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Influence of the Alternate Complement Pathway on Opsonization of Several Bacterial Species

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In normal human serum chelated with magnesium (10 mM) and ethylene glycotetraacetic acid (10 mM) (MgEGTA), the classical pathway of complement activation is blocked; however, the alternate pathway of complement activation is intact. *Diplococcus pneumoniae*, *Staphylococcus albus*, *Streptococcus viridans*, *Streptococcus faecalis*, and *Serratia marcescens* were opsonized in normal human serum containing 10 mM MgEGTA. In contrast, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were poorly opsonized in human serum chelated with 10 mM MgEDTA. Therefore certain bacterial species appear to require the classical pathway of complement activation to be opsonized and other bacterial species utilize the alternate pathway of complement activation.

Heat-labile opsonic activity is present in serum from patients with agammaglobulinemia (7) and C2 deficiency (5) and in serum depleted of Cl_q (4), suggesting that complement components activated via the alternate pathway are opsonins for bacteria. *Escherichia coli* were opsonized in these sera, but the opsonization of *Staphylococcus aureus* was decreased in Cl_q-depleted sera, suggesting that *S. aureus* cannot activate heat-labile opsonins via the alternate pathway and may depend on classic complement components for this activation.

It has been reported that 10 mM ethylene glycotetraacetic acid (EGTA) and 10 mM $MgCl_2$ (10 mM MgEGTA) completely blocks the classical complement system in normal sera through binding necessary calcium ions, but does not inhibit the alternate pathway which requires magnesium ions but not calcium ions for activation (1). Recent investigations in this laboratory have confirmed these observations. When serum was chelated with 10 mM MgEGTA, there was no inhibition of the activation of properdin, C3PA, and C3 by cobra venom factor, endotoxin, inulin, and zymosan (A. Forsgren, R H. McLean, A. F. Michael, and P. G. Quie, submitted for publication). We have also observed that when the classical pathway was inhibited by chelation of calcium with 10 mM MgEGTA, *E. coli* were opsonized normally, but no opsonization of *S. aureus* occurred (2). These findings support the observation cited above that the alternate pathway may be involved in the opsonization of *E. coli*, whereas opsonization of *S. aureus* requires an intact classical pathway for complement activation to provide heat-labile opsonins.

We investigated the requirement of classical and alternate pathway for opsonization of a variety of bacterial species.

Normal human serum pooled from five donors and kept frozen at −70 C until just before use was used as the opsonic source. Normal human polymorphonuclear leukocytes were used in all experiments. Hanks balanced salt solution without calcium and magnesium was used for serum dilutions and cell suspension. Phagocytosis studies were performed according to Maaløe (6) as modified by Hirsch and Strauss (3). Bacterial species studied were *Diplococcus pneumoniae* (type 25, kindly obtained from R. Austrian and type 7 isolated from patient B.W.), *Staphylococcus albus* (Baird-Parker biotype 1), *Streptococcus viridans*, *Streptococcus faecalis*, *Serratia marcescens*, and *Pseudomonas aeruginosa* strains freshly isolated from patients.

Control experiments were performed by using as opsonic source serum chelated with 10 mM ethylenediaminetetraacetic acid (EDTA) instead of 10 mM MgEGTA; i.e., both the classical and the alternate complement systems were blocked. In those experiments no phagocytosis and killing of any of the bacteria tested could be detected, showing the importance of complement for heat-labile opsonization. The possibility of a difference between the organisms stud-

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ied in their capacity to inactivate complement was investigated by measuring hemolytic complement levels (CH 50) in serum after adsorption with the organisms (10^9 to 10×10^9 bacteria per ml of serum). It was demonstrated that *D. pneumoniae*, *Staphylococcus albus*, *Streptococcus faecalis*, *Streptococcus viridans*, and *Serratia marcescens* completely inactivated complement in serum chelated with 10 mM MgEGTA, as earlier has been shown for *E. coli* (2). However, for the strains of *P. aeruginosa* tested, 10 times or more bacteria was required to completely inactivate complement in serum chelated with 10 mM MgEGTA as in unchelated serum. Similar results have earlier been demonstrated for *Staphylococcus aureus* (2).

