INFECTION AND IMMUNITY, July 1979, p. 175-186
0019-9567/79/07-0175/12$02/00/0
Vol. 25, No. 1

Immunological Specificity of Heat-Stable Opsonins in Immune and Nonimmune Sera and Their Interaction with Non-Encapsulated and Encapsulated Strains of *Staphylococcus aureus*

WALTER W. KARAKAWA* AND DEBORAH A. YOUNG

Department of Biochemistry and Biophysics, The Pennsylvania State University, Paul M. Althouse Laboratory, University Park, Pennsylvania 16802

Received for publication 21 February 1979

The in vitro interactions between strains of *Staphylococcus aureus* and human polymorphonuclear leukocytes in the presence of immune and nonimmune sera were studied. Evidence indicated that phagocytosis of encapsulated strains occurred in the presence of specific homologous antiserum, whereas non-encapsulated strains were readily phagocytized by polymorphonuclear leukocytes in the presence of both normal and immune sera. Immunological analyses demonstrated that normal serum opsonins, which reacted with the non-encapsulated strains, were specifically directed against exposed mucopeptide moieties of the organisms. Sera rich in antimucopeptide antibodies were obtained from rabbits immunized with heterologous bacteria such as *Escherichia coli* and group A-variant streptococci and were shown to be effective in opsonizing the non-encapsulated strains of *S. aureus*. Fresh clinical isolates of *S. aureus* were noticeably more resistant to the opsonizing effects of the antimucopeptide antibodies. Results were presented which suggest that the surface structures of these clinical isolates are more diverse than laboratory-propagated strains and that these antiphagocytic surface antigens may be significant factors in masking the opsonizing effects of the mucopeptide opsonins which are present in most sera.

Many attempts have been made to induce antibacterial immunity against the conventional strains of *Staphylococcus aureus* in experimental animals; however, most studies have been plagued by a series of difficult problems (5). One of these problems has been the lack of a suitable experimental animal for use in the infectivity studies. For example, experimental animals have a high degree of natural resistance to staphylococcal infections, and, therefore, the relatively high doses of *S. aureus* organisms frequently required in the animal protection studies often result in ambiguous observations which are of doubtful significance (21). Secondly, immunological resistance in these experimental animals to a challenge of *S. aureus* was considered nonspecific and could be induced by immunization with heterologous bacteria (23). Thirdly, experimental strains were frequently propagated in the laboratory and were often devoid of antiphagocytic surface antigens (26). In spite of these inherent difficulties, evidence has been presented which suggests that low levels of specific immunity to *S. aureus* can be induced in experimental animals (5, 10, 13; W. Karakawa and D. A. Young, J. Clin. Microbiol., in press).

In a continued effort to elucidate the perplexing problems encompassing host resistance to *S. aureus*, a number of investigators have recently focused on two major factors which could influence the opsonic response in an individual to staphylococcal infections (18). Some of the factors which influence staphylococcal pathogenesis are the antigenic diversity of the cell surfaces of the organisms under in vivo conditions of the host and the relative levels of natural antibodies or heat-stable opsonins in the host (5, 10, 13, 19; Karakawa and Young, in press). In the case of the cell surface mosaic of *S. aureus*, it has been shown that fresh *S. aureus* isolates are different from the corresponding laboratory-propagated strains in that the fresh isolates are more resistant to in vitro phagocytosis by polymorphonuclear leukocytes (PMN) (11; Karakawa and Young, in press). In an extension of these studies, Peterson et al. have suggested that encapsulated *S. aureus* strains were resistant to phagocytosis by human PMN by virtue of the fact that the surface capsules interfered with effective opsonization in the presence of heat-stable opsonins

present in nonimmune serum (18, 19). On the other hand, non-encapsulated strains, which lack the masking surface antigens, possess binding sites for the heat-stable factors found in nonimmune serum. These heat-stable opsonins were suggested to be directed against staphylococcal mucopeptide (18). In view of the common occurrence of antimucopeptide antibodies in sera, it is feasible to assume that these antibodies may play a significant role in the opsonization of non-encapsulated strains of *S. aureus* and may, in part, have influenced many of the ambiguous results obtained in previous studies on the immunity against staphylococci.

This report substantiates the view that antimucopeptide antibodies are effective in promoting the opsonization of non-encapsulated strains of *S. aureus*. Evidence is presented which indicates that these antibodies may be common features of both immune and nonimmune sera and thus may be factors in staphylococcal pathogenesis.

MATERIALS AND METHODS

Bacterial strains. Non-encapsulated *S. aureus* 57 was originally isolated from a burn patient and subsequently propagated on artificial media. Two other strains of *S. aureus*, provisionally designated Mardi and D, were isolated from patients with osteomyelitis (11; Karakawa and Young, in press). Variant strains, which were selected and isolated from cultures of the original or wild-type strains by a method previously described, were also included in these studies (Karakawa and Young, in press). The remaining strains of bacteria were obtained from the culture collection of the Immunochemistry Laboratory, Department of Biochemistry, Pennsylvania State University.

Preparation of surface antigens and mucopeptides. Surface antigens were extracted from the wild-type and variant strains by methods previously described (8). Mucopeptides were prepared from acid-treated cells by the method described by Krause and McCarty (14).

Chromatography and gel filtration. The techniques used for diethylaminoethyl-cellulose chromatography and Bio-Gel filtration were previously described (8).

Analytical methods. Analyses for hexosamines, amino acids, and hexoses were performed by methods previously described (1, 12, 25). Total hexose concentration was quantitated as previously described (22). Total phosphorus was measured by the method of Chen et al. (2). Uronic acid concentration was calculated by the carbazole method described by Davidson (3). Sodium borohydride reduction of polysaccharides was performed by the method of Deuel (4), and the constituents of the reduced and nonreduced polymers were analyzed by gas-liquid chromatography by the procedure described by Fraser and Mallette (6).

Serological methods. Quantitative precipitin analyses were performed by a modification of the method described by McCarty and Lancefield (15). The capillary precipitin test for the detection of mucopeptide antibodies was performed in a manner similar to that employed by McCarty and Lancefield for the detection of group-specific streptococcal antibodies (15).

