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Reactivity of Type-Specific Monoclonal Antibodies with *Staphylococcus aureus* Clinical Isolates and Purified Capsular Polysaccharide

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***Staphylococcus aureus* has been classified into at least eight different capsular types by using polyclonal rabbit antisera specific for their associated capsular polysaccharides. We produced and characterized monoclonal antibodies reactive with two serologically distinct capsular types, types 5 and 8, which account for more than 70% of all *S. aureus* bacteremias. These type-specific, monoclonal antibodies reacted with *S. aureus* clinical isolates possessing the homologous capsular type and exhibited no cross-reactivity against *S. aureus* clinical isolates possessing the heterologous capsular type, nontypeable *S. aureus* clinical isolates, *Staphylococcus epidermidis* clinical isolates, or a variety of gram-negative organisms. The anti-type 8 monoclonal antibodies also reacted with purified capsular polysaccharide derived from the prototype type 8 *S. aureus* strain.**

Infection due to *Staphylococcus aureus* remains a major clinical problem even in the era of aggressive use of potent antibiotic agents (17, 18). A number of bacterial products (e.g., toxins, extracellular enzymes) have been correlated with various staphylococcal diseases, but none appears to be related either to the occurrence of bacteremia or to the invasiveness of this organism (4, 18).

Capsular polysaccharides associated with many different bacteria (*Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Haemophilus influenzae* type b, *Neisseria meningitidis*, *Escherichia coli*, and group B *Streptococcus agalactiae*) (14) account, in part, for the invasiveness of these organisms. Although capsular polysaccharides have been demonstrated in several mucoid strains of *S. aureus* designated Smith (6, 19), M (16), T (20), K93-M (5), 7007 (7), D (10), and Mardi (9), organisms with these capsular types are rarely isolated from infected patients (8).

An *S. aureus* capsular polysaccharide typing classification has been developed (8). In that study, clinical isolates were classified into eight distinct capsular types by using polyclonal, type-specific antisera. A recent clinical survey indicated that two *S. aureus* capsular types, type 5 and type 8, account for over 70% of *S. aureus* bacteremias (1).

This report describes the production and characterization of monoclonal antibodies reactive with type 5 and type 8 strains of *S. aureus*.

MATERIALS AND METHODS

Bacteria. Bacteria were cultivated overnight at 37°C on Columbia agar supplemented with 1% NaCl under 5% CO_2. Organisms were removed from the agar plates, washed in phosphate-buffered saline (PBS), and either heat killed at 70°C for 1 h or treated with 3% Formalin overnight at 4°C, and then they were washed with 0.3% Formalin in PBS.

The methodology for *S. aureus* capsular polysaccharide typing, preparation of type-specific rabbit antisera, and the source and identification of the prototype strains used have been described previously (8). Isolates were typed originally by immunoelectrophoresis or immunodiffusion of culture extracts, or both. *S. aureus* strains possessing capsular types 1 (strain D), 2 (strain Smith), 4 (strain 7007), 6 (strain C), and 7 (strain 207) were described previously (8). The various type 5, type 8, and nontypeable *S. aureus* clinical isolates used in this study are listed in Table 1 along with their associated phage and capsular types. Reynold (type 5) and Becker (type 8) are the prototype strains for their respective capsular types (8). The remainder of the strains were *S. aureus* bacteremia isolates from the Veterans Administration Medical Center, Boston, Mass. *S. aureus* isolates were phage typed by the Staphylococcal Typing Laboratory, Bacterial Pathogens Branch, Centers for Disease Control, Atlanta, Ga., through the cooperation of P. B. Smith and G. Hancock.

