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Chemotaxigenesis by Encapsulated *Staphylococcus aureus* M

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Although encapsulated *Staphylococcus aureus* M is not opsonized by normal human serum, as a chemotaxigen this organism behaved similarly to an unencapsulated variant strain. For optimal chemotaxigenesis, an intact classical complement pathway was required, and C5a appeared to be the major chemotaxin.

Acute infection in humans is caused by a variety of pathogenic species of bacteria, many of which possess capsules or other surface factors which allow them to resist phagocytosis (19). Resistance to phagocytosis has previously been shown to be a characteristic of encapsulated *Staphylococcus aureus* strains (8, 9).

When staphylococci gain access to susceptible tissue, a complex sequence of events is triggered, involving both humoral and cellular components, and an acute inflammatory response is observed. One of the initial events in this host response entails the directional migration of phagocytic cells, i.e., chemotaxis, into the area of infection. Once phagocytic cells have arrived at the site of developing infection, the next crucial step in the process of elimination of the infecting organisms is opsonic recognition. The opsonization process facilitates phagocytosis and thus, finally, the destruction of the invading organisms.

Recent work in this laboratory has confirmed the observation that encapsulated staphylococci are able to resist phagocytosis, and this phenomenon has been attributed to ineffective opsonization of the encapsulated organisms by the serum complement (C) system in the absence of specific anticapsular antibodies (13). Encapsulated staphylococci have, however, been shown to be as efficient in activating C as their unencapsulated counterpart strains (12). The serum C system has recently been shown by Russell and his colleagues (14) to be the chief mediator of neutrophil chemotactic responsiveness to staphylococcal infection. Cell wall peptidoglycan (5, 6, 15) and other staphylococcal cell wall components (15) have been shown to behave as chemotaxigens, presumably by activating the serum complement system and thus generating the highly chemotactically active fragment C5a. It was of interest, therefore, to determine whether encapsulated and unencapsulated *S. aureus* strains were capable of the same degree of chemotaxigenesis, or whether the capsule may in some way mask the cell wall components previously shown to mediate chemotaxigenesis and thus allow the encapsulated organisms to act as "silent invaders" when causing infection in humans.

By using a previously described system for determining bacterial chemotaxigenesis (15), bacteria were incubated with fresh human serum, and this serum was removed and assayed for chemotactic activity. Briefly, the sera activated by bacteria were added in a 10% concentration to the lower compartment of a blind well chamber, and a 5-μm pore size membrane filter (Millipore Corp.) was inserted, followed by addition of 8×10^5 normal polymorphonuclear leukocytes to the upper compartment. The chambers were incubated for 2 h at 37°C, and the filters were then removed, fixed, and stained. Generation of chemotactic activity in serum was expressed as a chemotactic index: the ratio of the polymorphonuclear leukocytes having migrated into the filter when the attractant was test serum to the polymorphonuclear leukocytes having migrated into the filter when the attractant was control serum (i.e., serum incubated with buffer in place of bacteria). The encapsulated *S. aureus* M strain and its unencapsulated counterpart, *S. aureus* M variant, were studied (both strains were kindly provided by M. A. Melly, Vanderbilt School of Medicine, Nashville, Tenn.). The first step was to compare the encapsulated and unencapsulated strains, both living and heat killed, in this assay system using various inoculum sizes incubated for 60 min in normal pooled human serum. The results (Fig. 1) indicate that there was essentially no difference between the two strains, and there was little difference between living and heat-killed organisms in their chemotactigenic capacities, suggesting that extracellular bacterial factors play a minor role in chemotaxigenesis in this test system. It is also apparent from this figure that at least 10^9 organisms must be incubated in 1 ml of normal pooled human serum to produce sig-

nificant chemotactic activity. This inoculum size was therefore used for the remainder of this investigation.

Other investigators (4, 10) have emphasized the importance of studying the kinetics of chemotaxigenesis to determine whether the more rapid classical or the somewhat delayed alternative C pathway is being activated. Kinetic studies were therefore performed, using three serum sources: undiluted normal pooled human serum in which both the classical and alternative C pathways were intact, normal serum which had been treated with the chelating agent ethylene glycol-bis(β-aminoethyl ether)N,N^1-tetraacetic acid (EGTA) (10 mM with respect to serum) in the presence of $MgCl_2$ (1 mM with respect to serum) to block the classical pathway (2, 3), and C2-deficient serum obtained from a patient with a genetically determined complete and selective absence of C2, as assessed by hemolytic assays and by Ouchterlony analysis employing specific anti-C2 antibody (7). The kinetics of chemotaxigenesis by *S. aureus* M and M variant are very similar when normal pooled human serum is used, and chemotaxigenesis by both strains occurs at a slower rate and is not as extensive in either MgEGTA-treated or C2-deficient serum (Fig. 2). These kinetic results indicate a need for an intact classical C pathway for optimal chemotaxigenesis by either strain.

