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Polysaccharide of the Slime Glycolipoprotein of *Pseudomonas aeruginosa*

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The polysaccharide moiety was isolated by mild acid hydrolysis from the slime glycolipoprotein of *Pseudomonas aeruginosa* strain BI. After gel filtration, the polysaccharide obtained from the carbohydrate peak fractions was found to be lipid- and protein-free. Analyses indicated that the polysaccharide contained the carbohydrate components of the parent glycolipoprotein. Molecular size of the polysaccharide was estimated by gel filtration as 70,000 to 100,000. The polysaccharide showed no indications of toxicity in mice at doses far in excess of the lethal dose for the parent glycolipoprotein, nor did the mice develop the leukopenia that characteristically follows intraperitoneal injection of glycolipoprotein. The polysaccharide acted as an inhibitor of indirect hemagglutination of glycolipoprotein-coated erythrocytes in the presence of anti-glycolipoprotein serum; however, it was not antigenic itself in rabbits. Coupled with methylated bovine serum albumin, the polysaccharide continued to lack the leukopenic and toxic properties of the parent glycolipoprotein, but the coupled polysaccharide was capable of stimulating indirect hemagglutinating antibody against both the polysaccharide and the glycolipoprotein coating erythrocytes. Moreoever, the antibody to the coupled polysaccharide protected mice against challenge with lethal doses of viable *P. aeruginosa* with the same effectiveness as anti-glycolipoprotein serum.

Two decades ago, *Pseudomonas aeruginosa* was commonly regarded as a saprophyte of little clinical significance (14). Since then the organism has entered the ranks of formidable pathogens, at least in the context of certain patient conditions. The most common of these are severe burns, cancer, and cystic fibrosis (12). In addition, *Pseudomonas* infections develop occasionally in a wide range of other conditions with consequences of varying degrees of seriousness (12). Infection is complicated by the fact that, in many cases, available treatment is unreliable and often ineffectual (13).

The effects of *P. aeruginosa* itself on host defense remain obscure, and this deficiency in our knowledge effectively impedes progress in the development of an adequate therapy. Successful attempts have been made with other pathogenic organisms to characterize chemically the structural components that express immunogenic activity, for example, the group-specific carbohydrates of hemolytic streptococci (9) and the capsular polysaccharides of *Pneumococcus* (27). Furthermore, the mechanisms of bacterial interference with host defenses have been identified for many species (30). Similar attempts with *P. aeruginosa* remain on a primitive level. However, the studies on the glycolipoprotein (GLP) isolated from the extracellular slime of *P. aeruginosa* represent a modest step in clarifying the interaction of the surface of the infecting organism with the host (2, 10, 11, 20, 28, 29). The GLP has a demonstrated protective efficacy in passive and active immunization (2), and the antigenic specificity of the complex appears to be located in the carbohydrate moiety (29). Furthermore, GLP has a deleterious effect on the host defenses, and this effect has been attributed to an interaction with the circulating polymorphonuclear leukocytes that effectively removes the neutrophil from participation in the first line of host defense (20).

The present study attempts a further clarification of aspects of the influence of the invading *P. aeruginosa* on the host response and of the degree to which the GLP is responsible for the course of events. A polysaccharide fraction was isolated from the GLP by mild acid hydrolysis and characterized. When used as a primary immunogen, the polysaccharide failed to elicit a detectable humoral response; however, as a conjugate of methylated bovine serum albumin (MBSA), the polysaccharide fraction acted to stimulate the production of protective anti-

bodies. The possible significance of these results as an explanation of irregularities in the host response to infection by *P. aeruginosa* is discussed.

MATERIALS AND METHODS

Organism. The bacterium used in this study, *P. aeruginosa* strain BI, was originally isolated from a clinical specimen and has been previously described (3).

GLP. As previously described (2, 28), the GLP of the extracellular slime layer of strain BI was extracted from 18-h cultures, precipitated with ethanol, and dialyzed against distilled water. The dialyzed material was centrifuged at 105,000 × *g* for 3 h, and the supernatant fluid, which contained the GLP fraction, was lyophilized. Purity and homogeneity were demonstrated by chromatography, sedimentation pattern, and immunodiffusion (28).