Production of type-specific anti-*S. aureus* monoclonal antibodies. BALB/c strain mice (Jackson Laboratory, Bar Harbor, Maine) were immunized three times with Formalin-fixed *S. aureus* bacterial vaccines, with the last immunization given 3 days before fusion. Spleen cells derived from immune mice were fused with the mouse myeloma fusion partner, X63-Ag8.653 (11) at a 6:1 lymphocyte:myeloma ratio in polyethylene glycol (PEG 1000; J. T. Baker Chemical Co., Phillipsburg, N.J.) according to the method of Gefter et al. (3). Cell suspensions were plated in 96-well microtiter trays, and hybrids were selected in medium containing hypoxanthine-aminopterin-thymidine by the method of Littlefield (12). Hybridomas were cloned by limiting dilution.

Hybridoma cell culture supernatants were screened for anti-*S. aureus* antibody activity by bacterial agglutination. Immunoglobulin subclass determinations were performed by enzyme-linked immunosorbent assay (ELISA) with reagents

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TABLE 1. Phage and capsular types associated with *S. aureus* clinical isolates

S. aureus isolate[a]	Phage type	Type-specific capsular polysaccharide[b]
1	47/53/83A/84	Type 5
3	96	Type 5
13	47/54/75/83A[c]	Type 5
Reynold	96	Type 5
2	1136	Type 8
6	3C	Type 8
9	95	Type 8
15B	29/52/79	Type 8
Becker	95	Type 8
5	79/85/95	NT[d]
7	NR[e]	NT
69	52A/79/53	NT

[a] Reynold and Becker are the prototype strains for their respective capsular types (8).
[b] Capsular polysaccharide typing was done with polyclonal antisera as described in the text.
[c] Multiple antibiotic-resistant strain.
[d] NT, nontypeable in agglutination and gel assays with available polyclonal reagents.
[e] NR, No reaction.

purchased from Zymed Laboratories, Inc. (South San Francisco, Calif.).

Purification of *S. aureus* capsular polysaccharides. Type 8 capsular polysaccharides were extracted and purified as described by Fournier et al. (2). Purified type 8 capsular polysaccharide antigen was derivatized with biotin by the method of Schneerson et al. (15).

Bacterial agglutination assays. Bacterial agglutination assays were performed in round-bottomed 96-well microtiter plates with suspensions of heat-killed, trypsinized organisms. Bacterial cell suspensions were diluted in PBS to an optical density of 1.0 at 550 nm, and 25 μl was mixed with 25 μl of hybridoma culture supernatants. The plates were incubated for 1 h at 37°C and then overnight at 4°C. The agglutination patterns were read the next day, and results were scored on a scale from 0 (the complete absence of agglutination) to 4+ (complete agglutination).

ELISA. The ELISA with whole bacteria was performed in 96-well polyvinyl chloride microtiter trays (Costar, Cambridge, Mass.). Formalin-fixed bacterial suspensions were diluted in PBS to an optical density of 1.0 and were incubated in the wells at room temperature overnight. The next day, the bacteria were pelleted by centrifugation, nonadherent bacteria were removed by being washed with PBS, and the wells were blocked with PBS containing 1% normal goat serum. Hybridoma culture supernatants (50 μl) were added to the wells and incubated for 3 h at room temperature. The wells were washed with PBS, and the monoclonal antibodies which bound to the solid phase were detected with horseradish peroxidase-conjugated anti-mouse immunoglobulin (New England Nuclear Corp., Boston, Mass.). Ortho-phenylene diamine was used as the substrate, and color development was terminated with 4.5 M H_2SO_4. Plates were read in a Dynatech MR600 reader at 490 nm.

The ELISA with type 8 capsular polysaccharide was performed in 96-well polystyrene microtiter plates (Immulon I; Dynatech Laboratories, Inc., Alexandria, Va.). Wells were coated with avidin (10 μg/ml; Pierce Chemical Co., Rockford, Ill.) at room temperature overnight. The wells were washed with PBS and blocked with 1% bovine serum albumin in PBS. Biotin-derivatized type 8 capsular polysaccharide (2 μg/ml) was added to the plates, and incubation was at room temperature for 6 h. After being washed with PBS, hybridoma culture supernatants (50 μl) were added to the wells and were incubated at room temperature overnight. Antibody bound to purified capsular polysaccharide was detected with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin and ortho-phenylene diamine as described above.

