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Complement Activation and C3 Binding by Serum-Sensitive and Serum-Resistant Strains of *Pseudomonas aeruginosa*

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The relationship among complement consumption, C3 deposition, and C3 fragmentation pattern was compared for serum-sensitive (Ser^s) and serum-resistant (Ser^r) strains of *Pseudomonas aeruginosa*. The Ser^s strains, which were mucoid strains derived from patients with cystic fibrosis, had lipopolysaccharide deficient in O-antigen side chains. These organisms generally activated much less complement per organism than their Ser^r counterparts, characterized by the presence of lipopolysaccharide with long lipopolysaccharide O side chains. Surprisingly, however, although the Ser^r strains consumed more total hemolytic complement, less C3 was deposited onto the surface of these strains than onto that of the Ser^s strains. Maximal C3 binding required the participation of both the classical and alternative complement pathways, although classical complement pathway involvement was more important for Ser^r strains. Finally, while more than half of the C3 deposited on most Ser^s strains was in the form of C3b, most of the C3 on the Ser^r strains was in the form of iC3b, indicating a more rapid and extensive conversion of C3b to iC3b on the surface of these strains. Limited complement activation by Ser^s mucoid strains of *P. aeruginosa* may confer a selective survival advantage to these organisms in colonizing the airways of patients with cystic fibrosis.

Complement plays a central role in host defense against many bacterial pathogens. The human complement system, composed of at least 25 serum proteins which interact in a carefully regulated sequence, can be activated via either of two pathways (see reference 17 for a review). The classical complement pathway (CCP) is usually initiated by the interaction of antibody with antigen, whereas the alternative complement pathway (ACP) may be initiated in the absence of antibody by complex polysaccharides, such as the O antigen of lipopolysaccharide (LPS). Activation by either pathway leads to the generation of C3b, which binds covalently to the activating surface. Once attached, C3b serves as the impetus for the major biological consequences of complement activation, which include the following: (i) opsonization of bacteria; (ii) generation of C5a, a potent diffusible chemotaxin which can draw neutrophils to the site of complement activation, and (iii) formation of the terminal membrane attack complex (composed of C5b, C6, C7, C8, and C9), which can insert into the outer membrane of susceptible gram-negative bacteria, causing their death. Alternatively, C3b can be proteolytically degraded to iC3b, C3dg, or C3d, which cannot participate in C5a or C5b-9 generation (15). Various cell membrane complement receptors recognize these C3 cleavage fragments, but phagocytic uptake is mediated primarily via complement receptor types 1 (CR1) and 3 (CR3), which bind predominantly C3b- and iC3b-coated microorganisms, respectively (24). Therefore, the form of C3 localized on the bacterial surface determines in large part the role that complement will play in the interaction of the organism with the host.

Pseudomonas aeruginosa is an opportunistic gram-negative pathogen which causes serious infections in patients with severe burn or wound injuries, neoplastic disease, granulocytopenia, and immunological deficiencies and in patients receiving immunosuppressive chemotherapy (1). In addition, *P. aeruginosa* remains the major cause of morbidity and mortality in patients with cystic fibrosis (CF) (7). Interestingly, in contrast to strains from non-CF patients, strains of *P. aeruginosa* isolated from the sputa of CF patients are generally mucoid, nontypable, deficient in LPS O side chains, and serum sensitive (Ser^s) (13, 26, 30). Although most of these Ser^s strains are susceptible to direct complement killing mediated via either the CCP or the ACP, optimal killing requires the function of both pathways (27). Part of the bactericidal activity of pooled normal human serum (PNHS) is due to the binding of antibodies to the rough LPS on Ser^s strains, initiating activation of the CCP (27).