GLP polysaccharide. The GLP polysaccharide was obtained after subjecting GLP to mild acid hydrolysis (22). The method consisted of boiling purified GLP (1%, wt/vol) in 1% acetic acid for 90 min. After centrifugation at 27,000 × *g* for 20 min, the supernatant fluid was removed and the aqueous phase was then dialyzed extensively against distilled water and lyophilized. Total recovery of material from the GLP was approximately 90%. Forty-five milligrams of the lyophilized aqueous-phase material in 3 ml of 0.05 M phosphate-buffered saline (PBS), pH 7.4, was applied to a Sephadex G-100 column, and fractions were collected at the rate of 8 drops/min and monitored for carbohydrate and protein content. Fractions containing the peaks were pooled, dialyzed against distilled water, and lyophilized. The major carbohydrate peak, peak I, was designated GLP polysaccharide. For some experiments, the GLP polysaccharide was coupled to MBSA. MBSA was prepared by the method of Sueoka and Cheng (32). After methylation, the washed methanol precipitates were dissolved in water, neutralized, dialyzed against distilled water, and lyophilized. The dried product was stored in vacuo over KOH. Coupling was accomplished by mixing the protein carrier to the GLP polysaccharide in a ratio of 3:1 (wt/wt) according to the method of Kozel and Cazin (17).

Molecular weight. The molecular weight of the GLP polysaccharide was estimated by gel filtration as described by Andrews (1). Dextran standards (Pharmacia Fine Chemicals, Uppsala, Sweden) were applied to Sephadex G-200 in a column (2.5 by 91 cm). The samples were washed with a 0.5 M phosphate buffer, pH 7.4, at a flow rate of 8 drops/min at 4°C and monitored by the anthrone reaction.

Chemical analyses. Chemical determinations were made on at least three independently prepared lots of GLP and GLP polysaccharide; average values are presented. Hexoses were determined by the anthrone method with glucose standards (31), protein was determined by the method of Lowry et al. with bovine serum albumin standards (10), total amino sugars were determined by the method of Belcher et al. (5), lipid was determined by the method of Itaya and Ui with palmitic acid standards (16), *N*-acetyl was determined according to the procedure of Belcher and Godbert (4), and uronic acid was determined by the method of Bitter and Muir with glucuronolactone standards (6).

Animals. White male rabbits weighing 3 to 4 kg were housed individually. White male Swiss mice weighing 18 to 20 g were housed 5 to 10 per cage. All animals were supplied with water and Purina chow ad libitum.

Animal challenge. Bacterial cells were prepared for challenge as described previously (11). Briefly, 18-h cultures were washed from Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) slants, sedimented, washed once, and suspended finally in 0.01 M PBS (pH 7.2) at a turbidity equivalent to 10^{10} to 5×10^{10} viable cells per ml. Colony counts on Trypticase soy agar were made on each suspension to determine the numbers of viable bacteria. Groups of 10 mice were injected intraperitoneally with 0.5 ml of each of three twofold dilutions of bacteria or other material being administered. Mice were observed daily for up to 5 days. In passive immunization experiments, mice received 0.5 ml of antiserum intraperitoneally 2 h before bacterial challenge. The protective capacity of a serum is expressed as the percentage of survival 4 to 5 days after bacterial challenge. All experiments were repeated three times, and results are presented as arithmetic means.

Leukocyte counts. Leukocyte counts of mouse peripheral blood were established by standard techniques of dilution with 0.1 N HCl followed by enumeration of the total leukocyte count in a Neubauer hemacytometer (18). The results are presented as average leukocyte counts for groups of six mice.

Antisera. For the production of antisera, two immunization schedules were used. In schedule 1, which was used routinely for the production of antisera, on day 0 the rabbits received subcutaneous injections in four separate sites for a total of 2 mg of the appropriate preparation in an equal volume of 0.1 M PBS, pH 7.4, and Freund incomplete adjuvant. Groups of three rabbits were bled before immunization and 18 to 20 days afterwards. In schedule 2, the rabbits were given an initial intramuscular injection of 1.0 mg of the GLP polysaccharide in Freund incomplete adjuvant; additional intravenous injections of 1 mg of material in 1 ml of saline were given on days 7, 10, 13, 20, 23, and 26. Serum was collected 7 days after the last injection. Pooled sera were stored in 1-ml portions at −20°C.