RESULTS

Two groups of BALB/c strain mice were immunized with Formalin-fixed vaccines of *S. aureus*. One group was immunized with *S. aureus* isolate Reynold, which is the prototype for strains possessing type 5 capsular polysaccharides (8). The other group was immunized with *S. aureus* isolate Becker, which is the prototype for organisms possessing type 8 capsular polysaccharides. Hybridomas were derived from both groups of immune mice by the fusion of spleen lymphocytes with the mouse myeloma cell line, X63-Ag8.653, as described above. Hybridomas producing antibodies which agglutinated the homologous immunizing strain but not a strain possessing the heterologous capsular type were analyzed further. All of the type-specific, anti-*S. aureus* monoclonal antibodies possessed either the immunoglobulin M or the immunoglobulin G3 isotype. Most

TABLE 2. Reactivity of type-specific anti-*S. aureus* monoclonal antibodies in bacterial agglutination assays

Bacteria[a]	Agglutination reactivity[b]	
	Anti-type 5[c]	Anti-type 8[d]
S. aureus		
1[e]	19/19	0/9
3[e]	19/19	0/9
13[e]	19/19	0/9
Reynold[e]	19/19	0/9
2[f]	0/19	9/9
6[f]	0/19	9/9
9[f]	0/19	9/9
15B[f]	0/19	9/9
Becker[f]	0/19	9/9
5[g]	0/19	0/9
7[g]	0/19	0/9
69[g]	0/19	0/9
S. epidermidis		
99	0/19	0/9
141	0/19	0/9
142	0/19	0/9
E. coli O55:B55	0/19	0/9
Serratia marcescens	0/19	0/9
Proteus mirabilis	0/19	0/9
K. pneumoniae	0/19	0/9

[a] Heat-killed, trypsinized bacteria were used in agglutination assays as described in the text.
[b] Number of monoclonal antibodies positive for agglutination (2+ pattern of agglutination or better)/total number of antibodies tested.
[c] Hybridomas derived from mice immunized with *S. aureus* prototype type 5 strain Reynold.
[d] Hybridomas derived from mice immunized with *S. aureus* prototype type 8 strain Becker.
[e] Type 5 strain.
[f] Type 8 strain.
[g] Nontypeable strain.