In contrast, *P. aeruginosa* strains recovered from infections in non-CF patients are usually nonmucoid, have LPS with varying amounts of O side chains, and are serum resistant (Ser^r). We have previously described the mechanism of serum resistance for a mucoid strain of *P. aeruginosa* bearing O side chains (144M-SR), derived from a Ser^s mucoid CF isolate (144M) by serial passage in serum (29). This approach had permitted us to control for the contribution of the mucoid capsule to the observed results. The resistance of strain 144M-SR to the bactericidal activity of PNHS did not represent a failure to activate complement efficiently, but instead reflected failure of the assembled terminal complement complex C5b-9 to insert stably into the outer membrane of this organism (31). Furthermore, the Ser^r strain 144M-SR appeared to activate complement more rapidly and efficiently than its Ser^s counterpart, strain 144M, although less C3 was found deposited on the surface of 144M-SR. In this study, we have expanded these observations by examining a variety of Ser^s and Ser^r strains and have focused on the discrepancy between complement activation and C3 deposition.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. *P. aeruginosa* mucoid strains 144M, WcM#2, ByM, P1M, P7b, P10, and P11 were originally isolated from the sputum of CF patients. Strains 144M-SR and WcM#2-SR are Ser^r mucoid derivatives of 144M and WcM#2, respectively (29). Strains Mc208, Mc209, and Mc210 are nonmucoid Ser^r isolates recovered from the blood of non-CF patients with bacteremia. Strains were stored at −70°C in 1% proteose peptone (Difco Laboratories, Detroit, Mich.) containing 20% glycerol.

Each strain was thawed, plated on brain heart infusion agar (BBL Microbiology Systems, Cockeysville, Md.), and incubated overnight at 37°C in 5% CO_2. For each experiment, strains were grown to mid-log phase (4 to 5 h) in brain heart infusion broth at 37°C with agitation, harvested by centrifugation at 10,000 × *g* for 10 min at 4°C, and washed twice with Hanks balanced salt solution containing 0.40 g of KCl, 0.06 g of KH_2PO_4, 0.35 g of $NaHCO_3$, 0.048 g of Na_2HPO_4, 1.0 g of D-glucose, and 8.0 g of NaCl per liter of distilled H_2O, pH 7.4. The washed bacteria were then suspended in Hanks balanced salt solution containing 1.0 mM $MgCl_2$ and 0.15 mM $CaCl_2$ to the desired bacterial concentration (determined spectrophotometrically at an optical density of 600 nm). For those experiments in which the effect of EGTA [ethylene glycol-bis(β-aminoethyl ether)*N*, *N*,*N*′,*N*′-tetraacetic acid] was being examined, bacteria were suspended in Hanks balanced salt solution.

Serum. Blood was obtained by venipuncture of 20 to 30 normal healthy adult volunteers and was allowed to clot at room temperature for 20 min. After centrifugation at 1,000 × *g* for 15 min at 4°C, the serum was pooled, filter sterilized through a 0.22-μm-pore-size filter, and stored at −70°C in small amounts until use (PNHS).

Measurement of complement activity. Various concentrations of log-phase bacteria were incubated with equal volumes of PNHS at 37°C in a shaking water bath (incubation periods were defined by the experimental protocol). After removal of the bacteria by centrifugation at 15,000 × *g* and filtration through a 0.22-μm-pore-size filter, the residual complement hemolytic activity of the supernatants was determined by a micromodification of the 50% hemolytic titration for complement (CH_{50}) test (6). Complement consumption by these bacterial preparations was determined by comparison with the units of CH_{50} in the control tube (PNHS without bacteria). The percentage of complement consumed was calculated as $[1 - (CH_{50}\ \text{test}/CH_{50}\ \text{control})] \times 100\%$.

Determination of C3 binding. C3 was purified from fresh human plasma by using modifications of the procedure published by Hammer et al. (12) and was radiolabeled with ^{125}Na by using Iodobeads (Pierce Chemical Co., Rockford, Ill.) to a specific activity of 3.75×10^5 cpm/μg. For these experiments, approximately 3×10^8 to 5×10^8 log-phase bacteria were incubated at 37°C with 1.5 μg of ^{125}I-labeled C3 in 20% PNHS, 20% PNHS containing 2 mM Mg^{2+} and 10 mM EGTA, or 20% PNHS preheated to 56°C for 30 min. At designated intervals, samples were removed and quantitation of C3 binding was measured as described previously (19, 31).