Figure 1a shows a typical experiment with *D. pneumoniae* type 25. With a 4% serum concentration in the phagocytosis system, approxi-

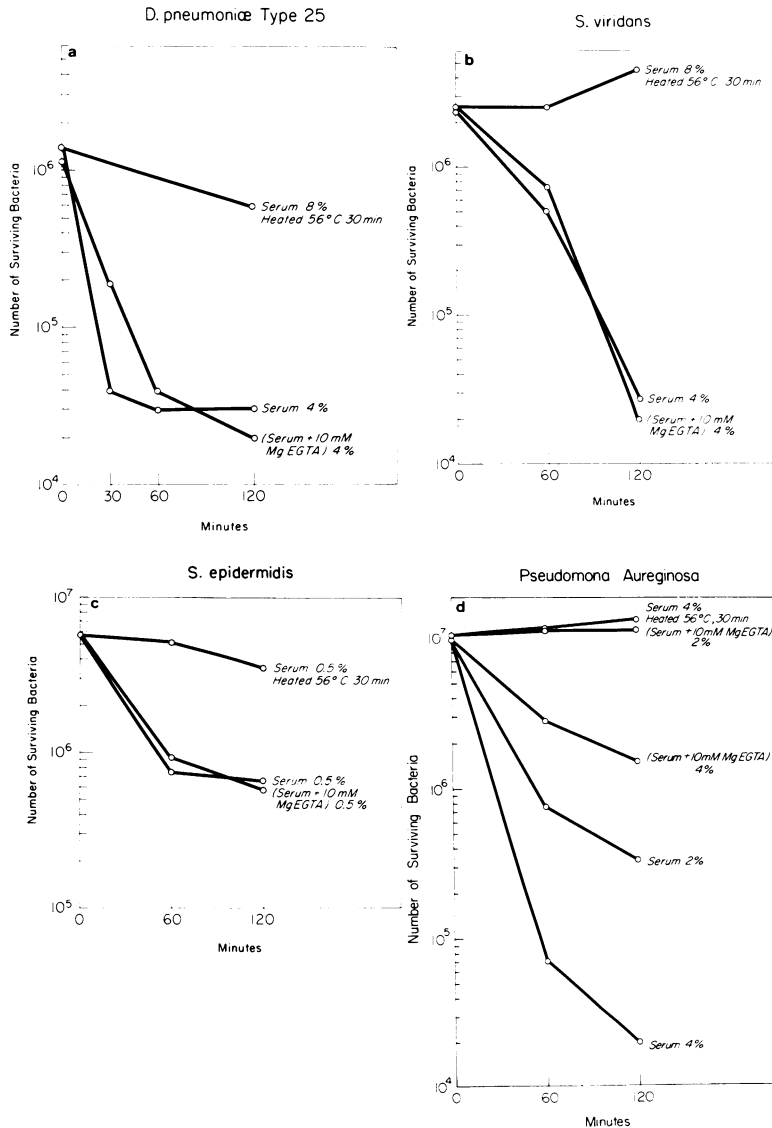

FIG. 1. *Effect of chelated and nonchelated serum. Heat-labile opsonic activity in normal human serum unchelated or chelated with 10 mM MgEGTA ($MgCl_2$ and EGTA). (a) Test organism D. pneumoniae, type 25; (b) test organism Streptococcus viridans; (c) test organism Staphylococcus epidermidis; (d) test organism P. aeruginosa.*

mately 95% of the organisms were killed within 120 min after incubation. The same degree of bacterial killing was recorded when 4% serum chelated with 10 mM MgEGTA was used as an opsonic source. Only a slight killing of the pneumococci was found when heat-inactivated serum (56 C for 30 min) was used as the opsonic source. A similar result was obtained with a strain of *D. pneumoniae* freshly isolated from a patient. Complement components activated by the alternate pathway were effective opsonins for *D. pneumoniae*.

Figure 1b shows a representative experiment with *Streptococcus viridans*. The heating of serum to 56 C for 30 min completely abolished opsonic activity; however, there was no reduction of opsonic activity in serum chelated with 10 mM MgEGTA. Similar results were obtained with *Streptococcus faecalis* and *Serratia marcescens*. Heat-labile components are activated by the alternate pathway, and there was normal opsonic activity even when the classical pathway was blocked.

Staphylococcus epidermidis strains were opsonized in chelated serum. Heat-labile opsonins activated by the alternate pathway were active as opsonins for *S. epidermidis*, and there was no opsonic activity in the heated serum at the concentration used (0.5%) (Fig. 1c).

Figure 1d shows a phagocytosis experiment with a strain of *P. aeruginosa*. Normal human serum provided sufficient opsonic activity so that greater than 99% of the organisms were phagocytized and killed within a 90-min incubation. *Pseudomonas* organisms were poorly opsonized in serum chelated with 10 mM MgEGTA, although some opsonic activity was present in chelated serum at higher concentration. The alternate pathway is not as efficient for providing heat-labile opsonins for *P. aeruginosa* as for other gram-positive and gram-negative organisms.

The alternate complement pathway appears to be important for providing heat-labile opsonins for *D. pneumoniae*, *Streptococcus viridans*, *Streptococcus faecalis*, *Staphylococcus epidermidis*, and *Serratia marcescens*. *Staphylococcus aureus* requires the classical complement components C142 for activation of heat-labile opsonins, and there is little activation of the alternate complement pathway (2). *P. aeruginosa* is in an intermediate position and may utilize both complement pathways for activation of heat-labile opsonins. It is possible that there may be differences in activation of the alternate complement pathway among strains of bacteria in the same species.

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