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Biologic Activities of Antibodies to the Neutral-Polysaccharide Component of the *Pseudomonas aeruginosa* Lipopolysaccharide Are Blocked by O Side Chains and Mucoid Exopolysaccharide (Alginate)

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Virulent strains of *Pseudomonas aeruginosa* are either of a nonmucoid, lipopolysaccharide (LPS)-smooth or mucoid, LPS-rough phenotype, and immunity to these different variants is efficiently mediated by antibodies specific to O antigens or mucoid exopolysaccharide (also called alginate), respectively. In addition to O side chains and core polysaccharide components, the LPS of *P. aeruginosa* also contains neutral-polysaccharide components that express antigenic determinants common to many clinical isolates. We evaluated antibodies specific to neutral polysaccharides for the ability to mediate opsonic killing and protective immunity. Antibodies to these antigens mediated opsonic killing of poorly virulent nonmucoid LPS-rough isolates but not of isogenic strains with either a LPS-smooth or a mucoid phenotype. Antibodies to neutral-polysaccharide antigens also failed to protect neutropenic mice from challenge with modest doses of LPS-smooth *P. aeruginosa* strains ($<10^3$ CFU per mouse), whereas O-antigen-specific antibodies were highly protective. Antibodies to neutral polysaccharides deposited significantly ($P = 0.002$) more C3 onto LPS-rough strains than did antibodies to O side chains, but this situation was reversed when isogenic LPS-smooth strains were tested. Given that protective immunity against *P. aeruginosa* must be directed against either nonmucoid LPS-smooth strains or mucoid LPS-rough strains, it appears that antibodies specific to neutral-polysaccharide antigens do not protect against *P. aeruginosa* infection. Lack of protection is likely due to the ability of both O side chains and mucoid exopolysaccharide (alginate) to interfere with the opsonic killing activity of neutral-polysaccharide-specific antibodies.

Two distinct phenotypes of *Pseudomonas aeruginosa* are associated with human infections: a nonmucoid, lipopolysaccharide (LPS)-smooth phenotype mostly isolated from patients with nosocomial and community-acquired infections and a mucoid, LPS-rough phenotype mostly isolated from cystic fibrosis (CF) patients with chronic respiratory infections. For LPS-smooth strains, numerous studies have documented the protective efficacy of opsonizing antibodies specific for the O-side-chain polysaccharide portion of the LPS antigen (4, 6, 24, 29, 34, 35). For mucoid LPS-rough strains, available evidence implicates protection associated with opsonic antibodies specific to the mucoid exopolysaccharide (MEP), or alginate, capsule surrounding bacterial cells (30, 31). One limitation in developing LPS-specific immunotherapies is the need for multivalent vaccines because of the structural diversity of the monosaccharide constituents of the O antigens of *P. aeruginosa*. Attempts to use antigens with less variability in immunotherapy for *P. aeruginosa* infection have focused on outer membrane proteins (9, 10), flagella (25, 38), and conserved epitopes associated with the LPS core (40). While animal protection studies with these vaccines have yielded promising results, few data thus far indicate an association between the presence of antibodies to these antigens and human immunity to *P. aeruginosa* infection. In contrast, a strong association between the presence of antibodies to O-side-chain antigens and the survival of humans with *P. aeruginosa* bacteremia has been documented (35).

In addition to MEP and O-side-chain and core antigens, *P. aeruginosa* strains commonly express another surface polysaccharide structure composed of uncharged pentoses and hexoses (17–19, 42, 44). Three of the latter structures have been described and are referred to as the immunotype 3 (IT-3), IT-4, and D-rhamnan neutral polysaccharides. The D-rhamnan antigen is also called "A band" by some investigators (2, 22). Serologic studies indicate that the IT-3 neutral-polysaccharide and D-rhamnan antigens are cross-reactive (15) and that they bind to a monoclonal antibody (MAb) designated E87 (15, 17). This antibody does not bind to the IT-4 neutral polysaccharide but does bind to the LPS isolated from IT-4 *P. aeruginosa*, indicating that multiple neutral-polysaccharide antigens may be associated with a single strain or its LPS. The neutral-polysaccharide antigens represent an additional component of the surface of *P. aeruginosa* that has potential as a target for therapeutic antibodies. They might be particularly useful vaccine reagents because of their limited serologic diversity and their occurrence on most clinical isolates. In this report we present data on the opsonic and protective efficacy of antibodies specific for the *P. aeruginosa* LPS neutral polysaccharides, and we present our analysis of the immunologic basis for the observed activity of these antibodies in relation to antibodies specific to the O-side-chain and MEP antigens.