Examination of the form of C3 on the bacterial surface. In these experiments, approximately 5×10^8 bacteria were incubated in 20% PNHS with 2 μg of ^{125}I-labeled C3 at 37°C. (For these studies, C3 was obtained from Cytotech, San Diego, Calif., and radiolabeled as described earlier.) After 30 min of incubation at 37°C, the bacteria were pelleted by centrifugation and washed three times in Hanks balanced salt solution with 1.0 mM $MgCl_2$ and 0.15 mM $CaCl_2$ and containing a 25 μM concentration of the serine esterase inhibitor *p*-nitrophenylguanidino benzoate (Sigma Chemical Co., St. Louis, Mo.). The bacterial pellet was then solubilized by boiling in 1% sodium dodecyl sulfate (SDS) for 5 min. After removal of the unsolubilized material, the solubilized supernatant was incubated in 1 M NH_2OH in 0.2 M Tris, pH 10, for 30 min at 37°C to cleave the oxyester linkage between C3 and its acceptor molecule (21). The mixture was then dialyzed overnight at 4°C against absolute methanol to precipitate released proteins, and the precipitate was pelleted, dissolved in SDS sample buffer (2% SDS, 10% glycerol, 62.5 mM Tris, pH 6.8, 0.002% bromphenol blue, 4% 2-mercaptoethanol), reboiled for 5 min, and then electrophoresed through a 10% SDS-polyacrylamide slab gel. The samples were electrophoresed at 30 mA per gel through the 3% stacking gel and then at 50 mA per gel through the resolving gel. After drying, the gels were examined by autoradiography, using XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) with Cronex Lightning-Plus intensifying screens (Du Pont Co., Wilmington, Del.).

RESULTS

Complement activating ability of Ser^s and Ser^r strains. Previous studies have shown that mucoid strains 144M, WcM#2, ByM, P1M, P7b, P10, and P11 are sensitive to the bactericidal activity of 10% PNHS (27). These strains are typical of most isolates from CF sputum in that they contain relatively few LPS O side chains (28, 29; N. L. Schiller, unpublished observations). In contrast, mucoid strains 144M-SR and WcM#2-SR are resistant to the effects of 50% PNHS and contain LPS with long O side chains (29). Similar studies have demonstrated that the nonmucoid bacteremic isolates Mc208, Mc209, and Mc210 are also serum resistant and contain LPS with long O side chains (data not shown). These strains were used to directly compare the extent of complement activation by Ser^s and Ser^r *P. aeruginosa* strains.

For these experiments, various concentrations of log-phase bacteria were incubated in 50% PNHS for 60 min at 37°C, after which the residual CH_{50} activity of the serum was determined and compared with that of 50% PNHS incubated without bacteria. With the exception of strain Mc209, each of the other four Ser^r strains (dashed lines) activated more complement per organism than the Ser^s strains (solid lines) (Fig. 1). Strains Mc208, 144M-SR, and WcM#2-SR were particularly effective in activating complement compared with all other strains examined. The data from these experiments were used to estimate the number of Ser^s and Ser^r bacteria needed to consume 50% of the complement present in 50% PNHS. Whereas strains Mc208, 144M-SR, and WcM#2-SR required $<2.5 \times 10^8$ bacteria, strains Mc209, Mc210, and P10 required between 2.5×10^8 and 5.0×10^8 bacteria, and the remaining six Ser^s strains needed $>6.0 \times 10^8$ bacteria to consume 50% of the available complement in 50% PNHS. These results show that the Ser^r strains generally activate complement more effectively than the Ser^s strains.

Comparison of the activation and binding of C3 to the cell surface of Ser^s and Ser^r strains. During the course of an earlier study (31), a discrepancy had been detected between the activation and binding of C3 on strains 144M and 144M-SR. To explore this apparent discrepancy further and to examine additional strains, the following experiment was conducted. Each strain was incubated at 37°C with ^{125}I-

