Antibody Response to *Serratia marcescens* Isolated from Patients with Malignant Diseases

E. KENNEDY GRIFFITHS,¹ M. J. SURGALLA,² J. E. FITZPATRICK,² AND E. NETER^{1*}

Department of Laboratory Medicine, Roswell Park Memorial Institute,² and the Laboratory of Bacteriology, Children's Hospital,¹ Buffalo, New York 14222

Received for publication 12 August 1977

Sixty-four patients with malignant diseases from whom *Serratia marcescens* was isolated from various sources were studied regarding their antibody responses to somatic O antigens of this microorganism. Antibodies were titrated by the passive hemagglutination test. An antibody response was considered present when either a fourfold or greater rise in antibody titers between two consecutive serum specimens was demonstrated, or when elevated titers (≥ 40 for serogroup O14 and ≥ 160 for all others) were present in the first available specimen. Overall, 31% of subjects mounted an immune response, but there were differences depending upon the infection site. Seventy-one percent of patients with *S. marcescens* bacteremia responded immunologically; whereas the percentage for patients with *Serratia* present in the respiratory tract was only 22%, in the urinary tract, 31%, and in wounds, 26%. Documentation of an immune response to the patient's own infecting strain of *Serratia* aids in the differentiation between infection and contamination and possibly also between clinical disease and colonization. In addition, immunoglobulin samples collected in different decades were examined to determine whether the background level of antibodies to *S. marcescens* had changed in the general population over the years. No difference in antibody titers to 13 O antigens was observed in immunoglobulin preparations from 1951, 1962, 1971, and 1975.

Opportunistic infections are playing an ever-increasing role in hospitalized patients. Among the opportunistic pathogens is *Serratia marcescens*, which accounted for 1.9% of all nosocomial infections in 1974 as reported to the Center for Disease Control (CDC) (13). *Serratia* infections have occurred in many different settings, including wards of general and veterans hospitals (4, 6, 12, 14, 15), intensive care (2), and inhalation therapy units (1, 9), and have also been traced from hospital to hospital (10). Patients with malignant diseases are particularly susceptible hosts, and often serious infections due to gram-negative microorganisms, including *Serratia*, contribute to death (5). Differentiation between clinical infection and colonization is notoriously difficult when cultures yield more than one potential pathogen as well as with specimens from the respiratory tract or wounds. Documentation of a patient's immune response to the isolate, to some extent, may help resolve this question and, particularly, aid in excluding the specimen's contamination. Results of an investigation aimed at the humoral immune response to O antigens of *S. marcescens* of patients with malignant diseases, from whom this microorganism was isolated, are presented here. The epidemiol-

ogy of *Serratia* infections in this group of patients will be the subject of a separate report.

SUBJECTS AND METHODS

Subjects with malignant diseases, all patients at Roswell Park Memorial Institute, Buffalo, N.Y., were entered into this study upon isolation of *S. marcescens* from cultures of various body sites. In all, 64 patients, ranging in age from 22 to 91 years, were studied. Of these, 56 had solid tumors, 7 had malignant diseases of the bone marrow or reticuloendothelial system, and 1 patient had both.

Serratia isolates were identified by conventional methods and were serogrouped by means of bacterial agglutination, using antiserum purchased from Lee Laboratories Inc., Grayson, Ga.

The antibody response of each of these subjects to O antigen of their own infecting strain of *S. marcescens* was determined. Preparation of somatic antigens has been published in detail elsewhere (7, 8). At least two serum specimens were available from 54 patients and one specimen from 10 subjects, obtained 5 or more days after the first positive culture of *Serratia*.

The passive hemagglutination test, as described previously (7, 8), was used for titration of antibodies. An antibody response was considered present either when a fourfold or greater rise in antibody titers was observed between two consecutive serum specimens,

or when elevated titers were present in the first available serum specimen. For comparison, antibody titers against various serogroups of *Escherichia coli*, *Shigella*, and *Salmonella* were determined as described previously (7). Since the "normal" antibody level to *Serratia* in the population was not known, we examined two different lots of pooled plasma from 10 healthy blood donors and several lots of human serum immunoglobulin (purchased from Cutter Laboratories, Inc., Berkeley, Calif., or donated by George G. Wright of the Massachusetts Department of Public Health) for antibodies against various serogroups of *S. marcescens*.