Antimucopeptide sera were obtained from rabbits which had been immunized intravenously with whole staphylococcal organisms treated with pH 5.0 buffer at 100°C for 20 min to remove the acidic surface polysaccharides. The immunization schedule was similar to that used by McCarty and Lancefield (15). Certain streptococcal and *Escherichia coli* antisera which contained high concentrations of mucopeptide antibodies were obtained from the collection of sera of the Immunochemistry Laboratory, Pennsylvania State University. Agglutinins were detected by a direct tube agglutination technique previously described (9). Mucopeptide, which had been solubilized in a 20-kc sonic oscillator for 15 min, was employed in the quantitative analysis of mucopeptide antibodies.

In vitro phagocytosis tests. To obtain human PMN, heparinized human peripheral blood was sedimented for 45 min at room temperature with 3% dextran T-250 (Pharmacia, Uppsala, Sweden). The PMN were then centrifuged at $160 \times g$ for 5 min, washed with saline containing 100 U of heparin per ml (Sigma Chemical Co., St. Louis, St. Louis, Mo.), and suspended to a concentration of 1.0×10^7 viable cells per ml in RPMI medium (Microbiological Associates, Bethesda, Md.) supplemented with 5% heat-inactivated fetal calf serum. Leukocyte counts were made with a Neubauer hemacytometer, and viability was determined by trypan blue staining. The in vitro phagocytosis test is a modification of the method described by Roberts (20). All tests were performed in sterile, siliconized glass, screw-capped tubes (13 by 100 mm). Before each test, each *S. aureus* strain was grown for 5 h in Todd-Hewitt broth (Difco, Detroit, Mich.), centrifuged and washed in phosphate-buffered saline (pH 7.2), and adjusted to a final concentration of approximately 2.0×10^7 organisms per ml with a Gilford spectrophotometer. The incubation mixture, which contained a total volume of 1.0 ml, contained approximately 1.0×10^7 PMN, 10% antiserum, and approximately 2.0×10^7 *S. aureus* organisms. Control tubes were also included to determine the effects of serum or phagocytes alone on the organisms. All tubes were tumbled slowly on a rotating rack (Scientific Industries Inc., Springfield, Mass.) at 37°C. Samples were taken from the phagocytic mixture with an Eppendorf pipette at timed intervals and diluted in distilled water to lyse the leukocytes. The dilutions were plated on Staphylococcus Medium 110 (Difco, Detroit, Mich.) to determine the number of colony-forming units. From these data, the number of surviving bacteria was calculated and plotted against the incubation time on semilogarithmic graph paper.

RESULTS

Demonstration of heat-stable staphylococcal opsonins in sera. In a competitive in vivo environment, a phenotype of an organism which can resist the selective biological pressures of the host often emerges as the dominant

strain. Under laboratory conditions, these in vivo pressures are not operative, and, therefore, various phenotypes of the organism may be represented and may display a surface mosaic which is more suitable for propagation and maintenance rather than for survival in the host. For example, it has been observed that laboratory-propagated strains of *S. aureus* frequently were agglutinated in the presence of nonimmune sera, whereas encapsulated or fresh clinical isolates were not readily agglutinated by normal sera (5). These observations suggested that the surface mosaic of laboratory strains may differ considerably from fresh clinical isolates and may represent phenotypic variants which are devoid of antiphagocytic surface antigens.

The results of the in vitro phagocytosis studies which used heat-inactivated immune and nonimmune sera, washed PMN, and non-encapsulated *S. aureus* organisms are shown in Fig. 1. Normal fetal calf serum and normal rabbit serum 1 possess essentially no opsonins against the non-encapsulated strain. In contrast, serum obtained from a young human adult, pooled adult immunoglobulin, and serum obtained from an adult patient with a previous staphylococcal infection contained opsonins which were effective in promoting the in vitro phagocytosis of 99% of the viable bacterial population by PMN. It should be noted that serum from normal rabbit 2 also contained opsonins which were effective in promoting the phagocytosis of over 90% of the viable cells by PMN. These results indicate that heat-stable opsonins directed against the cell surface of non-encapsulated strains of *S. aureus* may be a common feature of both immune and nonimmune sera.

Immunochemical specificity of the serum opsonins. Previous reports by Peterson et al. suggested that mucopeptide antibodies may be

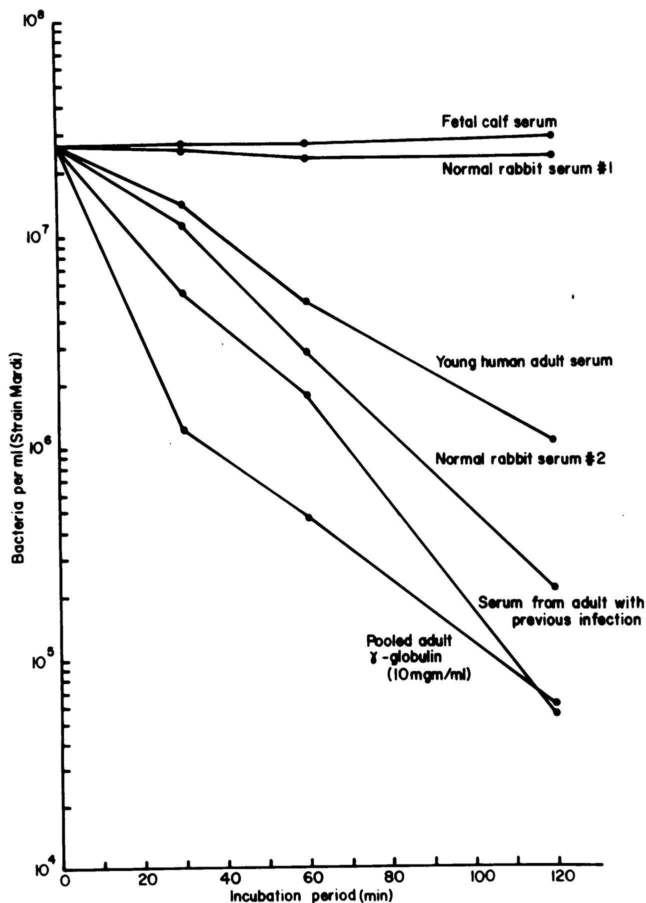

FIG. 1. *Interaction between S. aureus organisms and human PMN in the presence of fetal calf serum, normal rabbit serum 1, young human adult serum, normal rabbit serum 2, human adult serum, and pooled human immunoglobulin.*

