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Staphylococcus aureus Cell Surface: Capsule as a Barrier to Bacteriophage Adsorption

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Encapsulated *Staphylococcus aureus* strains M and Smith diffuse bound phage 84 and 52A much less efficiently than their unencapsulated counterparts, M variant and Smith compact. It is proposed that the capsule acts as a barrier excluding phage from interaction with their receptor (cell wall peptidoglycan and teichoic acid). Inefficient phage adsorption by encapsulated staphylococci may explain, in part, the poor phage typing of such strains.

The bacterial cell surface is an important determinant in the interaction of pathogenic bacteria with their hosts. For example, encapsulated bacteria are often more virulent than unencapsulated strains, and this is correlated with the ability of encapsulated organisms to "resist phagocytosis" (8, 11). In the case of *Staphylococcus aureus,* it is believed that phagocytosis of this organism is mediated by cell wall-directed opsonins present in normal human serum. More specifically, we have provided evidence that peptidoglycan is the key cell wall component promoting opsonization of *S. aureus* (10). Furthermore, we have proposed that encapsulated *S. aureus* strains do not become opsonized in the absence of type-specific antibodies because capsular material, external to the cell wall, masks the peptidoglycan (18).

The cell wall is also the receptor for *S. aureus* bacteriophages, and both peptidoglycan and teichoic acid contribute to the phage receptor site (2, 3). Often, the susceptibility of a bacterium to bacteriophage infection is primarily dependent on whether or not the bacteriophage can attach to specific receptors on the cell (7). However, in general this does not appear to be the basis of phage resistance in *S. aureus* because strains of this species inactivate typing phages irrespective of the ability of the phages to lyse these strains (12). Although the phage type of some encapsulated staphylococci has been reported (15-17) there have been a number of reports of the lack of susceptibility of encapsulated strains to bacteriophage (14, 20, 21). Hence, the present investigation was undertaken to see whether encapsulated *S. aureus* strains showed reduced ability to adsorb bacteriophage. *S. aureus* bacteriophages adsorbed only poorly to encapsulated organisms, and this indicates that the capsule excludes the phage from interaction with the cell wall of these organisms.

S. aureus strains M and Smith diffuse (encapsulated) and M variant and Smith compact (unencapsulated) were kindly provided by M. A. Melly, Vanderbilt University School of Medicine, Nashville, Tenn. The M strain is an encapsulated organism which produces a capsule visible in India ink preparations (13) or by electron microscopy after incubation in *S. aureus* M-immune rabbit serum (8). The M variant strain, which arose spontaneously as a nonmucoid colony on agar, does not produce a frank capsule (see reference 18 for electron micrographs) although it has some properties typical of encapsulated staphylococci, namely, clumping factor negative, diffuse in serum soft agar, mouse lethal, and agglutinable with *S. aureus* M-immune rabbit serum (M. A. Melly, personal communication). Strain Smith diffuse has been used as a prototype encapsulated, mouse-virulent staphylococcus (5, 8), and Smith compact is a relatively avirulent, colonial variant lacking capsular antigen. *S. aureus* phages 52A, 84, and 187 and their respective propagating strains of the international set of human *S. aureus* phages were used.

Bacteria were grown overnight in Trypticase soy broth (Baltimore Biological Laboratory, Cockeysville, Md.) supplemented with $CaCl_2$ (100 μg/ml). Live or heat-killed (60°C for 30 min) cells were used in phage binding studies. Irreversible phage adsorption was measured at 37°C by incubating phage lysate (0.1 ml, 10^6 plaque-forming units) with 0.9 ml of bacterial suspension (5×10^8 colony-forming units). Samples (0.1 ml) were removed at intervals and immediately diluted 100-fold in Trypticase soy

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broth-$CaCl_2$ broth to stop further adsorption, and which also permits elution of reversibly bound phage (1, 4). Samples (0.1 ml) of appropriate dilutions were assayed for plaque-forming units by mixing with 0.1 ml of live broth culture of the homologous propagating strain in 3 ml of molten soft agar-Trypticase soy broth-$CaCl_2$ supplemented with 0.7% (wt/vol) agar (Difco Laboratories, Detroit, Mich). This was overlayed on a nutrient agar plate (Difco), allowed to solidify, and incubated overnight at 30°C. Reversible phage adsorption was assayed by removing bacteria by centrifuging before determining plaque-forming units in the supernatant. Incubation mixtures (1 or 2 ml) were placed in the centrifuge (RC-5, Dupont Instruments, Sorvall, Newtown, Conn.) in the SS34 head and maximally accelerated to 39,000 × *g* and were held at this force for 1 min.