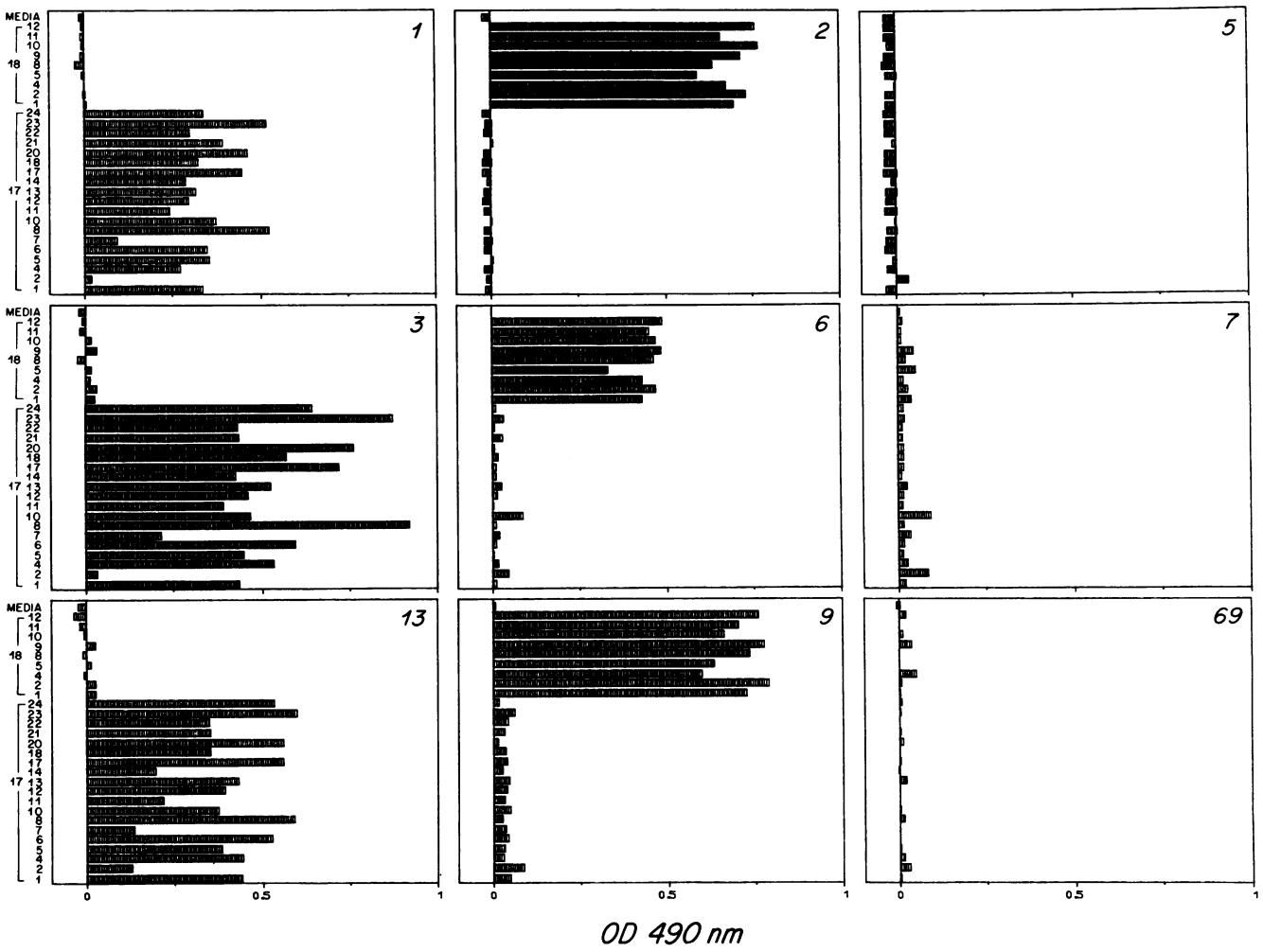

FIG. 1. ELISA was performed in 96-well polyvinyl chloride microtiter trays coated with Formalin-fixed *S. aureus*. Wells were incubated with hybridoma culture supernatants, and bound monoclonal antibody was detected with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin as described in the text. Monoclonal antibodies derived from fusions 17 and 18 were reactive with the prototype type 5 and type 8 strains of *S. aureus*, respectively. Isolates 1, 3, and 13 are type 5; isolates 2, 6, and 9 are type 8; isolates 5, 7, and 69 are nontypeable. OD 490 nm, Optical density at 490 nm.

(17 of 19) of the hybridomas derived from fusion 17 (anti-type 5) secreted antibodies of the immunoglobulin M isotype, whereas approximately half (5 of 9) of the hybridomas derived from fusion 18 (anti-type 8) secreted immunoglobulin G3 antibodies. A similar pattern of IgG isotype restriction has been observed in the murine antibody response to groups A and C streptococcal vaccines (13).

Reactivity patterns of type-specific, anti-*S. aureus* monoclonal antibodies with clinical isolates. The type-specific, anti-*S. aureus* monoclonal antibodies were characterized further to assess their reactivity with a variety of gram-positive and gram-negative bacteria. The agglutination data indicate that all 19 monoclonal antibodies derived from fusion 17 reacted with each of four different type 5 *S. aureus* clinical isolates but with none of the five *S. aureus* clinical isolates possessing type 8 capsular polysaccharides (Table 2). Conversely, all nine monoclonal antibodies derived from fusion 18 reacted with each of five type 8 *S. aureus* strains but not with any type 5 strains. Neither set of type-specific monoclonal antibodies agglutinated clinical isolates of nontypeable *S. aureus*, *Staphylococcus epidermidis*, or four species of gram-negative bacilli (Table 2).