Indirect hemagglutination. Hemagglutination activity of the sera was determined indirectly as described previously (11). Formalinized sheep erythrocytes (SRBC; Difco Laboratories, Detroit, Mich.) were sensitized by incubating a 5% suspension of cells in PBS with an equal volume of GLP (200 μg/ml) or GLP polysaccharide (1 mg/ml) at room temperature for 30 min. The sensitized cells were then washed three times in PBS and resuspended to a concentration of 5% in PBS. Sensitized cells (0.05 ml) were added to 0.5-ml serial dilutions of antiserum, mixed well, allowed to settle at room temperature for 2 h, and read. The titer was expressed as the reciprocal of the highest serum dilution resulting in a positive hemagglutination pattern.

Indirect hemagglutination inhibition (HAI). The preparation to be tested for inhibitory activity was added (10 μg in 0.1 ml of PBS) to 0.5-ml serial dilutions of antiserum. After incubation at room tem-

perature for 60 min, 0.05 ml of the sensitized SRBC was added to each tube, and the hemagglutination titer was determined after a 2-h incubation period at room temperature.

RESULTS

Isolation of the GLP polysaccharide. After mild acid hydrolysis of the GLP, a lipid-free, aqueous phase was collected, and the elution characteristics of this phase were studied on Sephadex G-100 (Fig. 1). A major (I) and a minor (II) peak were detectable. The major peak consisted entirely of carbohydrate, whereas the minor peak contained carbohydrate and all of the small amount of protein (0.7%, dry wt/wt) that remained in the aqueous phase. Calibration of the column by means of known substances allowed for an estimation of the molecular weight of the material in the two peaks. Accordingly, the molecular weight of peak I was estimated at from 70,000 to 100,000, and that of peak II was estimated as less than 15,000.

Because of the absence of lipid or protein from peak I, it offered the interesting possibility of studying the biological activity of the carbohydrate moiety of the GLP in isolation. A more precise analysis of the chemical components (Table 1) showed that peak I retained all the carbohydrate components of the parent molecule, indicating that it was indeed representative of the polysaccharide of the GLP. Thus, hexoses and hexosamines constituted the major carbohydrate components of the GLP polysaccharide, as they do of the parent GLP (2). An earlier study has shown that the hexoses are rhamnose, mannose, glucose, and galactose, and that the hexosamines are glucosamine and galactosamine (2).

Lethality and leukopenia. The injection of GLP intraperitoneally at appropriate concentrations produced lethal effects in mice (Fig. 2), as reported previously (28). The 50% lethal dose of the GLP of strain BI was approximately 30 μg per g of body weight, and this value agrees well with previously reported values. In contrast, the GLP polysaccharide showed no lethal effects throughout the range of values tested. The 50% lethal dose of the GLP expressed in terms of its total carbohydrate content (32%) was approximately 10 μg per g of body weight. Therefore, based on the total carbohydrate of the GLP polysaccharide (68%), a challenge dose of GLP polysaccharide equivalent to 10 50% lethal doses of GLP showed no lethality. Furthermore, the appearance of the mice injected with the highest dose of the GLP polysaccharide was indistinguishable from that of the control mice injected

TABLE 1. *General chemical analyses of the extracellular slime fractions of P. aeruginosa strain BI*

Component	Extracellular slime fraction (% dry wt)	
	GLP	Peak I (GLP polysaccharide)
Hexose	12	31
Hexosamine	20	37
Uronic acid	4	4
Protein	18	0
Lipid		
Colorimetric	22	0
Precipitate[a]	30	
N-acetyl	13	24

[a] Precipitate was collected after mild acid hydrolysis of GLP.