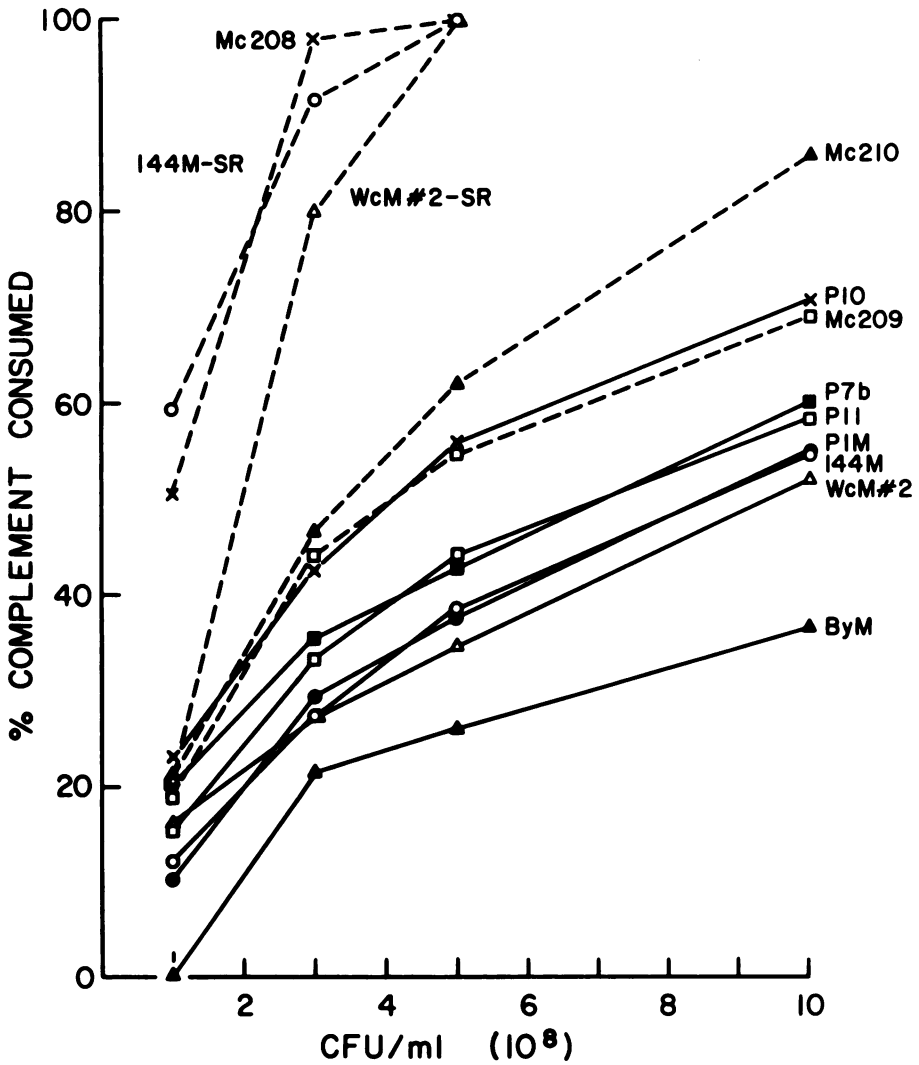

FIG. 1. Comparison of complement activation by Ser^s (solid lines) and Ser^r (dashed lines) *P. aeruginosa* strains. Various concentrations of log-phase bacteria were incubated with 50% PNHS for 60 min at 37°C, after which residual CH_{50} activity was determined and compared with that of 50% PNHS incubated without bacteria. The results are expressed as percent complement consumed {[1 − (CH_{50} units in test serum/CH_{50} units in control serum)] × 100%}.

labeled C3 in 20% PNHS, 20% PNHS containing 2 mM Mg^{2+} and 10 mM EGTA (MgEGTA) (which blocks the CCP), or 20% PNHS heated to 56°C for 30 min (which inactivates complement; this heated serum served as a control for nonspecific C3 binding, which was 0.3 to 0.6% of total input counts). At indicated times, samples were removed and specific C3 binding per CFU was determined by subtracting binding in the control tube from binding in the experimental tubes (Fig. 2). In most cases, C3 deposition occurred more slowly and was less extensive in PNHS containing MgEGTA (dashed lines) than in that without MgEGTA (solid lines), indicating the importance of a functioning CCP in the rapid activation or binding of C3 by these strains. The role of the CCP in C3 deposition varied from strain to strain but appeared more pronounced for the Ser^r strains, in which the average number of C3 molecules per CFU after 60 min in MgEGTA-treated serum was only 50.0% (range, 19.3 to 89.5%) of that seen with PNHS. Some Ser^r strains, such as Mc208 and Mc210, had <25% of the C3 bound in MgEGTA-treated serum compared with PNHS. In contrast, after 60 min of incubation, the Ser^s strains had an average of 91.4% (range, 66.9 to 127.6%) as much C3 bound in MgEGTA-treated serum as in PNHS (Fig. 2). In addition, the kinetics of C3 activation or deposition on most Ser^s strains was more rapid than that on most Ser^r strains (the exceptions being P1M and 144M-SR).

There is not a direct relationship between depletion of CH_{50} and C3 binding by these strains (Table 1). Rather, most Ser^r strains bind less C3 than most Ser^s strains despite consuming more total hemolytic complement.

Form of C3 bound to the surface of Ser^s and Ser^r strains. For these experiments, each strain was incubated in 20% PNHS with ^{125}I-labeled C3 at 37°C for 30 min, and the bacteria with C3 attached were then pelleted by centrifugation. Each bacteria-C3 pellet was then treated with hydroxylamine, which is known to break the oxyester bond between activated C3 and its bacterial acceptor (21). The proteins in the hydroxylamine-treated samples were then precipitated, solubilized, subjected to SDS-polyacrylamide gel electrophoresis, and autoradiographed, as described in Materials and Methods. The results are shown in Fig. 3.

