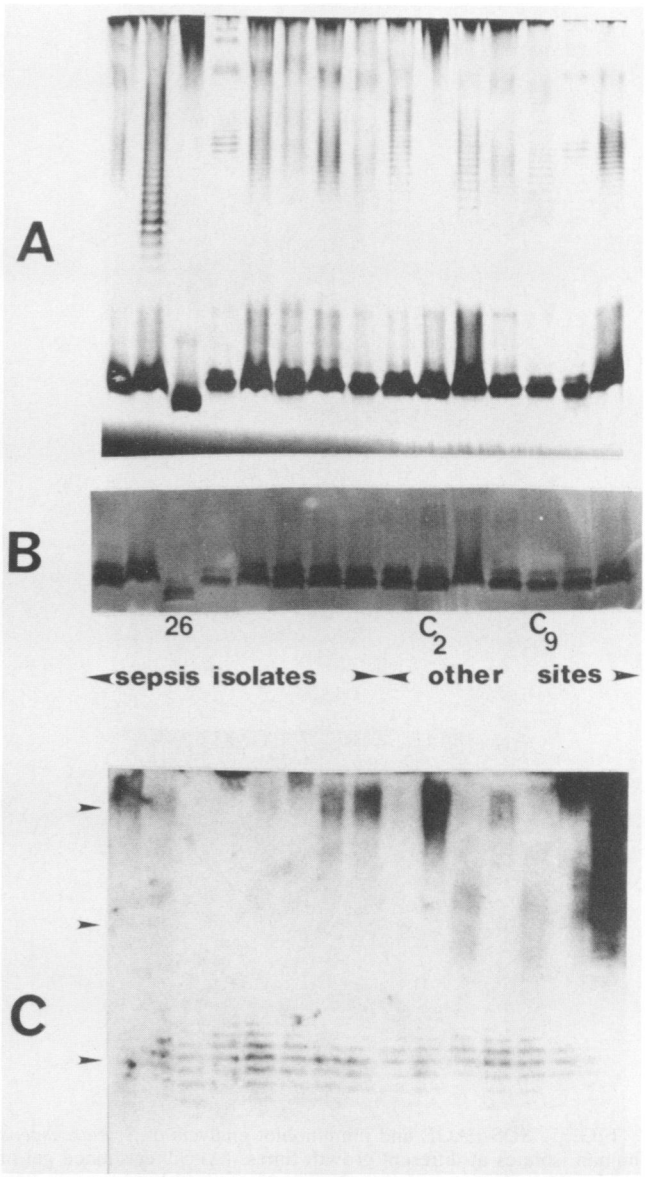

FIG. 4. SDS-PAGE and immunoblot of *S. marcescens* human isolates. Refer to the legend to Fig. 3 for description of panels. Note that strain C9 represents a different O-polysaccharide PAGE profile (O-PP4) than those noted in Fig. 3. Strains C2 and 26 have LPSs of the rough (low O copy number) type (O-PP5), also shown by strain C23 in Fig. 3. Strains 26 and C23 (see Fig. 3) are blood and urine isolates obtained from the same patient.

among strains was not therefore a function of when they were sampled in their growth cycle. There were slight changes that did occur in some strains (see below), but that did not result in reclassification. In general, only the length of O side chains changed over time for all strains, with shorter LPS at 2 and 48 h and longer ones at 6.5 and 24 h (Fig. 5A and 6A). However, the distance between the O-repeat units did not vary over time. The core LPS of 193 (Fig. 6B) developed a new component at 48 h (M_r, 4,000), and that of 7 (Fig. 6B) had two new components (M_r, 3,600 and 4,000).

(ii) Immunoblots with rabbit anti-21 antiserum. Although the overall pattern of binding of anti-21 was the same for each of the four blood and one wound isolate over growth time, the binding of anti-21 to the low-, middle-, and high-M_r LPS molecules decreased slightly at the 48-h time point (Fig. 5C and 6C).

(iii) Immunoblots with NHS. NHS bound to low-O-copy LPS determinants on all five strains (Fig. 7). It also bound to middle- and high-M_r determinants on *Serratia* 21, 7, 41, and 193. The wound isolate, strain C2, has a rough-type LPS, which explains why we did not observe NHS binding to the higher-M_r components of that strain.