involved in the in vitro phagocytosis of non-encapsulated *S. aureus* strains by PMN (18). In view of this report, the opsonins observed in immune sera were immunologically tested against purified streptococcal mucopeptide. The results of the quantitative precipitin reactions between purified heterologous streptococcal mucopeptide, which was shown to be devoid of teichoic or lipoteichuronic acids, and various staphylococcal antisera are shown in Fig. 2. In all instances, the solubilized streptococcal mucopeptide reacted strongly with antisera obtained from rabbits immunized with three different non-encapsulated strains of *S. aureus*. The observed reactivity was suggested to be due to the antigenic determinants common to both the streptococcal and the staphylococcal mucopeptides (9). The opsonic capability of the mucopeptide antibodies observed in these sera, as well as human sera, was determined by the in vitro phagocytosis tests which employed human PMN. Human serum from an individual with previous staphylococcal infections and the various anti-staphylococcal sera were effective in promoting the phagocytosis of over 90% of the viable non-encapsulated organisms by PMN (Fig. 3A). After absorbing these antisera with purified streptococcal mucopeptide, a large portion of the available opsonins were specifically removed by the heterologous mucopeptide (Fig. 3B). Note that, after absorption with mucopeptide, all of the anti-staphylococcal sera showed a marked reduction in opsonic activity, whereas the human serum, although reduced in opsonin titer, still showed significant levels of opsonins against the organisms. This residual level of opsonins could be attributed to antibodies directed against other cellular constituents of *S. aureus*. These results indicate that both non-immune and immune sera consist of opsonins with mucopeptide specificity.

Prevalence of opsonins in sera. The chem-

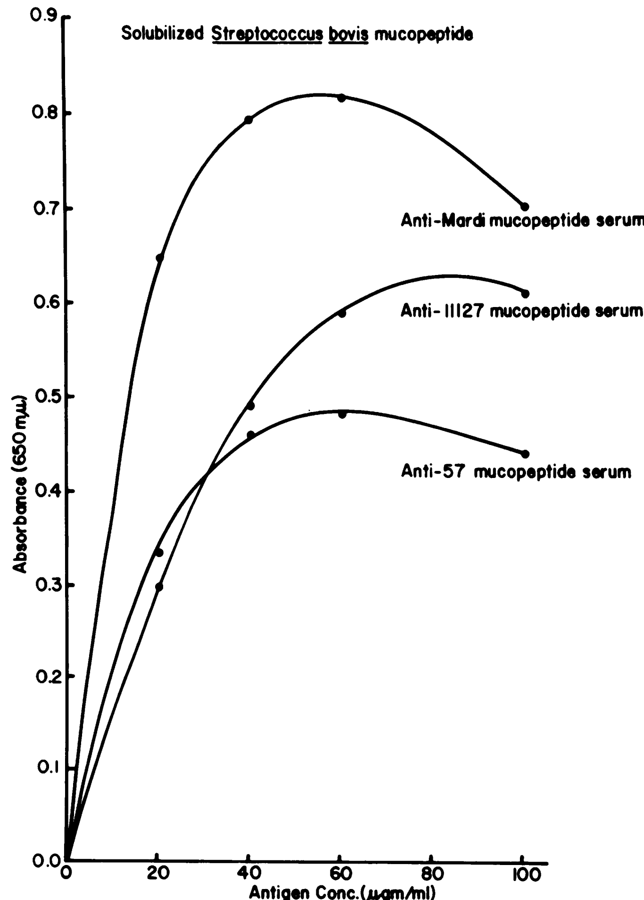

FIG. 2. *Quantitative precipitin reactions between solubilized Streptococcus bovis mucopeptide and anti-mucopeptide sera derived from rabbits immunized with particulate staphylococcal mucopeptide.*

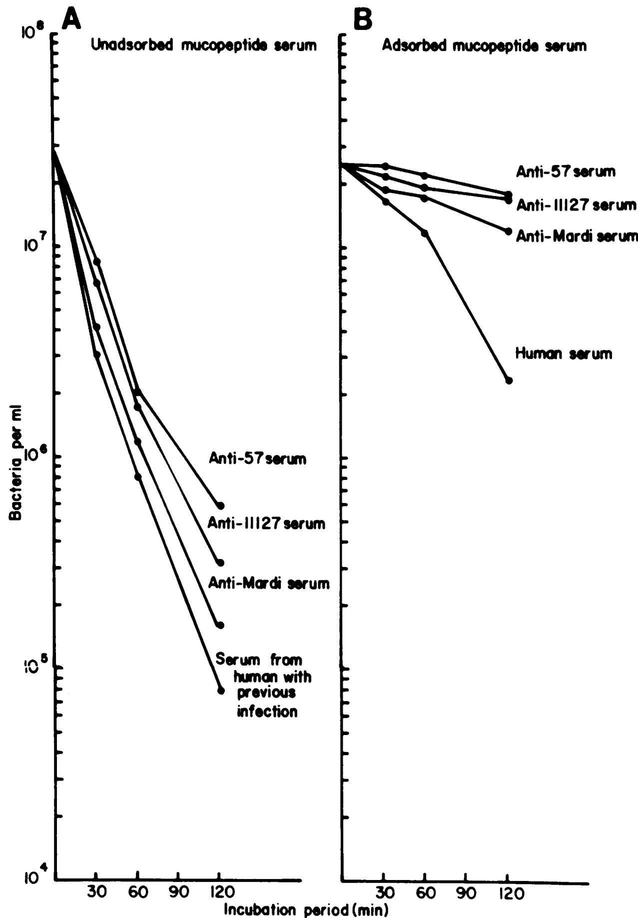

FIG. 3. *(A) Interaction between S. aureus and human PMN in the presence of antimucopeptide sera and adult human serum. (B) Interaction between S. aureus and human PMN in the presence of the same antimucopeptide sera which were absorbed with purified particulate streptococcal mucopeptide.*