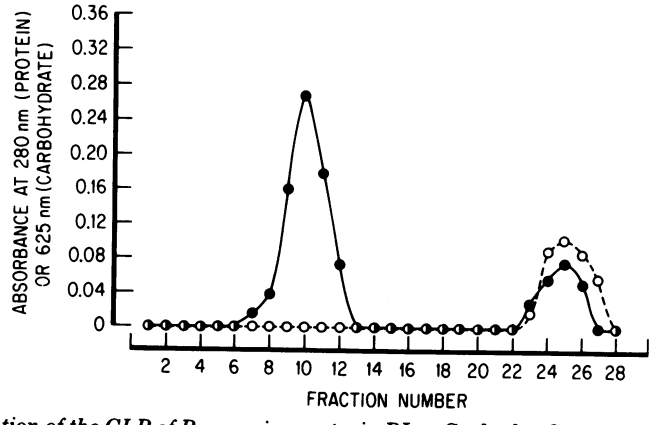

FIG. 1. *Fractionation of the GLP of P. aeruginosa strain BI on Sephadex G-100 after mild acid hydrolysis. Fractions were monitored for carbohydrate (●) and for protein (○). Fractions indicated by the horizontal bars were pooled.*

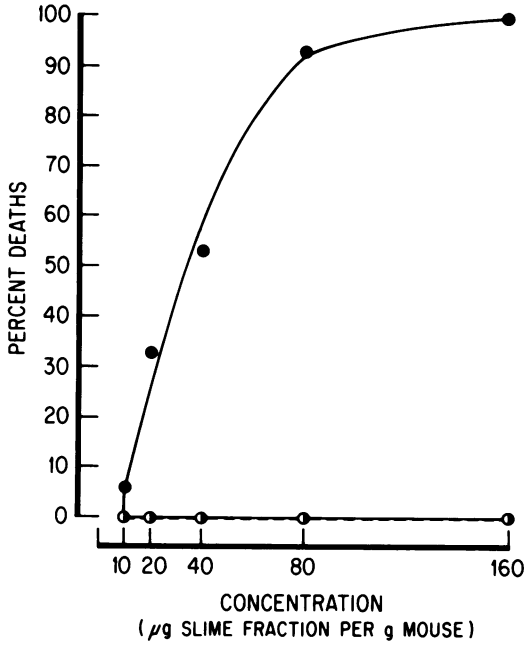

FIG. 2. *Lethality of extracellular slime fractions of P. aeruginosa strain BI. Groups of mice were challenged intraperitoneally at the indicated concentrations of GLP (●——●), GLP polysaccharide (●- - -●), and GLP polysaccharide coupled with MBSA (○).*

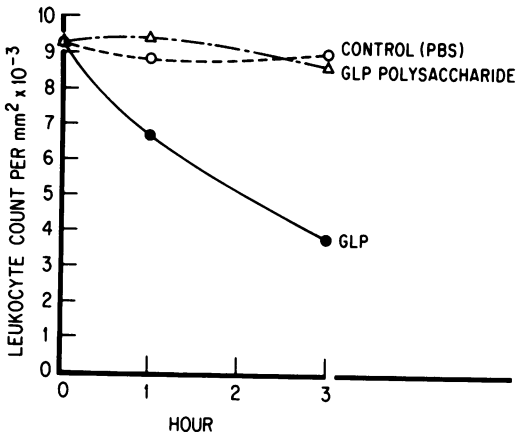

FIG. 3. *Leukopenia in the peripheral circulation of mice after intraperitoneal injection of extracellular slime fractions (50 μg per g of mouse) of P. aeruginosa strain BI.*

TABLE 2. *Antigenic relatedness of the extracellular slime fractions of P. aeruginosa strain BI, as determined by indirect hemagglutination*

Inhibitor	Indirect hemagglutination titer[a]	HAI index[b]
None (PBS)	320	1
GLP	320	160
GLP polysaccharide	320	160

[a] Reciprocal of the indirect hemagglutination titer without inhibitor.

[b] The index was calculated by dividing the reciprocal of the indirect hemagglutination titer by the reciprocal of the indirect HAI titer. Both titers were determined as described in the text.

with PBS alone; that is, the mice showed none of the clinical manifestations, such as ocular discharge, ruffled pelt, lethargy, diarrhea, and spastic movement, that many surviving mice showed after injection with sublethal doses of GLP.

Peripheral leukopenia has been well documented as a concomitant of injection of a lethal dose of GLP (28, 29). As expected from the results of the lethality studies, the intraperitoneal injection of up to 50 μg of the GLP polysaccharide per g of body weight did not result in leukopenia (Fig. 3), whereas the same dose of GLP produced the characteristic decline. In experiments not shown, concentrations of the GLP polysaccharide as high as 160 μg/g did not produce leukopenia.