(iv) Serum sensitivity. To compare the serum sensitivity of each of the strains with their LPS O-PP and core-PP, we next tested each of the strains in a bactericidal assay with NHS. The blood isolates (21, 41, 7, and 193) and wound isolate C2

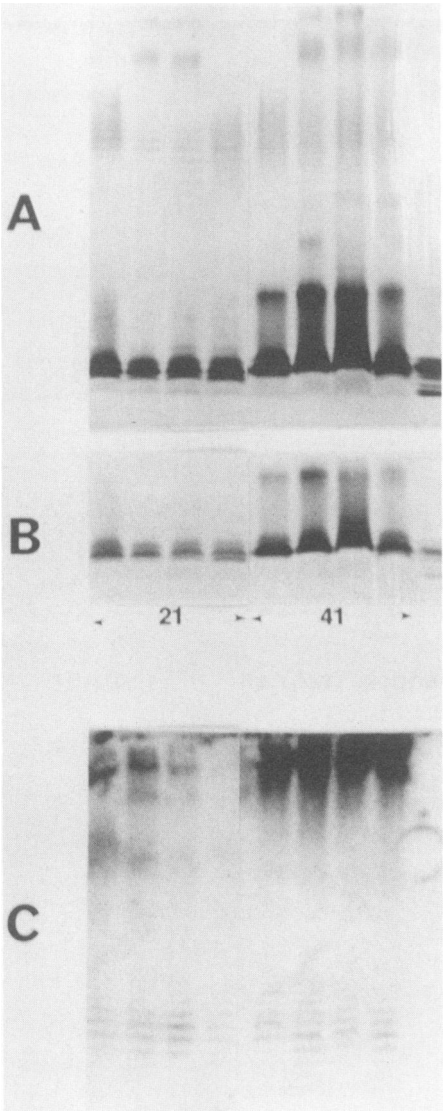

FIG. 5. SDS-PAGE and immunoblot analysis of *S. marcescens* human isolates at different growth times. (A) Silver-stained gel of strains 21 and 41 sampled at 2, 6.5, 24, and 48 h of growth. The O-polysaccharide PAGE profiles of strains 21 and 41 did not change over time except for longer LPS at 6.5 and 24 h and shorter LPS at 2 and 48 h. (B) Silver-stained core oligosaccharide components of strains 21 and 41 sampled at the above time points. The first sample on the right in panels A and B represents strain 8032, which we used as a low-M_r marker (see text). (C) Immunoblot of strains 21 and 41 with anti-21.

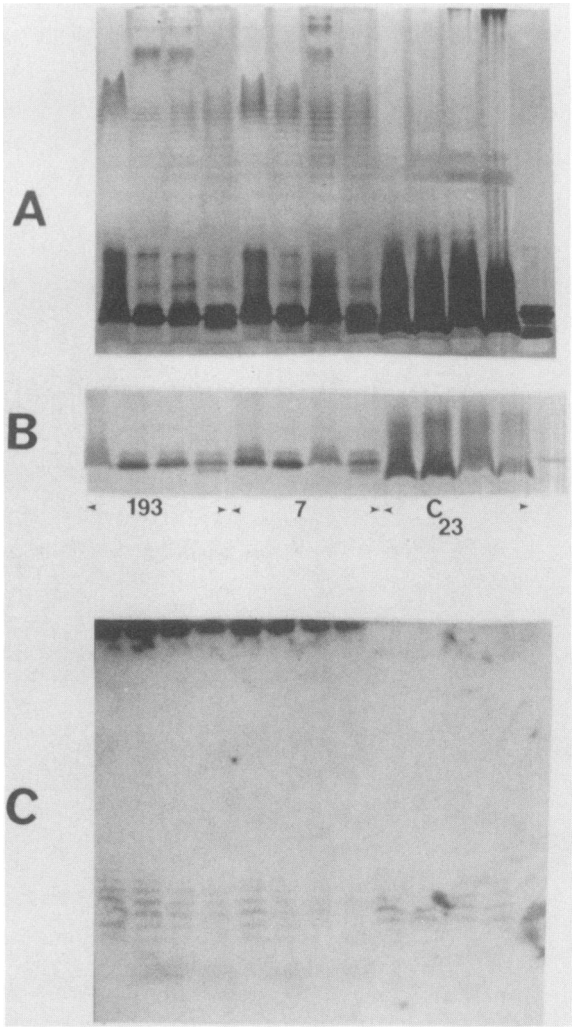

FIG. 6. SDS-PAGE and immunoblot analysis of *S. marcescens* human isolates at different growth times. Refer to the legend to Fig. 5 for description of panels. Strains 193, 7, and C23 are shown.

were sampled at each of the growth times and tested for serum sensitivity. Three patterns of serum sensitivity (Table 2) were noted: strains that were relatively serum resistant at all phases of growth (strain 21), relatively serum sensitive at all phases of growth (strains 7 and 41), and increasingly resistant at longer growth times (strains 193 and C2).