RESULTS

To obtain information on titers of antibodies to the various serogroups of *S. marcescens*, pooled plasma and serum immunoglobulin samples from healthy individuals were studied. Antibody titers to the serogroups (O1, O3, O4, O8, O12, O14) of *Serratia* encountered in this study, and to two randomly selected nontypable strains, ranged from <5 to a maximum of 80. Based on this information, elevated titers in our group of patients were defined as ≥ 160 (criterion I). Since antibodies to *S. marcescens* O14, which was the most frequently isolated serogroup, were not present even in a titer of 5, an additional criterion was established in which elevated titers to serogroup O14 were defined as ≥ 40 (criterion II). Antibody titers in plasma and immunoglobulin samples to mixtures of five O groups each of *Salmonella*, *Shigella*, and *E. coli* antigens ranged between 20 and 160.

The antibody responses of the 64 patients to O antigens of their own strains of *S. marcescens* were determined (Table 1).

It may be seen that a significant number of patients with malignancies had a specific immune response, despite the disease and/or chemotherapy possibly having immunosuppressive effects. The data shown in Table 1 also indicate that patients with bacteremia mount an immune response far more frequently than

all others. No significant differences were noted between the two criteria used for interpreting a significant antibody response. Three bacteremic patients who had antibody responses yielded *Serratia* from another site. One subject had simultaneous bacteremia and *Serratia* infection of the respiratory tract, and another supported bacteremia and a urinary tract infection. The bacteremia probably was more important than the local infection as the antigenic stimulus. This suggestion is supported because in the third patient, who had urinary infection and bacteremia separated by several months, there was no antibody response after the urinary tract infection, but 7 days after a positive blood culture was obtained, antibody titers rose 16-fold.

The data shown in Table 1 were recalculated so that each patient is listed only once, even when isolates were obtained from multiple sites. The percentages of subjects with antibody response differed by no more than one percentage point.

The question arose whether a relationship exists between the serogroup of the infecting microorganism and antibody response of the patients. *S. marcescens* O14 accounted for 51% of all isolates; whereas only a few strains of any other single serogroup were encountered. Therefore, a comparison was made between patients with *S. marcescens* O14 infection and those infected with all others, including nontypable strains. Analysis revealed that 37% of the former and 25% of the latter mounted an immune response, suggesting that striking differences did not exist between the two groups.

Analysis regarding the antibody response of patients with solid tumors and those with malignancy of the bone marrow or reticuloendothelial system revealed an antibody response in 33% of the former and 13% of the latter. Since only eight patients had malignancies of the bone marrow or reticuloendothelial system, differences, if any, cannot be determined definitively. However, statistical analysis indicates a trend for a more frequent antibody response in subjects with solid tumors ($P = 0.14$).

Samples of immunoglobulin (Massachusetts Department of Public Health) collected in different decades were made available to us. We hoped to determine whether the "normal" or background level of antibodies to *S. marcescens* had changed over a period of years. The results show that they had not. The hemagglutinin titers of immunoglobulin samples prepared in 1951, 1962, 1971, and 1975 to serogroups O1, O2, O3, O4, O6, O7, O8, O9, O10, O12, O13, O14, and O15 were essentially the same ($\pm$ twofold) for each year studied. Further, antibodies to serogroups O3, O6, O7, O8, O9, and O15 tended

TABLE 1. Antibody response of patients with malignancy and *S. marcescens* infection

Sites of <i>S. marcescens</i> isolation	No. of patients ^a with antibody response/total (%) for criteria: ^b	
	I	II
Blood	4/7 (57)	5/7 (71)
Urinary tract	7/29 (24)	9/29 (31)
Wound	3/19 (16)	5/19 (26)
Respiratory tract	4/18 (22)	4/18 (22)
Other (bile)	0/1 (0)	0/1 (0)
Total	18/74 (24)	23/74 (31)

^a Includes eight patients with isolates from multiple sites.

^b See text.

to occur in higher titers (40 to 80) than those to the other serogroups (2 to 20). Since antigen sensitivity was not standardized, it is not possible to state whether these differences reflect differences in exposure of the population at large to these serogroups.

DISCUSSION

Among the microorganisms causing opportunistic infections, *S. marcescens* plays a significant role (13). In addition to host factors, resistance of *Serratia* to commonly used antibiotics poses problems in the management of these infections (10, 12, 16). Patients with malignant diseases, often treated with immunosuppressive drugs, are among the susceptible hosts. The present study focuses on the immune response of such patients to O antigens of *S. marcescens*. The epidemiological aspects will be the subject of a separate report.

Despite the underlying disease, the majority (71%) of subjects with bacteremia produced antibodies against O antigens of their own strains. This figure is similar to that (70%) observed in a previous study (11) of patients with solid tumors and *E. coli* or *Klebsiella* bacteremia. Recently, Crowder et al. (3), using a gel-diffusion test, observed that 61% of patients with *Serratia* infections had antibodies in serum.