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MATERIALS AND METHODS

Bacterial strains and plasmids and recombinant *P. aeruginosa* strains. The Fisher IT-3 and IT-4 (International Antigenic Typing System O2 and O1 sero-

groups, respectively) strains of *P. aeruginosa* used to isolate polysaccharide antigens, along with additional LPS-smooth strains of *P. aeruginosa*, were initially obtained from cultures of blood from bacteremic patients and were supplied by the Clinical Microbiology Laboratory of Brigham and Women's Hospital. LPS-rough isolates were obtained initially as mucoid strains of *P. aeruginosa* from the sputa of colonized patients with CF and supplied by the Clinical Microbiology Laboratory of Children's Hospital in Boston, Mass. Nonmucoid derivatives of the strains from CF patients were derived by serial passage on tryptic soy agar. Strain AK1401 is an O-side-chain-deficient isolate selected from wild-type *P. aeruginosa* PAO1 by resistance to the O-side-chain-specific phage E79 (20) and was kindly supplied by J. Lam, Guelph, Ontario, Canada. Plasmid pLPS2, which contains the entire *rfb* locus from serogroup O11 (Fisher IT-2) *P. aeruginosa* in the broad-host-range plasmid pLAFR1 and induces the production of this antigen when transferred to *P. aeruginosa* strains (8), has been described (12). The nonmucoid LPS-rough strains which were complemented to an LPS-smooth phenotype by introduction of pLPS2 used in this study were the same as those previously described (8). Comparison of these complemented strains were made with the initial LPS-rough parental strains bearing only the cloning vector pLAFR1. Plasmid pLPS7 contains an insert of ~30 kb of DNA in pLAFR1 and was isolated from a gene bank derived from DNA from *P. aeruginosa* PAO1 (13) by complementation of the O-side-chain defect in strain AK1401. The methods used to derive pLPS7 were similar to those used to derive pLPS2 (12). A more detailed characterization of this plasmid will be published elsewhere.

Antibodies and antigens. Polyclonal antibodies to the IT-3 and IT-4 neutral-polysaccharide antigens were raised in rabbits by immunization with alkali (NaOH)-treated LPS as described elsewhere (17, 19). LPS was isolated by the phenol-water procedure (41) from IT-3 or IT-4 strains of *P. aeruginosa* and was purified further as described previously (15). Alkali treatment of LPS (0.1 N NaOH, 56°C, 4 to 18 h) has been shown to destroy the O antigen while leaving the neutral-polysaccharide antigens intact (15). Antibodies to the D-rhamnan polysaccharide were raised by hyperimmunization of rabbits with LPS isolated from strain AK1401 (15). Antibodies to O-side-chain antigens were raised by hyperimmunization of rabbits with purified high-molecular-weight O polysaccharides (33). These antisera have been previously shown to lack antibodies to the neutral-polysaccharide common antigens (15). Opsonic antibodies to MEP were raised in rabbits (1).

MAb E87 (murine immunoglobulin G) was kindly supplied by Teijin Pharmaceuticals (Tokyo, Japan), and MAb N1F10 (murine immunoglobulin M) was supplied by J. Lam. Both of these antibodies react with the D-rhamnan antigen (23, 44).

Serologic procedures. Enzyme-linked immunosorbent assays (ELISAs) with alkali-treated LPS from the Fisher IT-3 and IT-4 strains, LPS from strain AK1401, and purified D-rhamnan were performed as described elsewhere (15, 17). Purified D-rhamnan was kindly provided by Y. Knirel, Moscow, Russia (18). For calculation of the titer in serum, the mean of duplicate optical density (OD) values obtained after 30 min of incubation of the substrate with the bound-enzyme conjugate was entered on a data sheet in the Stat-View SE+Graphics program (Abacus Concepts, Berkeley, Calif.) on a Macintosh computer. A polynomial regression formula that described the best fit of the measured OD values to the corresponding dilutions of serum was then derived. For most sera, all of the values measured at dilutions of between 10 and 5,120 were used. The exceptions included those cases in which a correlation coefficient (r^2 value) of >0.95 was not obtained because the curve "bottomed out," i.e., the highest dilutions in serum all gave comparable readings above the background (no serum control). In these cases the regression formula was derived from all OD values except those for which the curve bottomed out. The smallest polynomial derivative between 2 and 5 was chosen to find the best-fit formulas; the final formula applied to a given set of OD values was the one that used the smallest polynomial derivative and yielded the highest correlation coefficient and the best *P* value, as determined by analysis of variance (ANOVA). After derivation of the best-fit formula for OD value versus dilution of serum, the equation was solved for an OD value of 0.200 to yield the titer, i.e., the dilution of serum giving an OD reading of 0.200 in 30 min.