Both groups of antibodies were also tested for reactivity against *S. aureus* strains possessing a variety of capsular types not encountered normally in clinical infections (data not shown). The anti-type 5 monoclonal antibodies were completely unreactive with *S. aureus* strains possessing capsular types 1, 2, 6, and 7. A number of the anti-type 5 monoclonal antibodies were reactive with the prototype type 4 strain. Similar cross-reactions were observed in the original study in which organisms were classified by using polyclonal typing sera (8). The anti-type 8 monoclonal antibodies were completely unreactive with *S. aureus* strains possessing capsular types 1, 2, 4, and 7. Surprisingly, they did react with the only strain in existence which has been classified as type 6. The biochemical composition of the type 6 capsular material has not been investigated. The cross-reactivity observed between the type 6 strain and anti-type 8 monoclonal antibodies may result from shared determinants between type 6 and type 8 strains which were not detected with polyclonal antisera.

Type-specific, anti-*S. aureus* monoclonal antibodies were also assayed for reactivity in ELISA against Formalin-fixed whole organisms (Fig. 1). The reactivity patterns were

TABLE 3. Reactivity of type-specific anti-*S. aureus* monoclonal antibodies with purified type 8 capsular polysaccharide[a]

Antibody[b]	Optical density value[c]
Anti-type 5	
17-1	0.006
17-2	0.001
17-4	0.002
17-5	0.008
17-6	0.006
17-7	0.007
17-8	0.006
17-10	0.002
17-11	0.001
17-12	0.010
17-13	0.004
17-14	0.004
17-17	0.010
17-18	0.009
17-20	0.005
17-21	0.002
17-22	0.076
17-23	0.016
17-24	0.007
Anti-type 8	
18-1	0.483
18-2	0.351
18-4	0.765
18-5	0.525
18-8	0.105
18-9	0.400
18-10	0.560
18-11	0.486
18-12	0.658

[a] Avidin-coated polystyrene microtiter trays (96 wells) were incubated with biotin-derivatized, purified *S. aureus* type 8 capsular polysaccharide as described in the text.

[b] Determined by bacterial agglutination (Table 2) and whole bacteria ELISA (Fig. 1).

[c] Hybridoma culture supernatants were added to type 8 capsular polysaccharide-coated wells and were incubated overnight at room temperature. Antibody binding was assessed with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin in ELISA as described in the text.

equivalent to those obtained by agglutination. Cell culture supernatants from fusion 17 reacted in ELISA with type 5 *S. aureus* strains. Monoclonal antibody 17-2 possessed marginal ELISA reactivity despite the fact that it had agglutinating reactivity. This might be the result of antibody 17-2 having a low affinity and being more likely to be washed off the microtiter plate during the ELISA. Low-affinity antibodies should not have this property in the agglutination assay, because no wash steps are involved. The anti-type 5 monoclonal antibodies did not react in ELISA with either type 8 or nontypeable *S. aureus* clinical isolates. In addition, the anti-type 5 antibodies did not react with *S. epidermidis* or with a variety of gram-negative controls (data not shown).

The nine anti-type 8 monoclonal antibodies derived from fusion 18 were also tested for activity in the ELISA (Fig. 1). As expected, the antibodies reacted with *S. aureus* strains possessing type 8 capsular polysaccharides but not with type 5 and nontypeable *S. aureus* strains. The anti-type 8 monoclonal antibodies also did not react with *S. epidermidis* or with a variety of gram-negative organisms (data not shown).