ical profile of the purified staphylococcal mucopeptide devoid of teichoic acid which was used in the subsequent experiments is shown in Fig. 4. The constituents depicted in this ion-exchange elution pattern represented approximately 98% by weight of the known components of a mucopeptide preparation, and, with the exception of serine, the elution pattern is consistent with the observations of other investigators (24). This chemically defined mucopeptide was mixed with representative anti-*S. aureus* serum, anti-*E. coli* serum, or normal porcine serum and incubated for 24 h at 4°C. The mucopeptide-antibody complexes were removed by centrifugation and washed with buffered saline, and the antibodies were subsequently eluted by the method previously described (9). The purified mucopeptide antibodies which were eluted from the mucopeptide-antibody complexes gave a strong reaction with a solubilized preparation of the staphylococcal mucopeptide (Fig. 5). Note that an antibody preparation derived from anti-*S. aureus*-mucopeptide complex at a concentration of 1 mg/ml gave a strong reaction with solubilized mucopeptide. Similarly, antibody preparation from anti-*E. coli* and normal porcine sera also gave a strong reaction with mucopeptide. These results substantiate the view that both immune and nonimmune sera contain opsonins with mucopeptide specificity.

Diversity of the surface antigens of *S. aureus* strains. Previous studies by a number of investigators have suggested that *S. aureus* strains containing capsules are markedly more resistant to phagocytosis by PMN than are the non-encapsulated strains (13, 16). In the case of the encapsulated strains, the presence of the capsular antigens can impede phagocytosis by PMN, whereas the non-encapsulated strains consisting of exposed mucopeptide are suscepti-

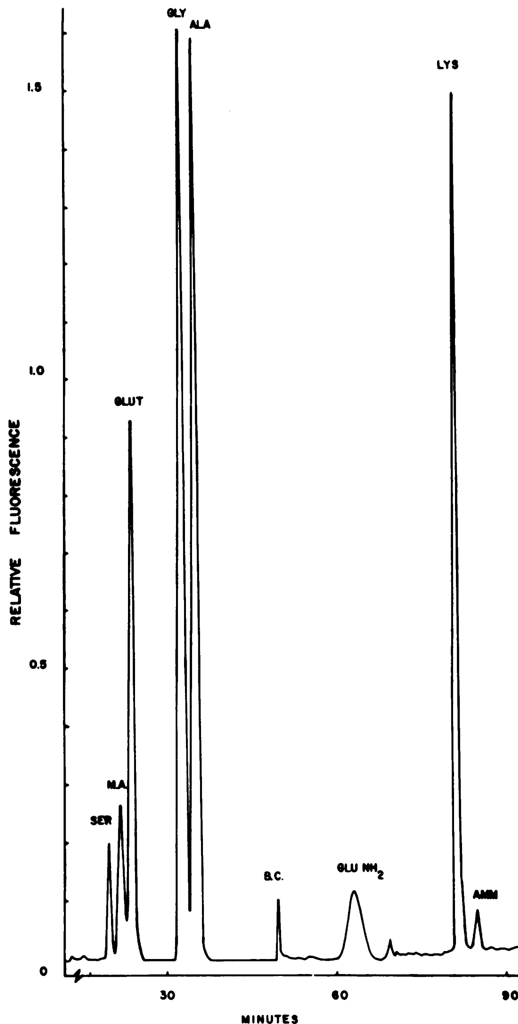

FIG. 4. *Analysis of an acid hydrolysate of formamide-extracted staphylococcal mucopeptide. Durrum DC-4A resin, 0.32- by 32-cm column, 50°C, Durrum Pico-buffer system II. Abbreviations: M.A., muramic acid; B.C., buffer change; AMM, ammonia.*

ble to the opsonizing activity of mucopeptide antibodies present in both immune and nonimmune sera. The results of the interaction between encapsulated and non-encapsulated *S. aureus* strains and human PMN in the presence of antimucopeptide sera are depicted in Fig. 6. Both encapsulated strains D and 93M were markedly resistant to opsonization in the presence of the mucopeptide antibodies contained in the staphylococcal and streptococcal antisera. On the other hand, both non-encapsulated strains 11127 and 57 were readily opsonized in the presence of the mucopeptide antibodies present in both the staphylococcal and the streptococcal antisera. In addition to these observations, in vitro phagocytosis studies which employed mucopeptide antibodies eluted from streptococcal mucopeptide complexes indicated that various strains of staphylococci differ considerably in their susceptibility to opsonization in the presence of a constant amount of mucopeptide antibodies. Encapsulated strain D, which possesses an aminogalacturonic acid-galactose capsule, was markedly resistant to opsonization in the presence of mucopeptide antibodies (Fig. 7). Fresh clinical isolates—strain Mardi, which possesses a galactose-glucuronic acid polymer, and strain 7, which possesses a fucosamine-aminomannuronic acid polymer—were moderately susceptible to opsonization in the presence of mucopeptide antibodies (Karakawa and Young, in press). Strain Wood, the standard erythrotoxin producer which has been propagated for many years on artificial medium, and strain 11127, another laboratory-propagated strain, were very susceptible to opsonization in the presence of mucopeptide antibodies. These results support the notion that *S. aureus* strains propagated on laboratory medium are prone to opsonization in the presence of nonimmune serum and that mucopeptide antibodies, in part, play a role in the opsonization of non-encapsulated strains. In addition, it appears that *S. aureus* strains can be placed into three groups based on their affinity for mucopeptide antibodies, namely, the fully encapsulated strains, the micro-encapsulated or fresh clinical strains, and the non-encapsulated laboratory strains. A picture emerges from these observations which suggests that *S. aureus* strains with exposed mucopeptide are highly susceptible to opsonization in the presence of mucopeptide antibodies found in most sera. In contrast, those strains possessing surface antigens are reasonably more fitted for in vivo survival by virtue of their surface antigens which can mask the binding sites for the ubiquitous mucopeptide antibodies and thus are resistant to phagocytosis by PMN.