Immunogenicity. GLP stimulates the production of hemagglutinating antibody, and the carbohydrate moiety of the GLP has been suggested as the active antigenic site (29). This hypothesis was supported by the finding that the GLP polysaccharide could act as an inhibitor of the interaction anti-GLP serum with the GLP antigen (Table 2). In these experiments, the presence of the isolated GLP polysaccaride was as effective in blocking the interaction of specific anti-GLP serum with GLP as the intact GLP. Peak II showed considerably less inhibitory capacity than the GLP polysaccharide (data not shown); consequently, this peak was not further studied. However, in spite of its inhibitory capacity, the GLP polysaccharide, when used as an antigen in the routine immunization schedule, failed to elicit detectable hemagglutinating antibody, as tested against sensitized SRBC coated with either GLP or the GLP polysaccharide (Table 3). When a more extensive immunization schedule was followed, the same negative results were produced. Conversely, anti-GLP serum agglutinated SRBC to the same degree whether they were coated with GLP or with GLP polysaccharide.

In a further attempt to investigate its antigenic capacity, the GLP polysaccharide was coupled with MBSA. The coupled GLP polysaccharide continued to lack the leukopenic, lethal, and toxic properties associated with the parent GLP; i.e., no evidence of these properties was mani-

TABLE 3. *Immunogenicity of the extracellular slime fractions of P. aeruginosa strain BI*

Antiserum raised to:	Immunization schedule[a]	Hemagglutination titer (SRBC coating)	
		GLP	GLP polysaccharide
GLP	1	320	320
GLP polysaccharide	1	0	0
GLP polysaccharide	2	0	0
GLP polysaccharide coupled with MBSA[b]	1	80	80

[a] Immunization schedule 1 was a routine procedure consisting of a one-time subcutaneous injection on day 0 and bleeding 18 to 20 days later; schedule 2 consisted of an intramuscular injection followed by a series of intravenous injections over a period of 26 days. Details are described in the text.

[b] Coupling of the GLP polysaccharide is described in the text.

fested in mice. However, the MBSA-coupled polysaccharide did stimulate the production of rabbit antiserum capable of agglutinating SRBC coated with either GLP or the GLP polysaccharide (Table 3).

Antiserum raised to the MBSA-coupled GLP polysaccharide was then used in mouse protection tests, using viable bacteria as a challenge. The results (Table 4) showed that MBSA-coupled GLP polysaccharide was capable of stimulating protective antibody, whereas the GLP polysaccharide alone failed to do so. Moreover, antiserum to MBSA-coupled GLP polysaccharide conferred levels of protection equivalent to those of antiserum to the parent GLP.

DISCUSSION

In the present work, a polysaccharide composed largely of hexoses and acetylated hexosamines was isolated from the GLP of *P. aeruginosa* strain BI by means of dilute acetic acid hydrolysis. This treatment cleaves ketosidic and, to a degree, pyrophosphate linkages (21). We have previously reported (8, 29) the presence of low amounts of a 2-keto-3-deoxy sugar acid in GLP, as indicated by its positive reaction with thiobarbituric acid. It is highly likely that the reactive material represents the ketosidic linkage cleaved by this treatment, possibly a linkage between the lipid and the carbohydrate moieties. In addition, the appearance of two peaks indicates that pyrophosphate bonds may also have been cleaved.

Earlier studies from this laboratory have suggested that the carbohydrate moiety is the basis of GLP antigenic specificity (29). Although the GLP polysaccharide was of relatively high molecular weight, it was incapable of inducing an antibody response in rabbits. However, coupled

TABLE 4. *Passive protection of mice challenged with viable cells of P. aeruginosa strain BI*

Antiserum raised to[a]:	Survivors (%)
GLP	80
GLP polysaccharide	0
GLP polysaccharide coupled with MBSA[b]	80
Normal rabbit serum	0

[a] Mice received 0.5 ml of antiserum intraperitoneally 2 h before challenge. Experimental details are described in the text.