Reactivity of type-specific anti-*S. aureus* monoclonal antibodies with purified capsular polysaccharides. Reactivity of type-specific, anti-*S. aureus* monoclonal antibodies was assessed further by using purified capsular polysaccharide in ELISA (Table 3). The monoclonal antibodies specific for type 8 *S. aureus* reacted strongly with type 8 capsular polysaccharide, giving ELISA optical density values ranging from 0.105 to 0.765. The antibodies were completely unreactive with purified teichoic acid derived from a nontypeable strain of *S. aureus* (data not shown). Monoclonal antibodies specific for type 5 *S. aureus* did not react with purified type 8 capsular polysaccharides in ELISA.

DISCUSSION

The serologic reactivities of the type-specific, anti-*S. aureus* monoclonal antibodies described in this report are concordant with those of previous studies, (1, 8) with polyclonal rabbit antisera. Nineteen different monoclonal antibodies derived from mice immunized with the prototype type 5 strain were reactive with each of four different clinical isolates of *S. aureus* possessing type 5 capsular polysaccharides. The antibodies did not react with types 1, 2, 6, 7, and 8 *S. aureus*, nontypeable *S. aureus*, *S. epidermidis*, or a variety of gram-negative organisms.

The serologic reactivities of the nine different anti-type 8 monoclonal antibodies were similarly concordant with data obtained by using polyclonal typing reagents. These antibodies reacted with five different *S. aureus* strains that possessed type 8 capsular polysaccharides. No reactivity was seen with types 1, 2, 4, 5, and 7 *S. aureus*, nontypeable *S. aureus*, *S. epidermidis*, or a variety of gram-negative organisms.

Several lines of evidence support the conclusion that the monoclonal antibodies described in this report are reactive with the type-specific capsular polysaccharides described by Karakawa and Vann (8). First, both sets of type-specific monoclonal antibodies are reactive with heat-stable, trypsin-resistant, bacterial cell surface antigens, as demonstrated by the agglutination assays (Table 2). Second, the serologic reactivity and specificity of the monoclonal antibodies in both the agglutination assays (Table 2) and whole organism ELISA (Fig. 1) correlate with the capsular types assigned by using polyclonal typing reagents. Third, at least with regard to the monoclonal antibodies specific for type 8 strains of *S. aureus*, the antibodies react in ELISA with purified type 8 capsular polysaccharides (Table 3). Thus far, we have been unable to show that any of the anti-type 5 monoclonal antibodies react with purified type 5 capsular polysaccharide antigen. The extraction or purification procedure, or both, may modify or destroy the epitopes to which the anti-type 5 antibodies are directed. We are currently investigating alternate methods of purification for the type 5 capsular polysaccharides.

The bacterial antigens considered in this and previous studies (1, 2, 8) are cell surface polysaccharides. Biochemically, they are acidic polymers containing uronic acids (2). Functionally, these bacterial cell surface molecules mask cell wall antigens such as teichoic acid and peptidoglycan, as documented by agglutination studies (8). In these characteristics, they are similar to previously well-defined capsular polysaccharides of other organisms such as *S. pneumoniae* and *H. influenzae* (14). However, as has been stressed previously, the strains studied here do not appear grossly mucoid on routine in vitro culture (1). Further studies are required to define the role of these polysaccharides in the pathogenesis of invasive staphylococcal infection and the potential for protection by type-specific antibody.

The type-specific, anti-*S. aureus* monoclonal antibodies

described in this report will be used in various studies of *S. aureus* capsular polysaccharides. In particular, the use of these antibodies should facilitate the biochemical purification and further characterization of these polysaccharides. In addition, these reagents can be used to investigate the role of capsular polysaccharides in the invasiveness and virulence of encapsulated strains of *S. aureus*. Knowledge gained from these studies will be applicable to the development of a suitable vaccine. These antibodies will also be evaluated as diagnostic reagents for use in staphylococcal disease.