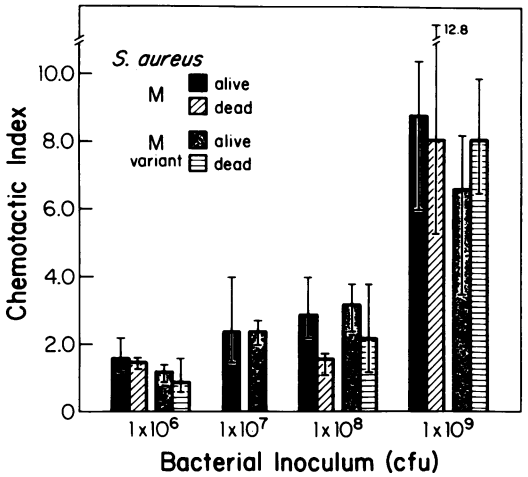

FIG. 1. *Dose response of chemotaxigenesis. Living and heat-killed organisms were tested at the indicated concentrations by incubation in 1 ml of normal serum for 60 min at 37°C. Each value represents average of three experiments with indicated ranges.*

Synderman and his colleagues (16, 17) have pointed out that the major neutrophil chemotactic factor resulting from in vitro C activation is a heat-stable, low-molecular-weight (17,500 daltons) cleavage product of the fifth component of complement-designated C5a. We have previously suggested that C5a is the major chemotaxin formed upon incubation of various staphylococcal cell wall components in human sera (15). This proposal was based on evidence of the presence of C5a derived from granulocyte aggregometry studies (1, 11) where the aggregating activity was found to closely parallel chemotactic activity of the same serum. Through the generosity of R. McLean, Department of Pediatrics, University of Connecticut, Farmington, we had the fortune of obtaining serum from a patient with a selective absence of C5 for use in the current study. The assumption was made that if C5a is the major chemotaxin generated

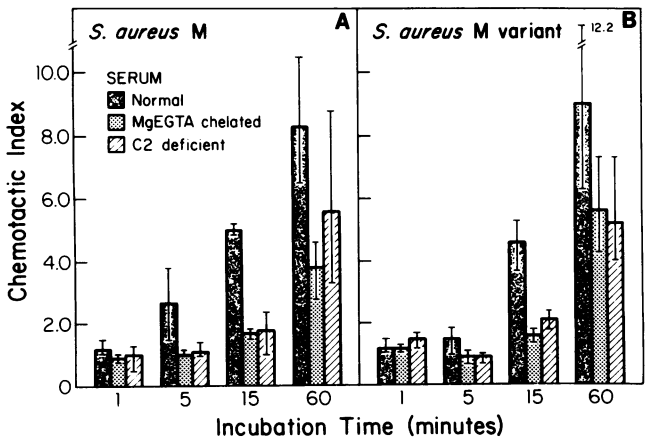

FIG. 2. *Comparison of the kinetics of chemotaxigenesis elicited by encapsulated S. aureus M (A) or unencapsulated S. aureus M variant (B) in normal, MgEGTA-chelated, or C2-deficient serum. Chemotaxigenesis was measured after incubation at 37°C for the indicated times. Values represent averages of three experiments.*

in serum by the two staphylococcal strains, then there should be no chemotaxigenesis by either strain in C5 deficient serum. This was found to be the case (Fig. 3A). There was, again, good chemotaxigenesis by both bacteria in normal pooled human serum, but no chemotaxigenesis was elicited by either strain when incubated in C5 deficient serum. This observation provides strong support for the proposal that C5a is the chemotactic factor generated in normal serum by these bacteria.

It was also of interest to assess the opsonic capacity of the C5-deficient serum used in the above experiment, since if C3b is the major C component involved in opsonic recognition (18), opsonization by this serum should be similar to that of normal serum. Opsonization studies were carried out by using a previously described method (13) in which 1×10^8 colony-forming units of ^{3}H-labeled *S. aureus* M and M variant strains were incubated for 60 min in 1 ml portions of undiluted serum before being added to polymorphonuclear leukocytes and determining leukocyte uptake at 12 min. The unencapsulated M variant strain was opsonized equally well in C5 deficient and normal serum, whereas the encapsulated M strain was, as expected, not effectively opsonized in either serum (Fig. 3B).

In summary, the results of this study indicate that although encapsulation markedly inhibits opsonic recognition of *S. aureus* M when undiluted normal serum is used as an opsonic source,

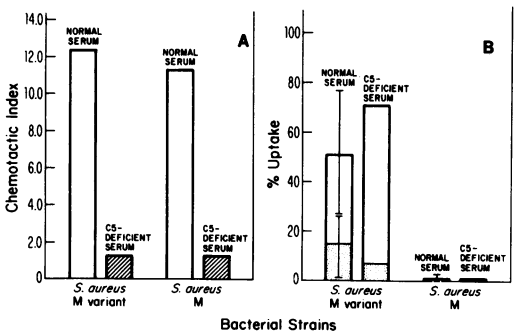

FIG. 3. *Comparison of chemotaxigenesis (A) and opsonization (B) of both staphylococcal strains by normal and C5-deficient serum. Chemotaxigenesis in 1 ml of undiluted serum was measured after 60 min incubation with staphylococci, and opsonization of this bacterial inoculum was assessed by measuring phagocytic uptake at 12 min of incubation with polymorphonuclear leukocytes. Shaded bars in panel B indicate heat-inactivated (56°C, 30 min) serum. Vertical lines indicate the means and ranges of phagocytosis experiments with serum from eight individual healthy donors. Values shown are representative of experiments run on at least 3 separate days.*

the capsule does not significantly interfere with chemotactic recognition generated in normal serum. Optimal chemotaxigenesis requires an intact classical pathway, and C5a appears to be the major chemotaxin produced upon the interaction of staphylococci with the serum C system.

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