Opsonic assays with *P. aeruginosa* (2×10^6/ml), dextran-purified human leukocytes (2×10^6/ml), fresh human serum as a complement source, and polyclonal antibodies or MAbs were conducted as has already been described (1, 26). In cases in which LPS-rough strains were evaluated, the dilution of human complement used was below the level at which direct bactericidal effects of complement—in the absence of phagocytic cells—were observed (generally a concentration of fresh serum of ≤2.0%). For LPS-smooth strains, higher levels of the complement source were used (range, 2.5 to 7.5%).

Deposition of C3 onto the bacterial surface was accomplished as described previously (27), except that the complement was labeled with ^{125}I (Iodobeads; Pierce Chemical Co., Rockford, Ill.). The number of C3 molecules per CFU was calculated as described previously (14). The data reported for C3 deposition in the presence of specifically immune rabbit sera reflect subtraction of the value for C3 molecules per CFU deposited in the presence of normal rabbit serum.

Animal protection studies. We used the neutropenic-mouse model of *P. aeruginosa* infection as originally described by Cryz et al. (5), except that we administered the bacterial challenge dose by the intraperitoneal route (32). Outbred CD-1 mice (Charles River Laboratories, Wilmington, Mass.) were rendered neutropenic by three injections of cyclophosphamide (200 mg/kg of body weight) given every other day. The typical kinetics of leukopenia in similar animals have been described (32). Two to 4 days after the final dose of cyclophosphamide, the mice were challenged by injection of bacteria in 0.2 ml of phosphate-buffered saline (PBS). The 50% lethal doses for the LPS-smooth challenge strains were quite low in this model, ranging from 10 to 75 CFU per mouse. Mice in groups of 10 were challenged with 200 to 700 CFU of different strains and were observed for up to 10 days for death.

Statistical analyses. Differences in opsonic killing activity were determined by ANOVA, and differences in C3 deposition mediated by antibodies with different antigenic specificities were assessed in a paired *t* test. These analyses were conducted with the Stat-View SE+Graphics software package on a Macintosh computer. Differences in survival rates among mice challenged after receiving different antibody preparations were calculated by using the Fisher exact formula.

TABLE 1. Titers of antibodies to *P. aeruginosa* LPS neutral polysaccharides[a]

Specificity of antiserum	Titer against target antigen			
	IT-3 NaOH LPS	IT-4 NaOH LPS	AK1401 LPS	D-Rhamnan
IT-3 NaOH LPS	11,198	11,942	4,050	2,877
IT-4 NaOH LPS	466	1,455	1,369	2,076
AK1401 LPS	4,401	2,748	1,375	6,263
NRS[b]	<10	59	26	160

[a] Titers calculated as described in Materials and Methods. IT-3 NaOH LPS and IT-4 NaOH LPS, IT-3 and IT-4 LPSs, respectively, treated with NaOH.
[b] NRS, normal rabbit serum.

RESULTS

Titers of antibodies to LPS neutral-polysaccharide antigens. Table 1 lists the titers of polyclonal antibodies raised to intact LPS from strain AK1401 and to alkali-treated LPS from *P. aeruginosa* Fisher IT-3 and IT-4 strains against these three antigens and the purified D-rhamnan antigen. We used alkali-treated LPS antigens to ensure the measurement of all antibody species that could bind to neutral-polysaccharide antigens and core determinants, and we used purified D-rhamnan to measure titers of antibodies to the most commonly occurring cross-reactive neutral-polysaccharide epitope detected among clinical isolates of *P. aeruginosa* (22). Antisera raised to IT-3 NaOH-treated LPS and AK1401 LPS were highly reactive with all of the target antigens, while the antiserum raised to IT-4 NaOH-treated LPS was the least reactive. The titers appeared to be properties of the individual antisera, since the target antigens all display cross-reactive epitopes associated with the neutral polysaccharides and the LPS core. Some antisera, such as that raised to AK1401 LPS, contained higher titers against heterologous antigens than against the immunizing antigen; this result perhaps reflected increased density or expression of cross-reactive epitopes on the heterologous antigen.