DISCUSSION

In general, the antigenic heterogeneity of the 29 *S. marcescens* strains was not marked, and it was independent of the site of isolation or the hospital from which the strains were obtained. SPRIA inhibition, SDS-PAGE, and immunoblot analysis indicated only a few distinct O and K determinants present among the *S. marcescens* strains. The rabbit antiserum used in these experiments recognized several common O and K antigens. Our methods allowed for the identification of common antigens, unlike serotyping, which emphasizes identification of differences between strains.

By SPRIA inhibition, the *Serratia* strains could be grouped into four distinct O-antigenic groups and five distinct K-antigenic groups. Blood isolates were widely distributed among these groups and did not display any characteristic pattern of homology. It appeared that the highest-titered rabbit antibody raised against *S. marcescens* 21 antigens was specific for common determinants rather than determinants unique to local or systemic isolates. Other polyclonal hyperimmune sera or monoclonal antibodies or both may be needed to identify antigenic differences between isolates from various sites.

PP analysis proved to be a rapid and reproducible method to examine a large number of *S. marcescens* strains. Organisms recovered from the urine and blood of the same patient had identical O- and core-PP, indicating that this analysis is useful as an epidemiologic tool to study the phenotypic

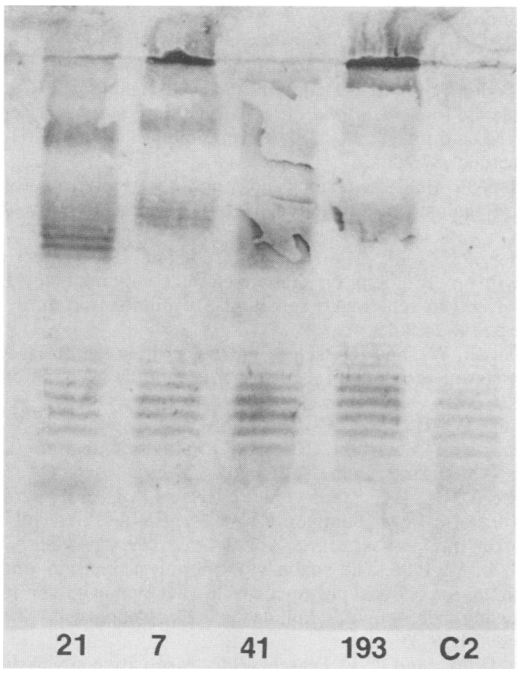

FIG. 7. Immunoblot of five *S. marcescens* human isolates with NHS. Strains are given at the bottom. NHS binds to low-O-copy LPS determinants on all five strains and middle- and high-M_r determinants on strains 21, 7, 41, and 193.

characteristics of *Serratia* spp. Five different O-PP and core-PP were noted (Fig. 3 and 4), but the stability over growth time (Fig. 5 and 6) indicated that the PP were not simply a function of sampling these organisms at different times in their growth cycles.

Three proteinase K-resistant antigens were shared among the 29 strains. The first was a group of molecules migrating in the middle of the gel and did not reduce silver or stain with Coomassie blue. These most likely represent the enterobacterial common antigen, which has been reported to have these properties (14, 15) and to be different from the LPS. Enterobacterial common antigen was present in all isolates we examined, including smooth and rough strains, blood isolates, and isolates recovered from other sites. The second and third shared antigens were located on the LPS molecules that migrate at the middle and top range of the gel, respectively.

In studies with human sera, Simberkoff et al. (19) and Traub (23) showed that, in addition to the presence of leukocytes, both antibodies and complement, preferably in combination, were necessary for effective opsonisation of *Serratia* spp. SDS-PAGE and immunoblot analysis with 5 of the 29 *Serratia* strains, including four blood isolates and one wound isolate, showed that an NHS had antibodies to the LPS of each of the strains. In the patients from whom these strains were obtained, antibodies must either have been absent or nonprotective.