As shown in the two end lanes, native C3 migrates as two

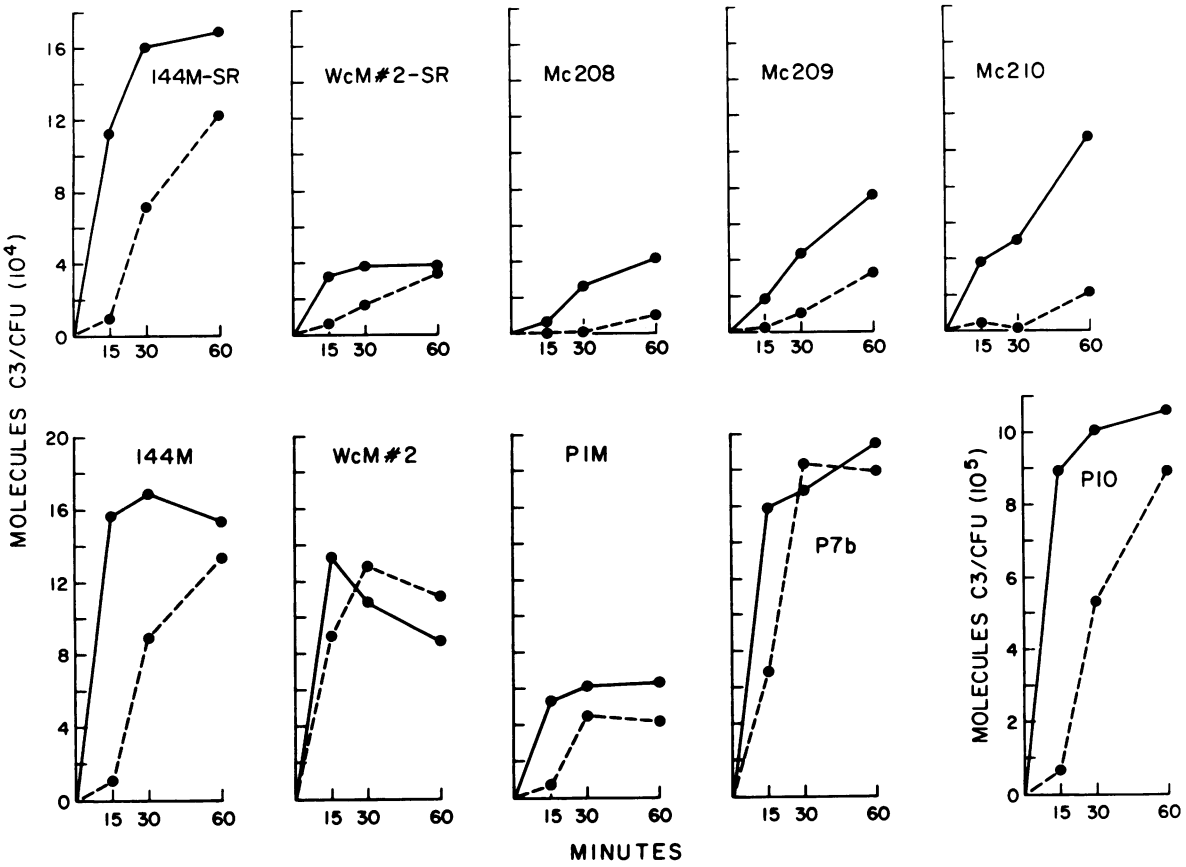

FIG. 2. Comparison of C3 binding by Ser^r (top) and Ser^s (bottom) strains incubated in PNHS with or without MgEGTA. Each strain was incubated at 37°C with ^{125}I-labeled C3 in either 20% PNHS (solid lines) or 20% PNHS containing 2 mM Mg^{2+} and 10 mM EGTA (dashed lines). At indicated times, samples were removed and specific C3 binding per CFU was determined.