Patients with malignancy and *S. marcescens* infection of the respiratory or urinary tracts or of wounds developed O antibodies to their isolates far less frequently (22 to 31%). In all likelihood, this is not due to inability to produce these immunoglobulins. Rather, it may be related to inadequate amounts of antigens reaching the immune system, perhaps because some of these patients were colonized rather than clinically infected.

Documentation of an immune response certainly aids in differentiating between colonization and infection, on the one hand, and laboratory contamination on the other.

If *S. marcescens* has become more widespread in nature over the last three decades, it might be anticipated that the titers of antibodies against their O antigens in serum of healthy individuals would show a parallel increase. To test this hypothesis, human immunoglobulin samples prepared in 1951, 1962, 1971, and 1975 were tested with O antigens of 13 different serogroups of *S. marcescens*. All antibody titers for each O antigen were identical within a two-fold variation. Thus, evidence for more frequent exposure of the population to these antigens was not obtained.

ACKNOWLEDGMENTS

We are grateful to Helga von Langendorff, Robert Botzer, Verna Mordaunt, and the late Betty Patterson for excellent

assistance. We also express our appreciation to George G. Wright of the Massachusetts Department of Public Health for the donation of immunoglobulin samples.

The study was supported by U.S. Public Health Service grant RR-05648-10 of the Division of Research Resources, National Institutes of Health and by grant AI-00658 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Cabrera, H. A. 1969. An outbreak of *Serratia marcescens*, and its control. Arch. Intern. Med. 123:650-655.
2. Cardos, S. F., A. L. Florman, M. S. Simberkoff, and L. Lanier. 1973. *Serratia marcescens*: use of detailed characterization of strains to evaluate an increase of isolates in an intensive care unit. Am. J. Med. Sci. 266:447-452.
3. Crowder, J. G., J. P. Rissing, J. W. Smith, and A. White. 1977. A serologic response in human infection with *Enterobacteriaceae*. Am. J. Med. Sci. 273:141-148.
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. Inagaki, J., V. Rodriguez, and G. P. Bodey. 1974. Causes of death in cancer patients. Cancer 33:568-573.
6. Maki, D. G., C. G. Hennekens, C. W. Phillips, W. V. Shaw, and J. V. Bennett. 1973. Nosocomial urinary tract infection with *Serratia marcescens*: an epidemiologic study. J. Infect. Dis. 128:579-587.
7. Neter, E., E. A. Gorzynski, R. M. Gino, O. Westphal, and O. Lüderitz. 1956. The enterobacterial hemagglutination test and its diagnostic potentialities. Can. J. Microbiol. 2:232-244.
8. Neter, E., O. Westphal, O. Lüderitz, and E. A. Gorzynski. 1956. The bacterial hemagglutination test for the demonstration of antibodies to enterobacteriaceae. Ann. N.Y. Acad. Sci. 66:141-156.
9. Sanders, C. V., Jr., J. P. Luby, W. G. Johanson, Jr., J. A. Barnett, and J. P. Sanford. 1970. *Serratia marcescens* infections from inhalation therapy medications: nosocomial outbreak. Ann. Intern. Med. 73:15-21.
10. Schaberg, D. R., R. H. Alford, R. Anderson, J. J. Farmer III, M. A. Melly, and W. Schaffner. 1976. An outbreak of nosocomial infection due to multiply resistant *Serratia marcescens*: evidence of interhospital spread. J. Infect. Dis. 134:181-188.
11. Surgalla, M. J., E. Neter, and J. E. Fitzpatrick. 1975. Antibody response of patients with malignancies to bacteremia with gram-negative bacteria. J. Clin. Microbiol. 1:298-301.
12. Thomas, F. E., Jr., R. T. Jackson, M. A. Melly, and R. H. Alford. 1977. Sequential hospital wide outbreaks of resistant *Serratia* and *Klebsiella* infections. Arch. Intern. Med. 137:581-584.
13. U.S. Public Health Service. 1977. National nosocomial infections study report, annual summary 1974, publication no. (CDC)77-8257. U.S. Department of Health, Education, and Welfare, Washington, D.C.
14. Wilfert, J. N., F. F. Barrett, W. H. Ewing, M. Finland, and E. H. Kass. 1970. *Serratia marcescens*: biochemical, serological, and epidemiological characteristics and antibiotic susceptibility of strains isolated at Boston City Hospital. Appl. Microbiol. 19:345-352.
15. Wilfert, J. N., F. F. Barrett, and E. H. Kass. 1968. Bacteremia due to *Serratia marcescens*. N. Engl. J. Med. 279:286-289.
16. Wilkowske, C. J., J. A. Washington II, 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.