TABLE 2. NHS bactericidal activity against five representative *S. marcescens* strains from different growth times

Strain	% Kill at growth time of		
	6.5 h	24 h	48 h
21	0	7	19
41	59	77	68
193	77	50	0
7	77	79	71
C2	95	59	26

The bactericidal activity of NHS against *Serratia* strains also differed. Both serum-resistant and serum-sensitive organisms were found among sepsis-causing strains, contrary to previous observations that only serum-resistant strains were recovered from blood (19). This suggests that, although the organism was serum resistant in the patient with sepsis, it was serum sensitive in other NHS or the organism changed from serum resistant to serum sensitive during in vitro passage or both. In addition, we found serum-sensitive strains with smooth LPS, indicating that the length of the LPS was not the only factor determining the serum resistance of *Serratia* spp. Strain 21 was serum resistant even though the test NHS contained antibodies to 21 (Fig. 7). This result may be related to our observations that NHS contains specific immunoglobulin G antibodies directed against galactose-α1→3-galactose determinants which block complement-mediated lysis of strain 21 (R. M. Hamadeh et al., Clin. Res. 457A, 1988). The bacteriolytic activity of NHS against 21 was restored after the serum was depleted of this blocking antibody.

Other strains, initially serum sensitive, varied in serum sensitivity over growth time. For example, strains 193 and C2 became progressively more serum resistant over time under nutrient-limiting growth conditions. Changes in serum sensitivity during in vitro growth have been described for *E. coli* urinary isolates that were serum sensitive when grown under magnesium-limited conditions but became serum resistant under carbon-limited conditions (21). Many cell surface changes associated with alterations in the growth environment were observed in these *E. coli*. In addition, these and other gram-negative bacteria were more readily killed by serum when in the early logarithmic phase than either the lag or stationary phase (18). These studies showed that both the nature of the medium and the phase of growth at which the cells were harvested may influence sensitivity to serum. The measurement of true division rates in vivo has indicated that the growth rate in mammalian tissues was slow compared with the maximum growth rate (20), suggesting that, in vivo, the growth rate was limited by the availability of substrates. The changes in serum susceptibility observed for some *S. marcescens* strains under nutrient-limiting conditions in vitro may be relevant to what occurs in vivo when similar limitations in substrates occur.

The pathogenesis of sepsis due to *S. marcescens* must involve both host and bacterial factors. There were no bacterial outer membrane antigens unique to blood isolates or to serum-resistant strains. Changes in serum sensitivity secondary to limited substrate availability in vivo is an interesting concept, and further investigation of the cell surface changes that occur under these conditions is warranted. Host factors such as the lack of specific bactericidal antibodies or the presence of blocking antibodies also may contribute to *Serratia* sepsis. Also, the relative homogeneity among human isolates of *S. marcescens* should be investigated further to identify protective antigens. This may aid in identifying representative strains that could be used in developing a polyvalent vaccine.

ACKNOWLEDGMENTS

This work was supported in part by the Veterans Administration and Public Health Service grant AI 21620 (J.M.G.) from the National Institutes of Health.