Isolation of antimucopeptide-resistant colonies of *S. aureus*. It has been shown that the wild-type (clinical isolates) *S. aureus* strains, when grown for 3 h in the presence of human PMN and antimucopeptide serum, frequently form colony variants which are resistant to phagocytosis by PMN in the presence of nonimmune serum (Karakawa and Young, in press). In a continuation of that study, three laboratory-propagated ("wild type") strains of *S. aureus*, namely, Mardi, 11127, and 57, were grown in the presence of PMN and antimucopeptide serum. In all instances, the cells which were obtained

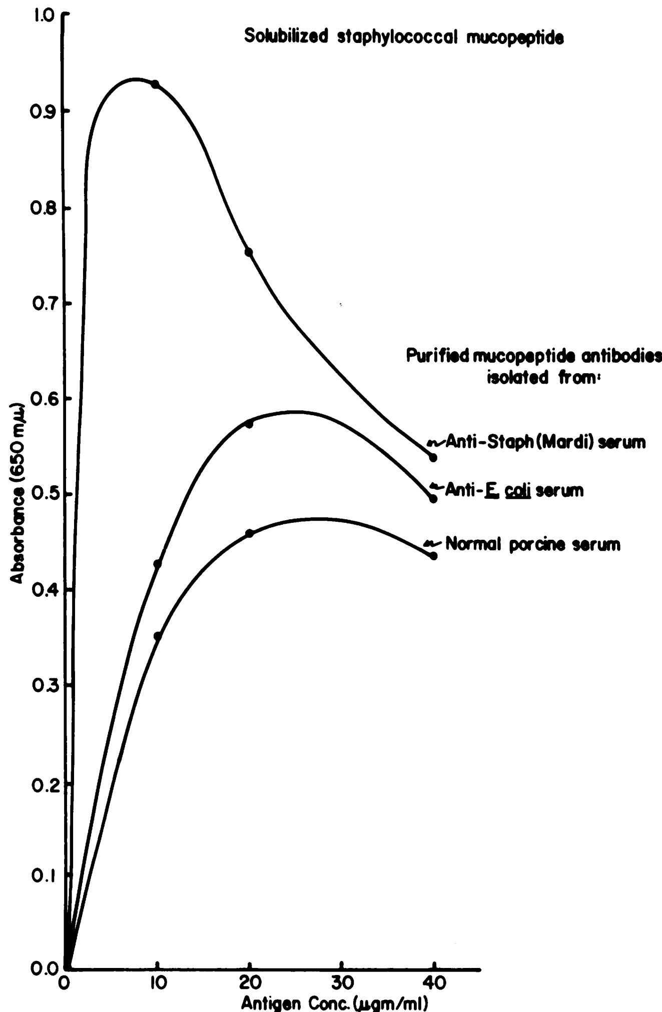

FIG. 5. *Quantitative precipitin reactions between solubilized staphylococcal mucopeptide and purified mucopeptide antibodies isolated from anti-S. aureus, anti-E. coli, and normal porcine sera.*

were resistant to opsonization in the presence of mucopeptide antibodies as determined by the in vitro phagocytosis studies. Whether these resistant cells are phenotypic variants or mutants is not known at this time. Surface antigens of the resistant and wild-type strains were isolated by the pH 7.0 extraction procedure previously described (11). The results of the quantitative precipitin analyses of the surface antigens of the resistant ("mutant or variant") strains with homologous variant and wild-type antisera are shown in Fig. 8. In all instances, the variant antigens gave a stronger reaction with their homologous variant antisera than with the corresponding wild-type antisera. Purification of the antigen preparations of variants Mardi, 11127, and 57 by gel filtration and diethylaminoethylcellulose chromatography suggested that all three polymers were acidic. In an attempt to identify the acidic moieties in the variant polymers, the polymers were reduced with borohydride and the alditol acetate derivatives of the reduced and nonreduced polymers were analyzed by gas-liquid chromatography. The results of the gas-liquid chromatography analyses of the reduced polymers are shown in Fig. 9. The variant 11127 polymer consisted of galactose and aminoglucuronic acid, the variant Mardi polymer consisted of galactose and aminogalacturonic acid, and the variant 57 polymer consisted of galactose and glucuronic acid. Quantitative analysis was attempted; however, due to the acid lability of these polymers, recovery of the constituents was less than 50%. Immunochemical

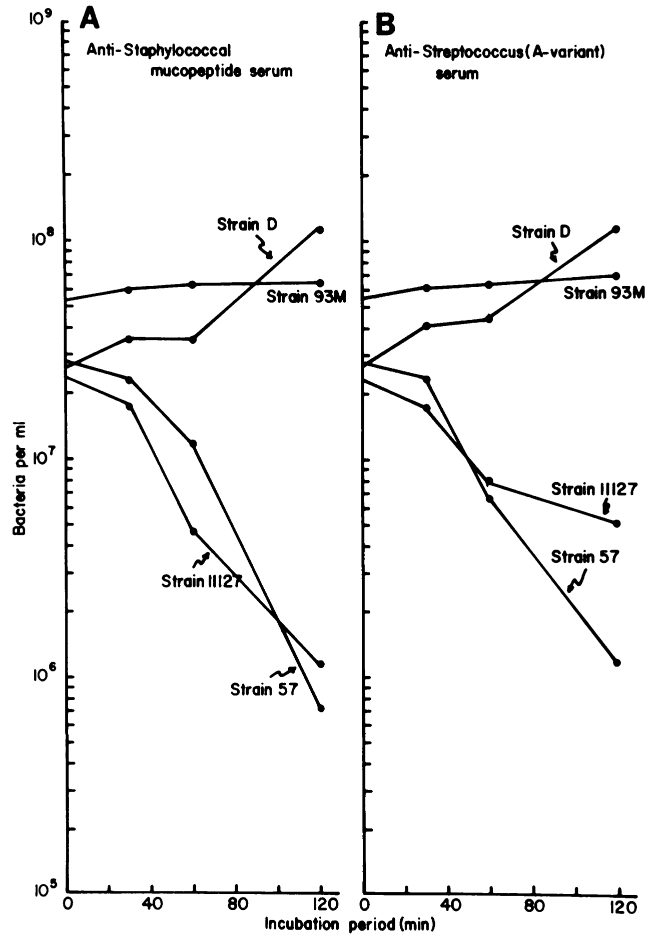

FIG. 6. *(A) Interaction between S. aureus strains D and 93M (encapsulated) and 11127 and 57 (non-encapsulated) and human PMN in the presence of anti-staphylococcal mucopeptide sera. (B) Interaction between S. aureus strains D and 93M (encapsulated) and 11127 and 57 (non-encapsulated) and human PMN in the presence of anti-streptococcal mucopeptide sera.*

and chemical studies of these polymers are now under investigation. These preliminary observations underscore the possible potential of *S. aureus* strains to form variants with an altered surface antigenic mosaic under conditions simulating an in vivo environment.