Opsonic activity of neutral-polysaccharide-specific antibodies in the absence or presence of O antigens on *P. aeruginosa* strains. To determine whether the antibodies to neutral polysaccharides had in vitro opsonic killing activity against *P. aeruginosa*, we compared the opsonic activities in 1:10 dilutions of polyclonal antisera to AK1401 LPS antigen or to LPS O side chains against a set of seven nonmucoid, LPS-rough strains and isogenic LPS-smooth strains, complemented to this phenotype by introduction of pLPS2 (8). An eighth strain, AK1401, was also used along with an LPS-smooth derivative complemented to this phenotype by the introduction of pLPS7. Additional comparisons were made with eight LPS-smooth clinical isolates. All of the strains tested bound to MAbs E87 and N1F10, which are specific for the D-rhamnan antigen.

As shown in Fig. 1, LPS-rough isolates were killed by anti-

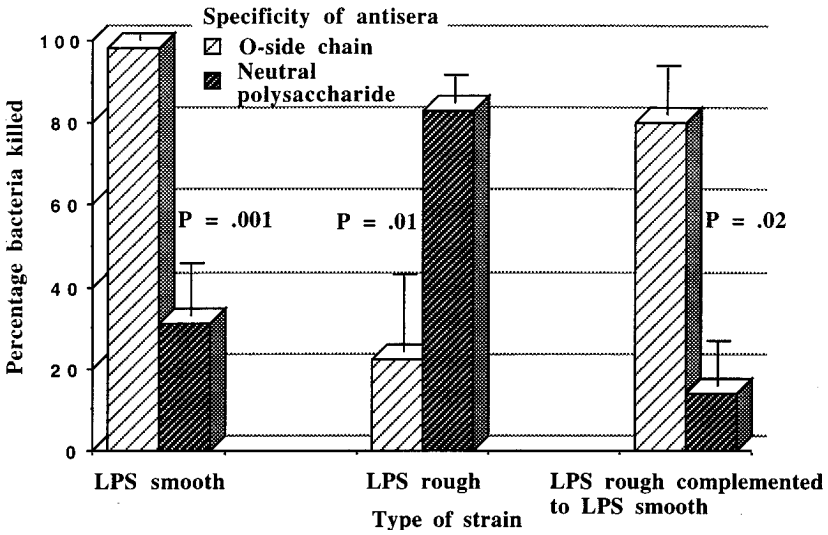

FIG. 1. Opsonic killing of *P. aeruginosa* expressing the indicated LPS phenotype by antibodies specific for O side chains (raised to purified high-molecular-weight O polysaccharide) or for neutral polysaccharides (raised to LPS from strain AK1401). Bars indicate the mean percentages of bacteria killed in studies with eight strains of each phenotype, and error bars indicate the standard errors of the means. *P* values were determined in a paired *t* test comparing the percentages of the bacteria that were killed by the two different antisera.

bodies to neutral polysaccharides present in antisera raised to strain AK1401 LPS when tested under conditions in which complement levels were below the direct bactericidal level (final concentration of fresh serum as the complement source, 0.1 to 2%). This level of killing was significantly higher ($P = 0.01$ by paired *t* test) than that mediated by antibodies to the poorly expressed O side chains. However, when these strains were complemented to an LPS-smooth phenotype by the introduction of pLPS2 or pLPS7, they were no longer susceptible to the opsonic activity of neutral-polysaccharide-specific antibodies; rather, they were now killed at a significantly higher rate ($P = 0.02$ by paired *t* test) by antibodies to homologous O side chains than by antibodies to neutral polysaccharides. This pattern of opsonic killing was also observed for naturally occurring LPS-smooth clinical isolates of *P. aeruginosa*. In all of the experiments in which killing of LPS-rough *P. aeruginosa* occurred, the presence of complement and leukocytes—along with the specific antibodies—was required for killing. Thus, under the conditions employed, antibodies and complement alone had no direct bactericidal effects on LPS-rough strains. When antisera raised to alkali-treated IT-3 or IT-4 LPS were tested against LPS-smooth strains, no opsonic killing was observed (data not shown); in contrast, these antisera manifested opsonic activity against LPS-rough strains comparable to that shown in Fig. 1 for antisera raised to LPS from strain AK1401.