LITERATURE CITED

1. **Adams, G. A., and R. Young.** 1966. Constitution of extracellular and capsular polysaccharides of *Serratia marcescens*. Ann. N.Y. Acad. Sci. **133**:527–545.
2. **Bertram, M. A., J. M. Griffiss, and D. D. Broud.** 1976. Response to antigenic determinants of *Neisseria meningitidis* lipopolysaccharide investigated with a new radioactive antigen-binding assay. J. Immunol. **116**:842–846.
3. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
4. **Davis, J. T., E. Foltz, and W. S. Blakemore.** 1970. *Serratia marcescens*: a pathogen of increasing clinical importance. J. Am. Med. Assoc. **214**:2190–2192.
5. **Ewing, W. H., B. R. Davis, and R. W. Reaves.** 1959. Studies on the *Serratia* group. Center for Disease Control, Atlanta.
6. **Feingold, D. S.** 1969. The serum bactericidal reaction. IV. Phenotypic conversion of *Escherichia coli* from serum-resistance to serum-sensitivity by diphenylamine. J. Infect. Dis. **120**:437–444.
7. **Glynn, A. A., and C. J. Howard.** 1970. The sensitivity to complement of strains of *Escherichia coli* related to their K antigens. Immunology **18**:331–346.
8. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
9. **Kim, J. J., R. E. Mandrell, and J. M. Griffiss.** 1989. *Neisseria lactamica* and *Neisseria meningitidis* share lipooligosaccharide epitopes, but lack common capsular and class 1, 2, and 3 protein epitopes. Infect. Immun. **57**:602–608.
10. **Kim, J. J., R. E. Mandrell, Z. Hu, M. A. Apicella, J. T. Poolman, and J. M. Griffiss.** 1988. Electromorphic characterization and description of conserved epitopes of the lipooligosaccharides of group A *Neisseria meningitidis*. Infect. Immun. **56**:2631–2638.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Le Minor, S., F. Benazet, and L. Martin.** 1983. New O (O23) and H (H26) antigenic factors, in *Serratia marcescens*. Ann. Microbiol. (Paris) **134B**:447–449.
13. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. **Makela, P. H., and H. Mayer.** 1976. Enterobacterial common antigen. Bacteriol. Rev. **40**:591–632.
15. **Männel, D., and H. Mayer.** 1978. Serological and immunological properties of isolated enterobacterial common antigen. Eur. J. Biochem. **86**:371–379.
16. **Rapp, H. J., and T. Borsos.** 1970. Molecular basis of complement action, p. 75–78. Appleton-Century Crofts, New York.
17. **Reynolds, B. L., and H. Pruul.** 1971. Sensitization of complement-resistant smooth gram-negative bacterial strains. Infec. Immun. **3**:365–372.
18. **Rowley, D., and A. C. Wardlaw.** 1958. Lysis of Gram-negative bacteria by serum. J. Gen. Microbiol. **18**:529–533.
19. **Simberkoff, M. S., I. Ricupero, and J. J. Rahal, Jr.** 1976. Host resistance to *Serratia marcescens* infection: serum bactericidal activity and phagocytosis by normal blood leukocytes. J. Lab. Clin. Med. **87**:206–217.
20. **Taylor, P. W.** 1983. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol. Rev. **47**:46–83.
21. **Taylor, P. W., P. Messner, and R. Parton.** 1981. Effect of the growth environment on cell-envelope components of *Escherichia coli* in relation to sensitivity to human serum. J. Med. Microbiol. **14**:9–19.
22. **Taylor, P. W., and R. Parton.** 1977. A protein factor associated with serum resistance in *Escherichia coli*. J. Med. Microbiol. **10**:225–232.
23. **Traub, W. H.** 1982. Opsonization requirements of *Serratia marcescens*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **253**:204–224.
24. **Traub, W. H.** 1985. Serotyping of *Serratia marcescens*: identification of a new O-antigen (O24). Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **259**:485–488.
25. **Tsai, C. M.** 1986. The analysis of lipopolysaccharide (endotoxin) in meningococcal polysaccharide vaccines by silver staining following SDS-polyacrylamide gel electrophoresis. J. Biol. Stand. **14**:25–33.
26. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
27. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **5**:83–91.
28. **Wilfert, J. N., F. F. Barrett, and E. H. Kass.** 1968. Bacteremia due to *Serratia marcescens*. N. Engl. J. Med. **279**:286–289.
29. **Wilkowske, C. J., J. A. Washington, Jr., W. J. Martin, and R. E. Ritts, Jr.** 1970. *Serratia marcescens*. Biochemical characteristics, antibiotic susceptibility patterns, and clinical significance. J. Am. Med. Assoc. **214**:2157–2162.
30. **Wong, K. H., O. Barrera, A. Sutton, J. May, D. H. Hochstein, J. D. Robbins, P. D. Parkman, and E. B. Seligmann, Jr.** 1977. Standardization and control of meningococcal vaccines, group A and group C polysaccharide. J. Biol. Stand. **5**:197–215.
31. **Zollinger, W. D., B. L. Brandt, E. C. Tramont, and A. S. Dobek.** 1980. Immune response to *Neisseria meningitidis*, p. 446–453. *In* N. R. Rose and H. Freidman (ed.), Manual of clinical immunology, 2nd ed. American Society for Microbiology, Washington, D.C.
32. **Zollinger, W. D., and R. E. Mandrell.** 1977. Outer-membrane protein and lipopolysaccharide serotyping of *Neisseria meningitidis* by inhibition of a solid-phase radioimmunoassay. Infect. Immun. **18**:424–433.
33. **Zollinger, W. D., and R. E. Mandrell.** 1980. Type-specific antigens of group A *Neisseria meningitidis*: lipopolysaccharide and heat-modifiable outer membrane proteins. Infect. Immun. **28**:451–458.