DISCUSSION

Success in inducing humoral immunity to staphylococci with a variety of bacterial products has not been dramatic (5). Many of the shortcomings can be attributed to the deficient knowledge of the immunologically important surface antigens of clinical *S. aureus* strains and the serum factors involved in the phagocytic process. This study attempts to quantitatively evaluate the interaction between strains of *S. aureus* carrying various amounts of surface antigens and PMN in the presence of specific opsonins found in immune and nonimmune sera.

The findings indicate that the antigenic surface of *S. aureus* is an important factor in the interaction between *S. aureus* organisms and PMN. For example, non-encapsulated strains were shown to be highly susceptible to opsonization in the presence of either nonimmune or immune sera. In contrast, fully encapsulated strains required homologous immune sera for the initiation of phagocytosis by PMN. These observations focus attention on a possible explanation for the frequently observed opsonization in laboratory strains in the presence of nonimmune sera. Since these strains are devoid of surface antigens, the mucopeptide moieties are exposed and therefore are available for the mucopeptide opsonins which are a common feature

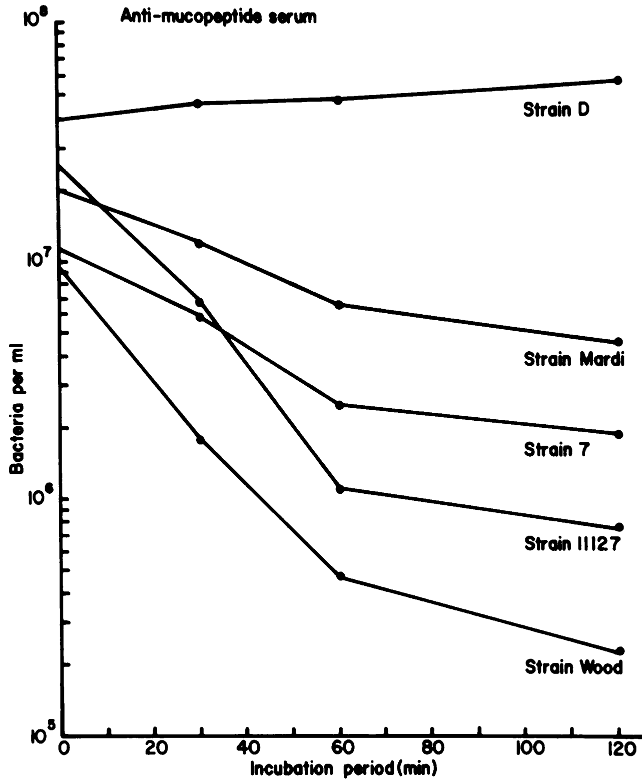

FIG. 7. *Interaction between S. aureus strain D (encapsulated), strains Mardi and 7 (micro-encapsulated), and strains 11127 and Wood (non-encapsulated) and human PMN in the presence of antimucopeptide sera.*

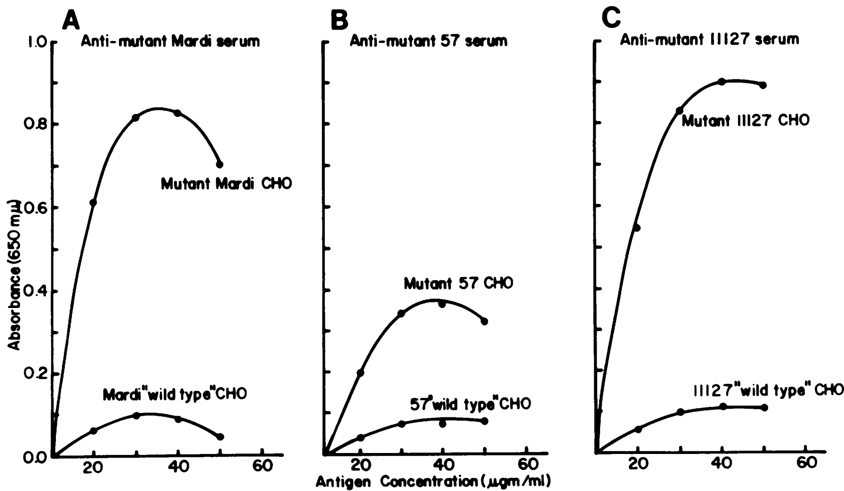

FIG. 8. *Quantitative precipitin reactions between the polysaccharide antigens isolated from the wild-type and variant S. aureus strains Mardi, 57, and 11127 and homologous variant antisera. (A) Strain Mardi; (B) strain 57; (C) strain 11127.*

of most sera. The mucopeptide specificity of these opsonins was supported by the fact that antibodies eluted from heterologous streptococcal mucopeptide-antibody complexes were effective in promoting opsonization of non-encapsulated strains of *S. aureus*. This reactivity was readily removed when the antibody preparation was absorbed with mucopeptide. These findings