Protection of neutropenic mice. Although antibodies to neutral polysaccharides did not mediate opsonic killing in vitro, it was still considered possible that they would possess in vivo protective activity, as these two measures of immune efficacy are often, but not always, correlated (28). Sera containing antibodies specific for either neutral polysaccharides (raised to alkali-treated LPS isolated from the IT-3 strain, which had the highest titers of antibodies to three of the four test antigens) or O side chains were evaluated for protective efficacy in a mouse model of *P. aeruginosa* infection during neutropenia. LPS-smooth strains were employed because this is the phenotype that infects immunocompromised patients and because LPS-rough strains are virtually avirulent in this model (5, 7). We used no challenge strains expressing the structurally related and cross-reactive LPS serogroup O2 and O5 antigens (16) because we wanted to avoid a potential confounding of the results by antibodies to these antigens which are potentially present at low levels in antisera raised to alkali-treated IT-3 LPS (serogroup O2); however, by use of colony blots, we ensured that the challenge strains all bound strongly to the neutral-polysaccharide-specific antibodies in the immune sera (17, 19). As shown in Fig. 2, protection was engendered only by passive administration of sera containing antibodies to homologous O-side-chain antigens, and this protection was significantly greater ($P < 0.001$ in all cases by Fisher's exact test) than that afforded by antibodies raised to neutral polysaccharides or by normal serum. There was no significant ($P > 0.4$ by Fisher's exact test) difference in the rates of survival of mice treated with antibodies to neutral polysaccharides and normal serum. Given that the challenge doses were fairly modest in these studies (200 to 700 CFU per mouse), it is unlikely that efficacy of antibodies to neutral polysaccharides was missed.

Effect of O antigens on the deposition of C3 onto bacterial surfaces. Since neutral-polysaccharide-specific antibodies mediated opsonic killing of LPS-rough strains only in the presence of complement, we investigated the effect of O-side-chain expression on the ability of these antibodies to deposit opsonically active C3 fragments onto bacteria. When LPS-rough strains were used, neutral-polysaccharide-specific antibodies deposited 8,693 ± 4,434 (mean ± standard error of the mean) molecules of C3 per CFU above background (molecules of C3 deposited per CFU by normal rabbit serum) onto these strains, whereas there was no increase above the background in the number of C3 molecules per CFU deposited by antibodies to O antigens ($P = 0.002$ by paired *t* test). In contrast, when recombinant, LPS-smooth bacteria were tested, antibodies to O side chains deposited 9,652 ± 2,021 (mean ± standard error of the mean) more molecules of C3 per CFU than did normal rabbit serum onto these isogenic strains, while the mean number of C3 molecules per CFU deposited by antibodies to neutral polysaccharides was no greater than the background ($P = 0.026$ by paired *t* test). It is unlikely that this effect was due to inhibition of the binding of the neutral-polysaccharide-specific antibodies to the bacteria, since it has been demonstrated by numerous investigators, using both colony blot and ELISA,