[b] Coupling of the GLP polysaccharide is described in the text.

to a bovine serum albumin carrier, the GLP polysaccharide elicited the production of antibody in rabbits. When passively transferred to mice, this antibody protected them against a lethal challenge by viable cells of the homologous strain. Furthermore, preliminary results (not presented) indicate that the coupled GLP polysaccharide may be capable of functioning as an active protective antigen.

Pier et al. (24, 25) have isolated a similar high-molecular-weight polysaccharide (which they called PS) from the slime of *P. aeruginosa*. However, GLP polysaccharide differed from it in at least one significant respect: GLP polysaccharide required a protein carrier before its antigenicity could be expressed, whereas PS alone was antigenic. Differing methods of extraction may have exerted a significant influence on the final products obtained. GLP is produced from slime that is gently separated from the viable cell after only 18 h of growth, when very little disruption of the cell wall has occurred. Consequently, contamination of the slime with lipopolysaccharide (LPS) would be very slight. In fact, GLP has been differentiated from LPS on the basis of electrophoresis, immunodiffusion, ultracentrifugation, and protective antibody (28). By contrast, the PS isolated by Pier and his co-workers is derived from old cultures (48 to 72 h) and is processed without attempting to avoid further cellular disruption. Consequently, their PS is extracted from a mixture of slime, LPS, and intracellular products. Although they present evidence indicating that PS is derived from LPS, the possibility exists that the LPS preparation may be in fact contaminated with slime. Supporting this view is the fact that PS disaggregates spontaneously from LPS, indicating a loose association. Moreover, as they observe, the chemical differences between PS and LPS suggest a different origin for the two molecules. Thus, GLP polysaccharide and PS may represent two forms of a polysaccharide derived in common from slime, differing sufficiently in configuration to affect their behavior as antigens.

The relationship between GLP and LPS has been studied in detail in this laboratory (28). First, the homogeneity of GLP has been demonstrated by several methods: GLP elutes as a single peak from diethylaminoethyl in a continuous KCl gradient of increasing molarity; after ultracentrifugation, GLP produces a single peak, as visualized by Schlieren optics; purified GLP produces a single precipitin band against antiserum raised to crude preparations of slime. In addition, we found that GLP is clearly distinguishable from LPS in terms of activity: when GLP and LPS are run together in double diffusion against anti-GLP serum, a band is produced by GLP but not by LPS; the protection conferred by anti-GLP serum against challenge with viable cells is removed by adsorption with GLP but not with LPS. Major chemical differences between GLP and LPS have also been reported (8), notably in sugar composition.

The GLP polysaccharide in uncoupled form was capable of inhibiting the indirect hemagglutination capacity of the anti-GLP serum. This interaction of GLP polysaccharide with specific antibody may have important implications for the explanation of several clinical phenomena related to host response to *P. aeruginosa* infection. A high level of antibody has been identified as the most common detectable host response leading to recovery of patients suffering from *P. aeruginosa* infection (34). Conversely, weak or no antibody response on the part of patients usually accompanies a fatal outcome (33). Weak antibody response, of course, can occur as a result of a defect in the patient's capability to respond, as in agammaglobulinemic patients or those under immunosuppressive therapy. However, interestingly, in chronically infected patients who will eventually demonstrate a strong antibody response, the development of precipitin antibody is usually slow (15). Furthermore, *P. aeruginosa*-infected patients are found who, although they show levels of antibody to *P. aeruginosa*, lack antibody to the serotype of the infecting strain (15). Even patients who do respond to immunization to *P. aeruginosa* may experience a precipitous decline in levels of circulating antibody in association with the onset of bacteremia. This has been observed in burn patients and in patients with neoplasms (33). Some investigators have suggested that a circulating antigen may cause these observed fluctuations in antibody titers (26, 34). A circulating antigen has been found, for example, in the *Haemophilus* infection of children (23). Although interaction of the circulating *Haemophilus* polysaccharide with antibody has not been demonstrated in vivo, its appearance in high levels in the bloodstream correlates strongly with the delayed appearance of antibody to the organism. We did not determine the occurrence of the GLP polysaccharide in vivo; however, it is conceivable that a circulating antigen such as the GLP polysaccharide may be released from the surface of the infecting *P. aeruginosa* into the circulation during the course of infection. The production of slime GLP in vivo has been demonstrated in the experimental infection (11). Its degradation by enzymes in vivo is highly probable; in that case, GLP polysaccharide may be released from the GLP into the circulation and effectively block the appearance of detectable antibody.