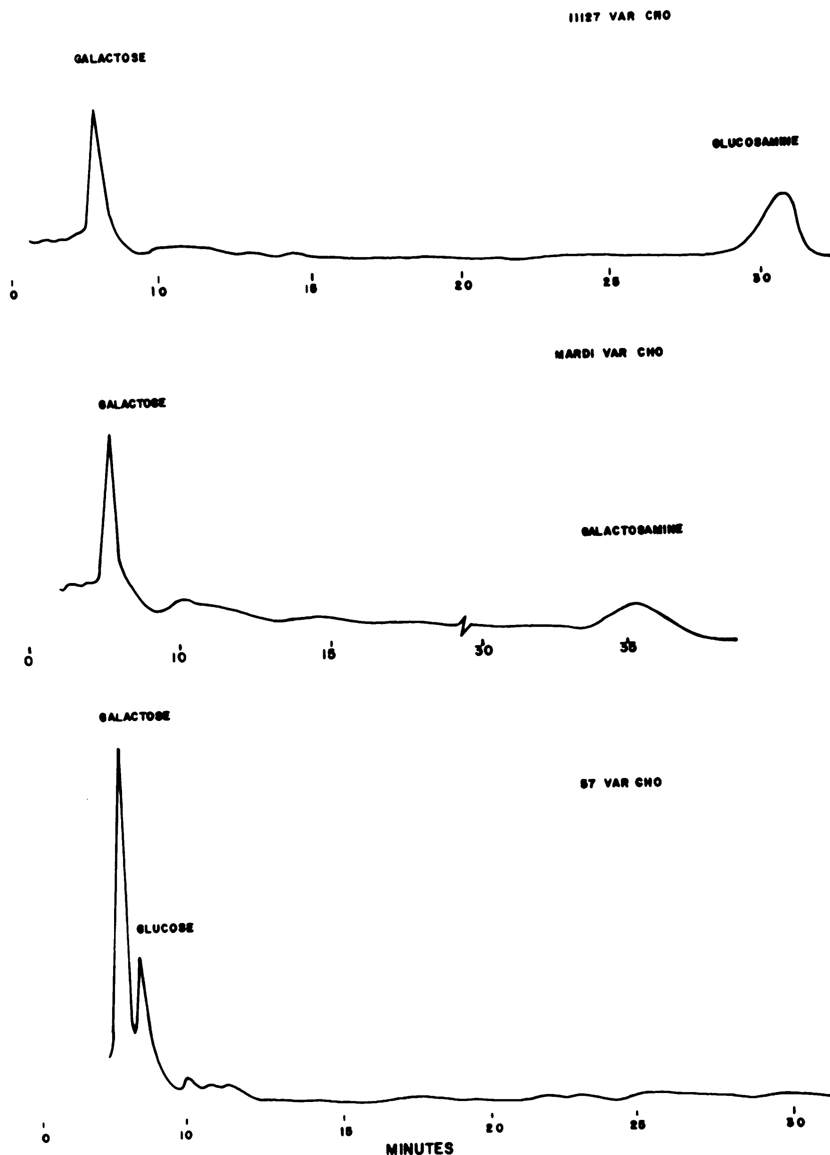

FIG. 9. *Gas-liquid chromatography of the alditol acetate derivatives of the $NaBH_4$-reduced surface antigens of the variant S. aureus strains 11127, Mardi, and 57. A 2-μl sample of alditol acetates in chloroform was analyzed on a glass column (4 feet [ca. 1.2 m] by 4 mm) of 3% OV-225 on Supelcoport 80/100 mesh at 220°C in 4 ml of N_2 per min.*

suggest that the availability of the mucopeptide matrix to the ubiquitous mucopeptide antibodies appears to be a key initiation factor in the phagocytosis of *S. aureus*. Therefore, organisms with surface antigens which can mask the mucopeptide moieties are more likely to resist phagocytosis and thus may be more virulent. This possibility appears feasible in light of the observations of Peterson et al., which clearly established that the mucopeptide of non-encapsulated *S. aureus* strains was involved in promoting opsonization in the presence of serum factors found in nonimmune sera (18). In contrast, the more virulent encapsulated strains were resistant to the nonimmune serum factors by virtue of the fact that the mucopeptide moieties were masked by surface antigens. The belief that surface antigens can mask mucopeptide sites is plausible since Melly et al. were able to demonstrate a direct correlation between the capsule size of *S. aureus*

strain M and its resistance to phagocytosis by PMN (16). These investigators were able to show that the organisms with larger capsules were more resistant to phagocytosis by PMN than were those organisms with little or no capsule, thus indicating that larger capsules may be effective in masking available mucopeptide sites. Another observation which supports the view that mucopeptide is a significant factor in the opsonization of *S. aureus* was described by Yoshida et al. (26). In these studies, they were able to isolate encapsulated strains from 4% of their isolates of *S. aureus* which, upon subsequent subculturing on artificial medium, reverted to non-encapsulated types which are essentially devoid of surface antigens and which were less virulent than the parent strains. In similar studies, it was shown that fresh clinical isolates were often more pathogenic for mice and frequently carried detectable levels of antiphagocytic surface antigens (Karakawa and Young, in press). As a result, these strains were generally more resistant to opsonization in the presence of nonimmune rabbit serum and therefore were able to resist in vitro phagocytosis by PMN by virtue of these surface antigens. These results can explain, in part, the frequent observation that fresh clinical isolates are often more pathogenic for mice than laboratory-propagated strains. Since most humans possess opsonins with mucopeptide specificity, non-encapsulated strains would be readily phagocytized by PMN under in vivo conditions, whereas encapsulated strains will have a survival advantage due to their antiphagocytic surface antigens. Because of the survival advantage of the encapsulated strains, the chance for isolating these strains would be greater than for the isolation of the non-encapsulated strains under in vivo conditions. On the other hand, the likelihood of isolating the encapsulated strains on artificial laboratory medium would be less since the production of antiphagocytic surface antigens is not required for maintenance and propagation of *S. aureus* strains. The observed decrease in the production of surface antigen by clinical isolates of *S. aureus* under laboratory conditions could possibly be the result of a phenotypic alteration of the surface antigen mosaic, thus causing the unmasking of the mucopeptide sites.

It has been suggested by a number of investigators that *S. aureus* strains have the genetic potential to form surface antigens under in vivo conditions. Park et al., for example, were able to isolate a variant strain of *S. aureus* which carried an acidic teichuronic acid polymer when the wild type was grown in the presence of staphylococcal teichoic acid antibodies (17). Karakawa and Young were able to isolate a variant from a wild-type strain which originated from a patient with osteomyelitis when the wild-type organism was grown in the presence of mucopeptide antibodies and human PMN (in press). This variant was very resistant to opsonization in the presence of mucopeptide antibodies by virtue of the fact that it carried an antiphagocytic galactose-aminogalacturonic acid polymer (Karakawa and Young, in press).