The M variant strain is susceptible to phage 84, whereas the M strain is not (M. A. Melly, personal communication). When the susceptibility of these strains to phages 52A, 84, and 187 was determined as in routine phage typing, only the M variant strain was found typable by phage 84, thus confirming Melly's observation. Figure 1a shows the irreversible adsorption of phage 84 by heat-killed M and M variant strains and propagating strain (PS) 84. The phage bound efficiently to the M variant strain but poorly to the encapsulated M strain. After 10 min the M variant had reduced the phage titer to 0.3% of the original, whereas 27% of the phage remained after incubation with the M strain. This suggests that the capsule acts as a barrier to binding of the phage to the cell wall, the presumed receptor site of phage 84. In the case of phage 52A, it has been well established that cell wall peptidoglycan and teichoic acid are integral components of its receptor (2). Figure 1b shows efficient adsorption of phage 52A to the unencapsulated M variant and Smith compact strains, even though these organisms are not lysed by this phage, but poor binding to the two encapsulated strains. This is again consistent with the idea that the capsule prevents the interaction of the phage with its receptor. With the encapsulated strains there was an initial fall in phage titer (measured after 15 min) with no further decrease on prolonged incubation. In fact, the phage titer appears to rise slightly with the encapsulated organisms. These observations (Fig. 1a and b) raise the possibility that the fall in phage titer observed with the encapsulated strains represents a nonspecific phenomenon.

When binding of phage 84 to live M and M variant strains was measured (data not shown) adsorption was very similar to that when heat-killed cells were used (see Fig. 1a). This indicates that heating does not impair the ability of the capsule to interfere with binding and argues against the unlikely possibility that defective binding to the M strain is due to selective destruction of a heat-labile receptor. The titer did not fall when phage 84 was incubated with the cell-free supernatant of heat-killed M or M variant strains. This indicates that there is no phage-inactivating activity in heated, spent medium which could explain the fall in phage titer.

S. aureus bacteriophage 187 is specific for certain unusual strains of this species which have *N*-acetyl-D-galactosamine residues in their wall teichoic acid rather than the more usual *N*-acetyl-D-glucosamine residues (6). When adsorption of phage 187 was measured (Fig. 1c), there was an apparent decrease in phage titer of about 50% with all four strains at 15 min. The phage titer increased on further incubation. These observations were similar to those made with the encapsulated strains and phage 52A (Fig. 1b). There was no difference in adsorption of phage 187 to encapsulated versus unencapsulated strains. This is presumably because the strains lack *N*-acetyl-D-galactosamine residues in their teichoic acids (cell wall analysis of M strain revealed no galactosamine [18]). These results suggest that the apparent decline in phage titer observed with phages 84 and 52A and the encapsulated strains represents a nonspecific artifact of the experimental system. Alternative explanations would be the presence of unencapsulated cells in the population of the encapsulated strain or partial penetration of the capsule by the phage. Capsule production by M strain is a stable characteristic, and populations of this strain are not grossly heterogeneous, because Smith et al. (14) found only two unencapsulated colonies on screening 19,087 M strain colonies.

Bacteriophage adsorption is thought to be a two-step process passing through a reversible phase before irreversible adsorption occurs (1, 4). Reversible phage adsorption can be assayed by centrifuging the mixture of bacteria and phage before determining plaque-forming units in the supernatant. Reversible phage adsorption to the M and M variant strains was measured (Fig. 1d) to check whether or not the capsule was allowing reversible but not irreversible adsorption. Although the phage titer was decreased somewhat more by both strains in reversible compared with irreversible adsorption (Fig. 1a), phage binding to the M strain was poor, whereas the M variant bound phage efficiently.

In summary, the *S. aureus* capsule interferes with both reversible and irreversible bacteriophage adsorption by the organism. This then is a possible explanation for the high incidence of