distinct polypeptide bands at 120,000 (α chain) and 75,000 (β chain) daltons (Da) in SDS-polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol. Comparison of the C3 deposited onto Ser^s strains 144M, WcM#2, P10, and P7b shows similar patterns, with major bands at 110,000 (α′), 75,000 (β), and 68,000 (α'_1) Da. Conversion of C3 to C3b would produce the α′ band at 110,000 Da while keeping the 75,000-Da band intact; further cleavage of the α′ band to α'_1 (68,000 Da) and 46,000 Da is indicative of iC3b formation. (The 46,000-Da fragment would not be apparent here since it is not iodinated in C3 labeled with Iodobeads.) Densitometric tracings of these bands reveals an α'/α'_1 (representing C3b/iC3b) ratio of 1.0 to 1.8:1. In contrast, Ser^r strains 144M-SR and WcM#2-SR had α'/α'_1 ratios of 1.0:7.7 to 10.5, whereas the other Ser^r strains, Mc208, Mc209, and Mc210, had essentially no detectable α′ band. Thus, most of the C3 on the Ser^r strains was in the form of iC3b, whereas the Ser^s isolates had approximately equal amounts of C3b and iC3b.

TABLE 1. Comparison of complement activation and C3 binding activity of Ser^s and Ser^r *P. aeruginosa* strains

Strain	% Complement consumed[a]	Molecules of C3 per CFU[b]
Ser^s		
144M	26.9	1.53×10^5
WcM#2	27.0	8.7×10^4
PIM	29.3	6.23×10^4
P7b	35.4	1.95×10^5
P10	45.0	1.06×10^6
Ser^r		
144M-SR	98.1	1.68×10^5
WcM#2-SR	80.0	3.8×10^4
Mc208	91.4	4.14×10^4
Mc209	44.3	7.57×10^4
Mc210	46.6	1.07×10^5

[a] Data are based on the incubation of approximately 3×10^8 bacteria per ml of 50% PNHS for 60 min at 37°C and represent the mean based on two or three separate experiments.

[b] After 60 min of incubation at 37°C in 20% PNHS. Data were derived from one series of experiments, using a single C3 preparation. Additional experiments with other C3 preparations gave comparable results.

PIM, another Ser^s isolate, differed from the other Ser^s isolates in two ways. First, there was an additional band at approximately 120,000 Da, which could represent either native C3 bound noncovalently to the bacteria or a C3 fragment covalently coupled to its bacterial acceptor not released by hydroxylamine. Second, the C3b/iC3b ratio for this strain was approximately 1:2.

Finally, it was noted that the hydroxylamine treatment was not equally effective for all strains. Radioactively labeled C3 appeared to be still attached to high-molecular-weight acceptors on Ser^s strains PIM and P7b and Ser^r strains Mc209 and Mc210. The identity of the C3 acceptor molecule(s) on these strains has not yet been determined.