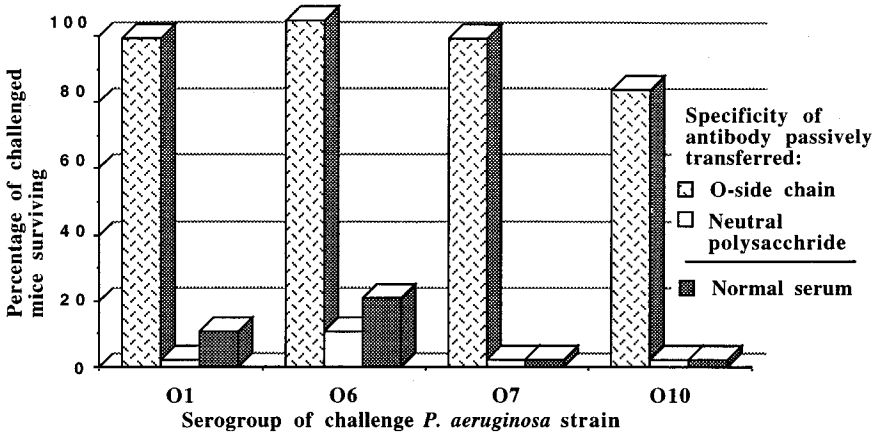

FIG. 2. Protection of neutropenic mice against challenge with LPS-smooth *P. aeruginosa* cells (200 to 700 CFU per mouse) after passive transfer of antibodies specific for the indicated antigens. The degree of protection conferred by sera containing O-side-chain-specific antibodies was significantly greater ($P < 0.001$ in all comparisons by Fisher's exact test) than that conferred by sera containing antibodies to neutral polysaccharides or by normal rabbit serum. Bars along the *x* axis that are just above the zero mark on the *y* axis indicate groups of mice with no survivors; these bars are shown for clarity of presentation of data.

that these antibodies can bind to LPS-smooth *P. aeruginosa* cells (17, 19, 21, 22).

Effect of MEP (alginate) on phagocytosis mediated by antibodies to neutral polysaccharides. The above results indicate that antibodies to neutral polysaccharides are unlikely to be protective against LPS-smooth strains of *P. aeruginosa*. However, mucoid LPS-rough strains are the principal pathogens in chronic lung infections in CF patients, and most isolates express epitopes reactive with MAb E87 (22). To determine the effect of MEP expression on the opsonic activity of antibodies to neutral polysaccharides, we tested six mucoid strains of *P. aeruginosa* (the parental strains of the nonmucoid LPS-rough strains described above) for susceptibility to phagocytic killing in the presence of antibodies to either neutral polysaccharides or MEP (1). Although the neutral-polysaccharide-specific antibodies killed nonmucoid LPS-rough strains (Fig. 1), they were no more effective than antibodies in normal serum at killing the mucoid parental strains (Fig. 3). In contrast, opsonic antibodies to MEP were significantly more effective at mediating opsonic killing ($P = 0.003$ by ANOVA) than either normal rabbit serum or antibodies to neutral polysaccharides.

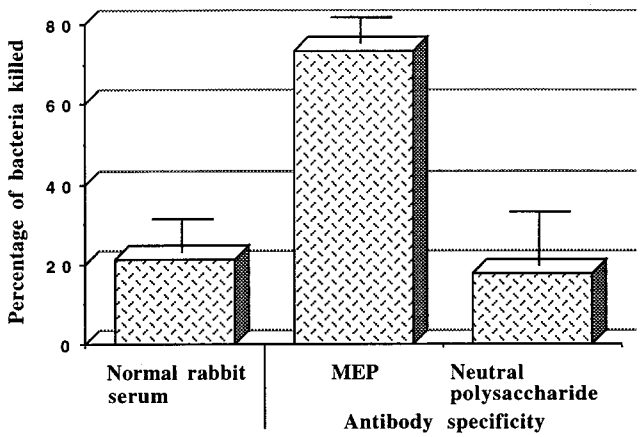

FIG. 3. Opsonic killing of mucoid *P. aeruginosa* by antibodies specific for MEP (alginate) or neutral polysaccharides (raised to LPS from strain AK1401). Bars indicate the mean percentages of bacteria killed for six strains, and error bars show the standard errors of the means. The level of opsonic killing by sera containing MEP-specific antibodies was significantly higher ($P = 0.003$ by ANOVA) than that obtained with sera containing antibodies to neutral polysaccharides (raised to LPS from strain AK1401) or obtained with normal rabbit serum.

DISCUSSION

The purpose of this study was to evaluate the opsonic and protective efficacy of antibodies to the neutral polysaccharides expressed on the LPS of *P. aeruginosa*. We found that antibodies to these antigens failed to mediate opsonic killing of LPS-smooth strains and failed to protect neutropenic mice from low-dose challenges with these strains. These failures were associated with the inability of the antibodies to deposit opsonically active C3 onto the bacterial surface in the presence of O side chains. Antibodies to neutral polysaccharides effectively mediated opsonic killing and C3 deposition onto nonmucoid LPS-rough strains, but these strains have virtually no pathogenic potential (7, 11). Mucoid LPS-rough strains, the principal pathogens of patients with CF, were not susceptible to opsonic killing by antibodies to neutral polysaccharides; opsonic killing of mucoid *P. aeruginosa* strains, mediated by antibodies specific for MEP, has been shown to be the principal immune effector associated with human (30) and animal (31) resistance to chronic lung infection by these organisms. Overall, our results suggest that O side chains and MEP function in similar ways to protect *P. aeruginosa* cells from the immunologic activity of antibodies to neutral polysaccharides.