Curtailed macrophage function has been implicated by Boxerbaum et al. (7) as a factor in the establishment of *P. aeruginosa* infection in the lungs of cystic fibrosis patients. Interestingly, these investigators found a selective incapacity for the phagocytosis of *P. aeruginosa* by macrophages in the presence of sera from cystic fibrosis patients who had *P. aeruginosa* in their respiratory tracts, the phagocytic capacity of the macrophages being otherwise normal. The authors suggest that the serum itself of these patients is inhibitory. Another interpretation is possible: the formation of a soluble polysaccharide released into the peripheral circulation or neighboring tissue by the infecting strain of *P. aeruginosa* could also function as such an inhibitor. Earlier studies from our laboratory have demonstrated the inhibitory activity of the GLP on phagocytosis (28). Furthermore, the existing evidence indicates that the inhibiting property depends on the presence of the carbohydrate portion of the GLP molecule (29). Derived as it is from the GLP molecule, the polysaccharide identified in this study may have the capability of acting as a circulating inhibitor of macrophage activity.

Further studies on the kinetics of interaction between the GLP polysaccharide and circulating antibody and phagocytic cells are planned. The outcome of these studies may clarify the molecular basis of the clinical phenomena. In addition, it may be possible to compare the chemical composition of GLP polysaccharides from mutant strains as a basis for defining the chemical determinants of the humoral response.

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LITERATURE CITED

1. **Andrews, P.** 1970. Estimation of molecular size and molecular weights of biological compounds by gel filtration. Methods Biochem. Anal. **18**:1–53.
2. **Bartell, P. F., T. E. Orr, and B. Chudio.** 1970. Purification and chemical composition of the protective slime

antigen of *Pseudomonas aeruginosa*. Infect. Immun. **2**: 543–548.