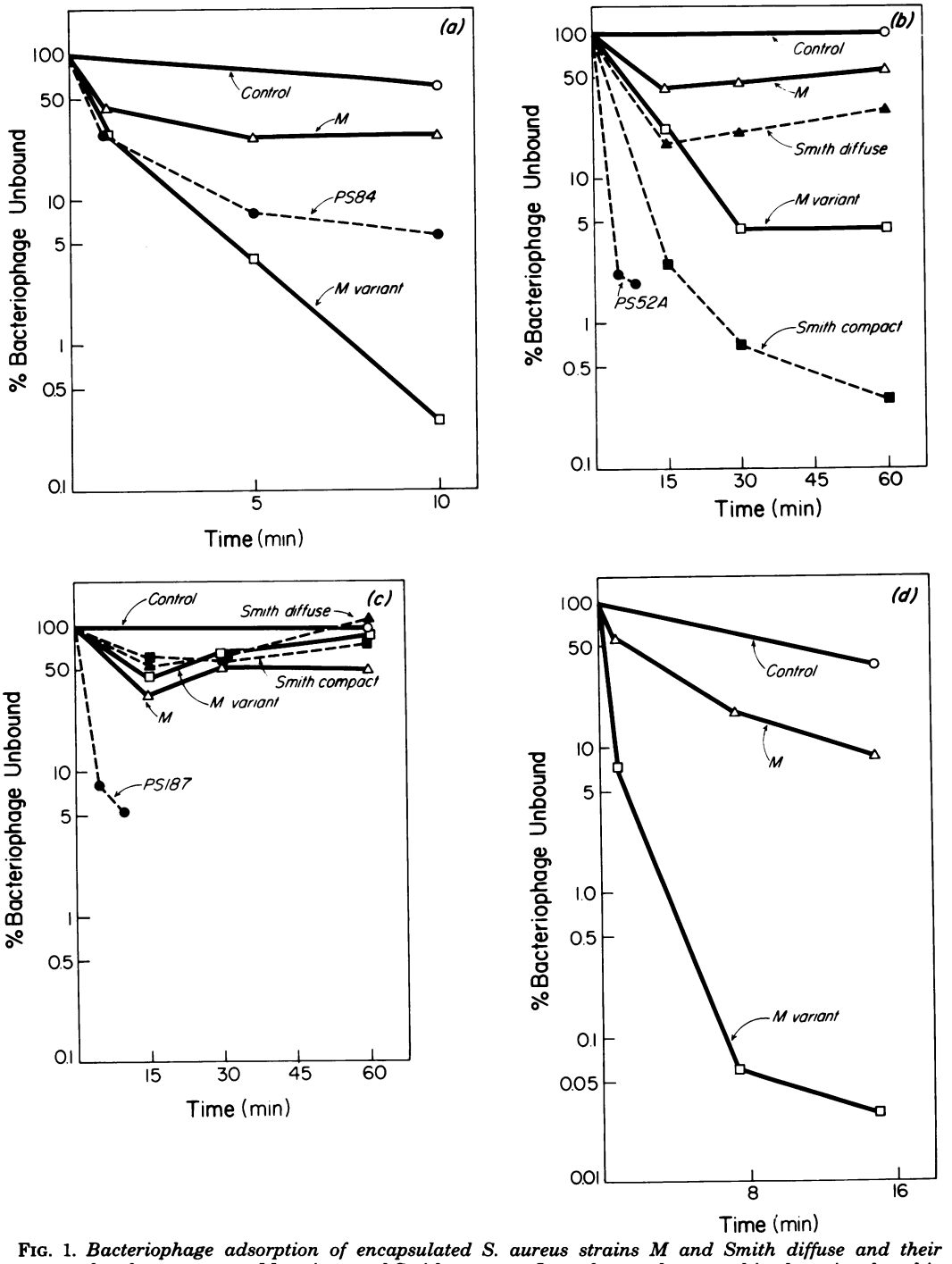

FIG. 1. *Bacteriophage adsorption of encapsulated S. aureus strains M and Smith diffuse and their unencapsulated counterparts M variant and Smith compact. In each case the control is phage incubated in Trypticase soy broth-$CaCl_2$ without bacteria. In (a), (b) and (c) adsorption of the phage by the homologous propagating strain is shown for comparison. (a) Irreversible adsorption of phage 84 by heat-killed cells of strains M, M variant and PS 84. (b) Irreversible adsorption of phage 52A by heat-killed cells of strains M, M variant, Smith diffuse, Smith compact, and PS 52A. (c) Irreversible adsorption of phage 187 by heat-killed cells of strains M, M variant, Smith diffuse, Smith compact, and PS 187. (d) Reversible adsorption of phage 84 by heat-killed cells of strains M and M variant.*

non-phage typability in encapsulated *S. aureus* isolates. The capsule appears to mask the bacteriophage receptor, the cell wall, from interaction with the phage. A similar conclusion has been reached with a phage-resistant mutant of *S. aureus* H, selected through being non-agglutinable with anti-teichoic acid antibody, which produces a cell surface polymer in addition to the normal wall teichoic acid (9). This strain does not, however, produce a classical capsule. From the experiments reported here, it seems possible that the capsule acts as a barrier to phage adsorption by excluding phage from penetration through this layer. If this were so, it might indicate that the physical nature of the surface polysaccharide is such that it is impermeable to a particle the size of a phage. The capsule, however, appears to pose no penetration problems to a small-molecular-weight protein preparation since the organism is readily lysed by lysostaphin (8, and confirmed by B. J. Wilkinson, unpublished data), which specifically dissolves staphylococcal cell walls. We favor the idea that the capsule excludes the bacteriophage and that the fall in phage titer observed with the encapsulated cells is not due to entrapment of phage in the capsule layer. Detailed electron microscopic examination might be used to more definitively resolve this issue.

However, Wiley and a co-worker (15–17) have reported the phage type of several *S. aureus* strains shown to be encapsulated by the specific capsular reaction test which involves light microscopic examination of organisms incubated overnight with homologous antiserum and methylene blue. Possible explanations to account for this include heterogeneous populations of organisms resulting in phage infection of unencapsulated cells and production of capsule lytic enzymes by the phage (19). This latter possibility does not appear to have been extensively investigated, if at all, in *S. aureus*. However, a proportionality has been reported between capsule thickness and phage resistance in *Escherichia coli* (see reference 19), and Wilkinson (19) implies that the phage head, but not the tail, may be excluded by the capsule. Perhaps the test for encapsulation used by Wiley is a particularly sensitive indicator of the presence of additional surface antigens. Interestingly, Wiley and Maverakis (17) reported that a heavily encapsulated strain of *S. aureus* was highly virulent and not phage typable.

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