In the present studies, preliminary evidence is presented which indicates that, when laboratory-propagated *S. aureus* strains are grown in the presence of antimucopeptide serum and PMN, resistant variants which carry an antiphagocytic acidic polymer can be found. In the case of strain 11127, the variant surface antigen consisted of a galactose-aminoglucuronic acid polymer. Strain 57 also carried an antiphagocytic polymer consisting of galactose and glucuronic acid. The immunochemistry of this polysaccharide is now under investigation.

This report underscores the significance of mucopeptide antibodies in the opsonization of non-encapsulated strains of *S. aureus*. Since many studies on staphylococcal immunity have used laboratory-propagated strains, attempts should be made to reevaluate the feasibility of active immunization against staphylococci. For example, some of the failures in previous immunization procedures with staphylococcal products may have been a reflection of the fact that the vaccines may have lacked the essential protective surface antigens (7). These surface antigens, which are usually synonymous with fresh clinical isolates of *S. aureus*, could be of utmost significance in answering certain important questions such as the following: how many different capsular types are associated with serious staphylococcal diseases; is there a specific predilection for a particular capsular type in a specific clinical infection in man; what is the prevalence of capsular antibodies in humans with repeated staphylococcal infections?

ACKNOWLEDGMENTS

This investigation was supported, in part, by Public Health Service Grant AI-11598 from the National Institute of Allergy and Infectious Disease.

LITERATURE CITED

1. **Benson, J. R.** 1973. Single column analysis of amino acids. *In* I. L. Simmons and G. W. Ewing (ed.), Applications of the newer techniques of analysis. Plenum Publishing Corp., New York.
2. **Chen, P. S., Jr., T. Y. Toribara, and H. Warner.** 1956. Microdetermination of phosphorus. Anal. Chem. **28:** 1756–1758.
3. **Davidson, E. A.** 1966. Analysis of sugars found in mucopolysaccharides. Methods Enzymol. **8:**55.

4. **Deuel, J.** 1947. Pectins. XXI. Glycol esters of pectic acid. Helv. Chim. Acta **30**:1523–1534.
5. **Ekstedt, R. D.** 1974. Immune response to surface antigens of *Staphylococcus aureus* and their role in resistance to staphylococcal disease. Ann. N.Y. Acad. Sci. **236**:203–220.
6. **Fraser, B. A., and M. F. Mallette.** 1973. An improved isolation method and new composition data for the Forrsman hapten from sheep erythrocytes. Immunochemistry **10**:745–753.
7. **Greenberg, L.** 1968. Staphylococcal vaccines. Bull. N.Y. Acad. Med. **44**:1222–1226.
8. **Kane, J. A., and W. W. Karakawa.** 1977. Multiple polysaccharide antigens of group B streptococcus, type Ia: emphasis on a sialic acid type-specific polysaccharide. J. Immunol. **118**:2155–2160.
9. **Karakawa, W. W., D. G. Braun, H. Lackland, and R. M. Krause.** 1968. Immunochemical studies on the cross-reactivity between streptococcal and staphylococcal mucopeptide. J. Exp. Med. **128**:325–340.
10. **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.
11. **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.
12. **Kesler, R. B.** 1967. Rapid quantitative anion-exchange chromatography of carbohydrates. Anal. Chem. **39**: 1416–1422.
13. **Koenig, M. G., and M. A. Melly.** 1965. The importance of surface antigens in staphylococcal virulence. Ann. N.Y. Acad. Sci. **128**:231–250.
14. **Krause, R. M., and M. McCarty.** 1961. Studies on the chemical structure of the streptococcal cell wall. I. The identification of a mucopeptide on the cell walls of group A and A-variant streptococci. J. Exp. Med. **114**: 127–140.
15. **McCarty, M., and R. C. Lancefield.** 1955. Variation in the group-specific carbohydrates of group A streptococci. I. Immunochemical studies on the carbohydrates of various strains. J. Exp. Med. **102**:11–28.
16. **Melly, M. A., L. J. Duke, D. F. Liau, and J. H. Hash.** 1974. Biological properties of encapsulated *Staphylococcus aureus*. Infect. Immun. **10**:389–397.
17. **Park, J. T., R. D. Shaw, A. N. Chatterjee, D. Mirelman, and T. Wu.** 1974. Mutants of staphylococci with altered cell walls. Ann. N. Y. Acad. Sci. **235**:54–61.
18. **Peterson, P. K., B. J. Wilkinson, Y. Kim, D. Schmeling, S. D. Douglas, P. G. Quie, and J. Verhoef.** 1978. The key role of mucopeptide in the opsonization of *Staphylococcus aureus*. J. Clin. Invest. **61**:596–609.
19. **Peterson, P. K., B. J. Wilkinson, Y. Kim, D. Schmeling, and P. G. Quie.** 1978. Influence of encapsulation on staphylococcal opsonization and phagocytosis by human polymorphonuclear leukocytes. Infect. Immun. **19**:943–949.
20. **Roberts, R. B.** 1970. The relationship between group A and group C meningococcal polysaccharides and serum opsonins in man. J. Exp. Med. **131**:499–513.
21. **Rogers, D. E., and M. A. Melly.** 1965. Speculation on the immunology of staphylococcal infections. Ann. N.Y. Acad. Sci. **128**:274–284.
22. **Scott, T. A., and E. H. Melvin.** 1953. Determination of dextrose with anthrone. Anal. Chem. **25**:1656–1661.
23. **Shayegani, M.** 1970. Failure of immune sera to enhance significantly the phagocytosis of *Staphylococcus aureus*: non-specific adsorption of phagocytosis-promoting factors. Infect. Immun. **2**:742–749.
24. **Tipper, D. J., J. L. Stominger, and J. C. Ensign.** 1967. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. VII. Mode of action of the peptidase from Myxobacter and the isolation of intact cell wall polysaccharide. Biochemistry **6**:906–920.
25. **Weber, P., and R. H. Winzler.** 1969. Determination of hexosaminitols by ion-exchange chromatography and its application to alkali-labile glycosidic linkages in glycoproteins. Arch. Biochem. Biophys. **129**:534–538.
26. **Yoshida, K., M. R. Smith, and Y. Naito.** 1970. Biological and immunological properties of encapsulated strains of *Staphylococcus aureus* from human sources. Infect. Immun. **2**:528–532.