3. **Bartell, P. F., T. E. Orr, and G. K. H. Lam.** 1966. Polysaccharide depolymerase associated with bacteriophage infection. J. Bacteriol. **92**:56–62.
4. **Belcher, P., and A. L. Godbert.** 1954. Semi-micro quantitative organic analysis, p. 42–44, 123–125. Longmans Green and Co., London.
5. **Belcher, R., A. J. Nutten, and C. M. Sambrook.** 1954. The determination of glucosamine. Analyst **79**:201–208.
6. **Bitter, T., and H. M. Muir.** 1962. A modified uronic acid carbozole reaction. Anal. Biochem. **4**:330–334.
7. **Boxerbaum, B., M. Kagumba, and L. W. Matthews.** 1973. Selective inhibition of phagocytic rabbit alveolar macrophages by cystic fibrosis serum. Am. Rev. Respir. Dis. **108**:777–783.
8. **Castillo, F. J., and P. F. Bartell.** 1974. Studies on the bacteriophage 2 receptors of *Pseudomonas aeruginosa*. J. Virol. **14**:904–909.
9. **Coligan, J. E., B. A. Fraser, and T. J. Kindt.** 1978. Immunochemistry of streptococcal Group C polysaccharide and the nature of its cross-reaction with the Forssman glycolipid. Prog. Clin. Biol. Res. **23**:601–612.
10. **Dimitracopoulos, G., and P. F. Bartell.** 1980. Slime glycolipoproteins and the pathogenicity of various strains of *Pseudomonas aeruginosa* in experimental infection. Infect. Immun. **30**:402–408.
11. **Dimitracopoulos, G., J. W. Sensakovic, and P. F. Bartell.** 1974. Slime of *Pseudomonas aeruginosa*: in vivo production. Infect. Immun. **10**:152–156.
12. **Doggett, R. G. (ed.).** 1979. *Pseudomonas aeruginosa*: clinical manifestations of infection and current therapy. Academic Press, Inc., New York.
13. **Flick, M. R., and L. E. Cluff.** 1976. *Pseudomonas* bacteremia: review of 108 cases. Am. J. Med. **60**:501–508.
14. **Forkner, E., E. Frei, J. H. Edgcomb, and J. P. Utz.** 1958. *Pseudomonas* septicemia: observations on 23 cases. Am. J. Med. **25**:877–889.
15. **Høiby, N., and S. Olling.** 1977. *Pseudomonas aeruginosa* infection in cystic fibrosis: bactericidal effect of serum on *P. aeruginosa* strains from patients with cystic fibrosis or other diseases. Acta Pathol. Microbiol. Scand. Sect. C **85**:107–114.
16. **Itaya, K., and M. Ui.** 1965. Colorimetric determination of free fatty acids in biological fluids. J. Lipid Res. **6**: 16–20.
17. **Kozel, T. R., and J. Cazin, Jr.** 1974. Induction of humoral antibody response by soluble polysaccharide of *Cryptococcus neoformans*. Mycopathol. Mycol. Appl. **54**:21–30.
18. **Linne, J. J., and K. M. Ringsrud.** 1970. Basic laboratory techniques for the medical laboratory technician, p. 121–134. McGraw-Hill Book Co., New York.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
20. **Lynn, M., J. W. Sensakovic, and P. F. Bartell.** 1977. In vivo distribution of *Pseudomonas aeruginosa* slime glycolipoprotein: association with leukocytes. Infect. Immun. **15**:109–114.
21. **Lüderitz, O., C. Galanos, V. Lehmann, M. Nurminen, E. T. Rietschel, G. Rosenfelder, M. Simon, and O. Westphal.** 1973. Lipid A: chemical structure and biological activity. J. Infect. Dis. **128**:s17–s29.
22. **Morgan, W. T. J.** 1936. Studies in immuno-chemistry. I. The preparation and properties of a specific polysaccharide from *B. dysenteriae* (Shiga). Biochem. J. **30**: 909–925.
23. **O'Reilly, R. J., P. Anderson, D. L. Ingram, G. Peter, and D. H. Smith.** 1975. Circulating polyribophosphate in *Hemophilus influenzae*, type b meningitis: correlation with clinical course and antibody response. J. Clin. Invest. **56**:1012–1022.
24. **Pier, G. B., H. F. Sidberry, and J. C. Sadoff.** 1978. Protective immunity induced in mice by immunization with high-molecular-weight polysaccharide from *Pseudomonas aeruginosa*. Infect. Immun. **22**:919–925.
25. **Pier, G. B., H. F. Sidberry, S. Zolyomi, and J. C. Sadoff.** 1978. Isolation and characterization of a high-molecular-weight polysaccharide from the slime of *Pseudomonas aeruginosa*. Infect. Immun. **22**:908–918.
26. **Reynolds, H. Y., A. S. Levine, R. E. Wood, C. H. Zierdt, O. C. Dale, and J. E. Pennington.** 1975. *Pseudomonas aeruginosa* infections: persisting problems and current research to find new therapies. Ann. Intern. Med. **82**:819–831.
27. **Robbins, J. B., C. Lee, S. C. Rostogi, G. Schiffman, and J. Henrichsen.** 1979. Comparative immunogenicity of group 6 pneumococcal type 6A(6) and type 6B(26) capsular polysaccharides. Infect. Immun. **26**: 1116–1122.
28. **Sensakovic, J. W., and P. F. Bartell.** 1974. The slime of *Pseudomonas aeruginosa*: biological characterization and possible role in experimental infection. J. Infect. Dis. **129**:101–109.
29. **Sensakovic, J. W., and P. F. Bartell.** 1975. Biological activity of fragments derived from the extracellular slime glycolipoprotein of *Pseudomonas aeruginosa*. Infect. Immun. **12**:808–812.
30. **Smith, H.** 1977. Microbial surfaces in relation to pathogenicity. Bacteriol. Rev. **41**:475–500.
31. **Spiro, R. G.** 1966. Analysis of sugars found in glycoproteins. Methods Enzymol. **8**:3–26.
32. **Sueoka, M., and T. Cheng.** 1962. Fractionation of nucleic acids with the methylated albumin column. J. Mol. Biol. **4**:161–172.
33. **Young, L. S.** 1973. *Pseudomonas aeruginosa* vaccine in cancer patients. Ann. Intern. Med. **79**:518–527.
34. **Young, L. S.** 1974. Role of antibody in infections due to *Pseudomonas aeruginosa*. J. Infect. Dis. **130**:s111–s118.