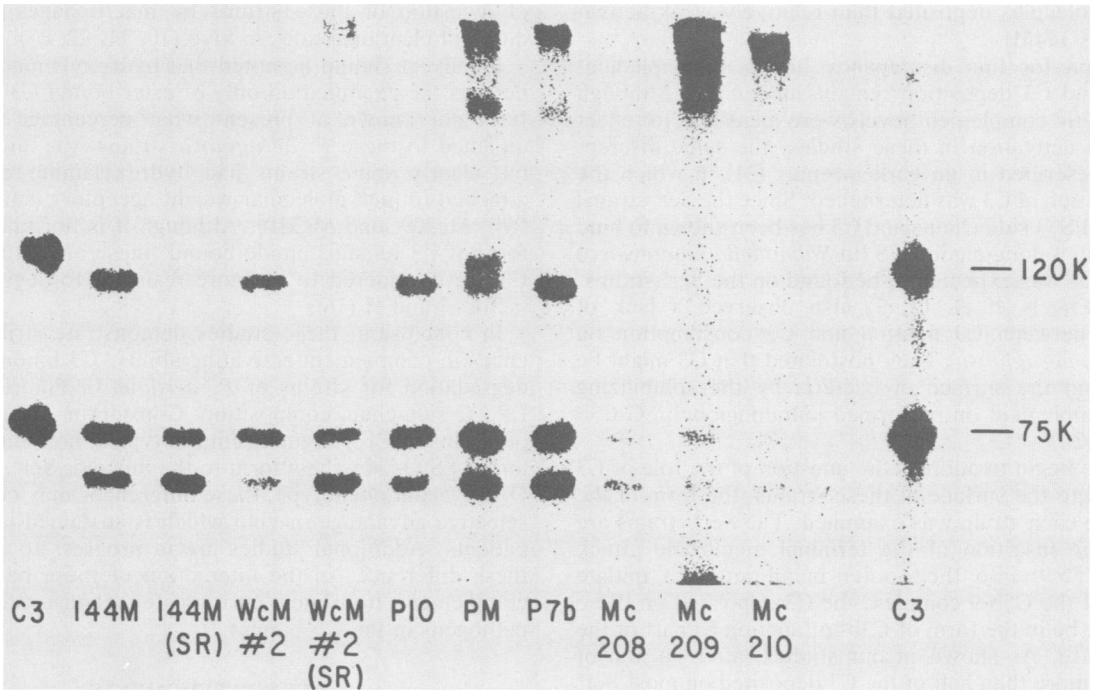

FIG. 3. Form of C3 deposited on Ser^s and Ser^r strains of *P. aeruginosa*. In this experiment, bacteria were incubated with radiolabeled C3 and 20% PNHS for 30 min at 37°C. See text for methods. (Note: Because the Ser^r strains generally bound fewer C3 molecules, these lanes were loaded with more sample than other lanes to improve the visibility of some of these bands.) Autoradiography was done on dried gels. The end lanes (1 and 12) contain native C3 without bacteria, revealing the α band at 120,000 Da and the β band at 75,000 Da. The form of C3 found on the surface of Ser^s strains is shown in lanes 2 (144M), 4 (WcM#2), 6 (P10), 7 (PIM), and 8 (P7b). The form of C3 found on the surface of Ser^r strains is shown in lanes 3 (144M-SR), 5 (WcM#2-SR), 9 (Mc208), 10 (Mc209), and 11 (Mc210).

DISCUSSION

The relationship among complement consumption, C3 deposition, and C3 fragmentation pattern was compared for Ser^s and Ser^r strains of *P. aeruginosa*. Ser^s strains 144M, WcM#2, and ByM activated much less complement per organism than their Ser^r derivatives 144M-SR, WcM#2-SR, and ByM-SR (data for ByM-SR not included). These results are probably attributable to the difference in LPS composition of these Ser^s and Ser^r strains. The LPS preparations from these ser^r strains were found to be heterogeneous, containing LPS with varying degrees of O-side-chain substitution, whereas the LPS of the Ser^s strains contained primarily lipid A-core polysaccharide components (29). In a similar manner, two additional Ser^r strains (Mc208 and Mc210) bearing long O-polysaccharide side chains also activated more complement per organism than four other Ser^s strains bearing rough LPS. The polysaccharide region of bacterial LPS has been found to activate complement via the ACP in the absence of antibody (24) and hence might contribute to the enhanced complement activation by these strains. Alternatively, antibodies to the polysaccharide regions of the LPS could activate the CCP, also depleting the total hemolytic complement levels. Non-LPS bacterial surface structures might also participate in complement activation, either directly or through antibody-mediated activation.

The observation that most Ser^s strains bearing LPS with short O side chains activate complement less effectively than their Ser^r, long LPS O-side-chain counterparts suggests that these Ser^s strains might be less inflammatory in vivo. Although most CF patients are initially colonized by Ser^r nonmucoid strains, these strains are eventually replaced by mucoid Ser^s strains. While the presence of a mucoid coat might prove to be a barrier to host defenses, the loss of the LPS O side chain might minimize the complement-dependent inflammatory response, thereby permitting local bacterial growth. This hypothesis would be consistent with the chronic nature of this pulmonary disease in CF patients.

To explore the relationship between complement and C3 binding, the kinetics of C3 deposition was examined, using PNHS in which both the CCP and ACP are functional as well as MgEGTA-treated serum in which only the ACP is active. Several conclusions can be drawn from our results. First, MgEGTA treatment of PNHS reduced either the amount or the rate of C3 deposition for most strains, implying the importance of a functioning CCP for maximal C3 deposition. Second, while the role of the CCP in C3 deposition varied from strain to strain, the effect of MgEGTA on C3 binding was more pronounced for the Ser^r strains, indicating that the CCP might be more important for optimal deposition of C3 for these strains than for the Ser^s strains. This observation is consistent with the finding that most of the C3 on these Ser^r strains was in the form of iC3b, an observation noted on strains which activate the CCP efficiently (25). Third, most Ser^s strains (except P1M) activated or deposited C3 more rapidly and bound more C3 per organism than the Ser^r strains (except 144M-SR). Although in our earlier study (31) more C3 was deposited on 144M than on 144M-SR when 10% PNHS was used, these strains had comparable levels of C3 attached when 20% PNHS was used. Finally, there is no apparent correlation between complement activation and C3 binding (as revealed in Table 1), since some of the best activating strains (such as WcM#2-SR or Mc208) had far

fewer C3 molecules deposited than relatively weak activators (such as 144M).