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LITERATURE CITED

1. **Arbeit, R. D., W. W. Karakawa, W. F. Vann, and J. B. Robbins.** 1984. Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **2**:85–91.
2. **Fournier, J.-M., W. F. Vann, and W. W. Karakawa.** 1984. Purification and characterization of *Staphylococcus aureus* type 8 capsular polysacchride. Infect. Immun. **45**:87–93.
3. **Gefter, M. L., D. H. Margulies, and M. D. Scharff.** 1977. A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. Somatic Cell Genet. **3**:231–236.
4. **Kaplan, M. H., and M. J. Tenebaum.** 1982. *Staphylococcus aureus*: cellular biology and clinical application. Am. J. Med. **72**: 248–258.
5. **Karakawa, W. W., and J. A. Kane.** 1972. Characterization of the surface antigens of *Staphylococcus aureus*, strain K-93M. J. Immunol. **108**:1199–1208.
6. **Karakawa, W. W., and J. A. Kane.** 1975. Immunochemical analysis of a Smith-like antigen isolated from two human strains of *Staphylococcus aureus*. J. Immunol. **115**:564–568.
7. **Karakawa, W. W., J. A. Kane, and M. R. Smith.** 1974. Isolation of an acidic surface antigen from a conventional strain of *Staphylococcus aureus*. Infect. Immun. **9**:511–518.
8. **Karakawa, W. W., and W. F. Vann.** 1982. Capsular polysaccharides of *Staphylococcus aureus*. Semin. Infect. Dis. **4**:285–293.
9. **Karakawa, W. W., and D. A. Young.** 1979. Immunochemical study of diverse surface antigens of a *Staphylococcus aureus* isolate from an osteomyelitis patient and their role in in vitro phagocytosis. J. Clin. Microbiol. **9**:399–408.
10. **Karakawa, W. W., D. A. Young, and J. A. Kane.** 1978. Structural analysis of the cellular constituents of a fresh clinical isolate of *Staphylococcus aureus*, and their role in the interaction between the organisms and polymorphonuclear leukocytes in the presence of serum factors. Infect. Immun. **21**:496–505.
11. **Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky.** 1979. A new mouse myeloma line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. **123**:1548–1550.
12. **Littlefield, J. W.** 1964. Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. Science **145**:709–710.
13. **Perlmutter, R. M., D. Hansburg, D. E. Briles, R. A. Nicolotti, and J. M. Davie.** 1978. Subclass restriction of murine anti-carbohydrate antibodies. J. Immunol. **121**:566–572.
14. **Robbins, J. B., R. Schneerson, W. B. Egan, W. Vann, and D. T. Liu.** 1981. Virulence properties of bacterial capsular polysaccharides—unanswered questions, p. 115–132. *In* H. Smith, J. J. Skehel, and M. J. Turner (ed.), The molecular basis of microbial pathogenicity. Verlag Chemie International, Inc., Weinheim, Federal Republic of Germany.
15. **Schneerson, R., O. Barrera, A. Sutton, and J. B. Robbins.** 1980. Preparation, characterization and immunogenicity of *Haemophilus influenzae* type b polysaccharide protein conjugates. J. Exp. Med. **152**:361–376.
16. **Scott, A. C.** 1969. A capsulate *Staphylococcus aureus*. J. Med. Microbiol. **2**:253–260.
17. **Sheagren, J. N.** 1984. *Staphylococcus aureus*: the persistent pathogen. N. Engl. J. Med. **310**:1368–1373.
18. **Smith, I. M.** 1979. *Staphylococcus aureus*, p. 1530–1552. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases. John Wiley & Sons, Inc., New York.
19. **Smith, I. M., and R. J. Dubos.** 1956. The behavior of virulent and avirulent staphylococci in the tissues of normal mice. J. Exp. Med. **103**:87–108.
20. **Wu, T. C. M., and J. T. Park.** 1971. Chemical characterization of a new surface antigenic polysaccharide from a mutant of *Staphylococcus aureus*. J. Bacteriol. **108**:874–884.