Three structurally distinct neutral polysaccharides have been isolated from *P. aeruginosa* (17–19, 37, 44). Two of these, the purified IT-3 neutral polysaccharide and D-rhamnan, react with MAb E87 (15, 39, 44). The purified IT-4 neutral polysaccharide does not react with this antibody; however, the intact and alkali-treated LPS from this strain does react, and this reaction indicates the presence of MAb-E87-reactive epitopes on this LPS. MAb E87 has been used to show that cross-reactive neutral-polysaccharide antigens are expressed by most clinical strains of *P. aeruginosa* (17, 22). Both Yokota et al. (44) and Hatano et al. (15) presented evidence that D-rhamnan is part of the traditional *P. aeruginosa* LPS structure. In contrast, Rivera et al. (36, 37) suggested that D-rhamnan polysaccharide is part of a second *P. aeruginosa* LPS, designated A-band LPS. However, to date, no group has reported the isolation of A-band LPS from an LPS-smooth strain that is free of the traditional O-side-chain-bearing LPS. This lack of evidence sup-

ports the conclusions of Yokota et al. (44) and Hatano et al. (15) that *P. aeruginosa* LPS is a multiantennary structure composed of lipid A, core polysaccharide, variable O side chains, and neutral polysaccharides that express cross-reactive epitopes.

The neutral polysaccharides have been reported to be up to 30 repeat units in size (in the case of D-rhamnan) (2) and to include a carbohydrate core that might link the repeat structure to lipid A. This suggests that the largest polymers of neutral polysaccharides would be <6 kDa in size. Since O side chains on *P. aeruginosa* range up to 25 to 30 kDa in size, they likely project out from the bacterial surface considerably farther than neutral polysaccharides. This projection may be the basis for the observed interference of O side chains with the deposition of an opsonically active complement by neutral-polysaccharide-specific antibodies, which may be analogous to the interference by the O-antigen capsule with complement-mediated killing of *Escherichia coli* (3).

Since neutral polysaccharides are expressed by LPS-rough *P. aeruginosa* isolates from patients with CF, it is conceivable that antibodies to these structures would be protective in this situation. However, data presented here suggest that the elaboration of MEP by clinical isolates from patients with CF interferes with the opsonic activity of antibodies to neutral polysaccharides. In addition, the high levels of antibodies to D-rhamnan in sera from CF patients (22) indicate that chronic *P. aeruginosa* infections can progress in those patients in the presence of neutral-polysaccharide-specific antibodies. This observation constitutes further evidence for a lack of protective efficacy of antibodies to neutral polysaccharides against mucoid LPS-rough strains of *P. aeruginosa*.

While antibodies to the neutral polysaccharides lacked protective efficacy in our study, Terashima et al. (40), using a neutropenic-mouse model of infection, have found protective efficacy of a human MAb specific to an L-rhamnose residue in the outer core of *P. aeruginosa* LPS. However, later studies suggested serologic variability in outer-core epitopes among clinical isolates of *P. aeruginosa* (43). This finding poses the same problem of serologic variability for immunotherapy for core epitopes as that encountered with therapies for O side chains of *P. aeruginosa*. We observed no protective efficacy in mice when we used polyclonal sera with high titers of antibodies to neutral polysaccharides, although we would reasonably expect antibodies to core epitopes to be elicited by these antigens. Perhaps the lack of protective efficacy was a result of the lack of the proper protective epitope (possibly due to destruction by alkali treatment) on the LPS antigens we used to raise the neutral-polysaccharide-specific antibodies. Alternatively, our immunization schedule may have failed to induce sufficiently high titers of antibodies to protective core epitopes. Overall, in this study we failed to document any potential efficacy for antibodies to *P. aeruginosa* LPS neutral-polysaccharide-specific antibodies. These antigens are thus unlikely to be an important component of a *P. aeruginosa* vaccine.

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