The reason for this discrepancy between complement activation and C3 deposition remains undefined. Although total hemolytic complement levels were measured to reflect complement activation in these studies, the same discrepancy was described in an earlier report (31) in which the direct activation of C3 was determined. Since the Ser^r strains have long LPS O side chains and C3 has been shown to bind preferentially to long-chain LPS (in *Salmonella montevideo*) (18), more C3 was expected to be found on the Ser^r strains. However, Engels et al. (2, 3) also observed a lack of correlation between C3 fixation and C3 consumption on strains of *P. aeruginosa*. They postulated that C3 might be released from the surface of bacteria by the solubilizing effect of complement on preformed immunoglobulin G-C3b complexes (2).

Finally, to begin to address the question of the role of C3 deposited onto the surface of these strains, the form of the C3 bound to each strain was examined. The Ser^s strains are killed by the insertion of the terminal membrane attack complex (C5b-9) into their outer membrane. To initiate formation of the C5b-9 complex, the C3 deposited on these strains must be in the form of C3b to function as part of the C5 convertase. As shown in our studies, after 30 min of incubation, more than half of the C3 deposited on most Ser^s strains was in the form of C3b. In contrast, with the Ser^r strains, most of the C3 was rapidly converted to iC3b, which cannot participate in C5 convertase formation or lead to generation of C5b-9.

The extent and rate of C3b degradation on the surface of microbes vary from one species of microorganisms to another and, as seen here, even from strain to strain within a species. In one study with *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* type 3, and *Haemophilus influenzae* type b, only 16 to 28% of the C3 deposited was in the form of iC3b even after 2 h of incubation (25). This is in contrast to a separate study with *Staphylococcus aureus* and *E. coli* in which a more rapid decay of C3b to iC3b and C3d was observed (9). Similarly, most of the C3 found on *Cryptococcus neoformans* was in the form of iC3b (20). Finally, Hostetter (14) demonstrated differences in the form of C3 located on different serotypes of *Streptococcus pneumoniae* and related these differences to serotypic variance in resistance to phagocytosis. Whereas serotypes 3 and 4, which are highly resistant to phagocytosis, revealed C3b, iC3b, and C3d on their capsular surfaces, serotypes 6A and 14, which are less resistant to phagocytosis, had only iC3b on their surfaces.

That different amounts of C3 as well as different forms of the C3 bound were found on the surfaces of the Ser^s and Ser^r *P. aeruginosa* strains in this study suggests that these strains might also differ with respect to their uptake and killing by phagocytic cells. Engels et al. (2) reported a high degree of correlation between C3 fixation and subsequent phagocytosis of *P. aeruginosa* strains by human polymorphonuclear leukocytes. C3b-coated microbes will interact with the C3b receptor on phagocytic cells (CR1) (4), whereas iC3b-coated microorganisms will interact predominantly with complement receptor type 3 (CR3) (8). Whether this difference conveys a selective advantage to the Ser^s strains ordinarily seen in the lungs of CF patients remains to be determined. However, previous studies with *Salmonella* strains differing in their LPS O-side-chain composition demonstrated that these differences not only caused different levels of complement activation, but also were responsible for different rates of ingestion of these strains by macrophages as well as different clearance rates in vivo (10, 11, 22, 23).

Finally, it should be noted that hydroxylamine treatment permits the examination only of ester-bound C3 fragments. It is not known at present what percentage of the C3 attached to these *P. aeruginosa* strains was amide linked, but clearly some strains had hydroxylamine-resistant C3 attached to high-molecular-weight acceptors (such as P1M, P7b, Mc209, and Mc210). Although it is not known which form of C3 remains amide bound, these amidated forms of C3 are considered to be more resistant to degradation by factors I and H (16).

In conclusion, these studies demonstrate striking differences in complement-activating ability, C3 binding, and C3 degradation for strains of *P. aeruginosa* differing in their LPS O-side-chain composition. Considering the conversion of strains in CF patients from the typical non-mucoid, Ser^r, long LPS O-side-chain form to the mucoid, Ser^s, short LPS O-side-chain phenotype, these differences may confer some selective advantage in vivo which is sustained in these CF patients. Additional studies are in progress to define how these differences in the interaction of these bacteria with complement translates into greater persistence of these pathogens in the CF patient.

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