Antilipopolysaccharide Antibodies and Differential Diagnosis of Chronic *Pseudomonas aeruginosa* Lung Infection in Cystic Fibrosis

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Chronic lung infection in cystic fibrosis is characteristically associated with polyagglutinable, serum-sensitive, mucoid strains of *Pseudomonas aeruginosa*. Enzyme-linked immunosorbent assay (ELISA) methods for standard-free quantitation of immunoglobulin G (IgG) and IgM antibodies to *P. aeruginosa* lipopolysaccharides (LPSs) have been developed. We now report the development of assays for quantitation of monomer and dimer total IgA and IgA anti-LPS antibodies. Use of these methods in diagnosis of early chronic *P. aeruginosa* lung infection was assessed. IgG and IgA anti-LPS levels increased significantly at the onset of chronic infection and continued to increase to very high levels in the later stages of infection. IgM anti-LPS levels also rose at the onset of chronic infection but did not increase further. The function of true- and false-positive rates was illustrated by using various concentrations of IgG, IgA, and IgM anti-LPS for discrimination of patients. Values that gave optimum separations were used for statistical evaluation of the diagnostic sensitivities and specificities of anti-LPS antibody concentrations. The results obtained in these assays were compared with a diagnosis, based on the number of precipitins in crossed immunoelectrophoresis, of serum samples from cystic fibrosis patients. In 64 paired serum samples taken before and immediately after the onset of chronic infection, as defined by crossed immunoelectrophoresis precipitins, the predictive values of a positive ELISA were 86% for IgG and 89% for IgA. The predictive values for a negative ELISA were 98% for IgG and 97% for IgA. Results of the IgM anti-LPS ELISA had a lower predictive value. Immunoblotting and absorption studies showed that IgG anti-LPS antibodies were directed specifically against LPS of *P. aeruginosa*. ELISAs were developed to determine the specific IgG subclasses involved. The increase in IgG anti-LPS involved all four subclasses. Highest anti-LPS titers were seen with IgG1 and IgG4, but the largest relative increases were seen with IgG2 and IgG3.

Chronic pulmonary infection with *Pseudomonas aeruginosa* is characteristic of cystic fibrosis (CF) patients and is the major factor determining the severity of illness and mortality (28). After a period of relatively harmless colonization of the mucosal lining by *P. aeruginosa*, the clinical condition begins to deteriorate and symptoms of respiratory infection increase. Although long-term eradication of *P. aeruginosa*, once the infection has become established, is seldom achieved, exacerbations can be brought into remission by regular intensive antibiotic treatment. Early and active treatment regimens improve the lung function, wellbeing, and long-term survival of patients (20). It is therefore important to identify the early stages of infection and the appropriate time to start intensive antimicrobial therapy.

The establishment of chronic invasive, tissue-destroying infection is associated with characteristic changes in the bacteria. Thus, *P. aeruginosa* isolated from sputum of CF patients with chronic pulmonary infection are often mucoid, serum sensitive, and polyagglutinable (14). We have previously shown that the lipopolysaccharide (LPS) (O antigen; endotoxin) of polyagglutinating clinical isolates is deficient in the O-polysaccharide chain but conserved in the core oligosaccharide and lipid A part of the molecule (5). The prevalence of O-deficient strains in which common antigenic sites are accessible to antibodies makes the LPS of such strains potentially useful in diagnostic assays of chronic lung infection. Serological assays determining antibodies to *P. aeruginosa* (1, 2, 19, 23) and *P. aeruginosa* products (12, 16, 21) have previously been found to be of value in monitoring both the course of infection and treatment. However, none of the tests using single antigens has been able to detect the antibody response in all *P. aeruginosa*-infected CF patients. The number of different kinds of precipitating antibodies (precipitins) against *P. aeruginosa* whole-cell sonic extracts rises during the infection and is used in combination with bacteriological criteria to diagnose the onset of chronic *P. aeruginosa* lung infection in Danish CF patients (15). However, more sensitive assays detecting antibodies of different classes and subclasses specific for defined antigens would be useful for characterization of early specific antibody responses in these patients. Information about specific antibody responses could be especially useful in the early diagnosis of cases in which there is a rapid development of the lung infection.

In a longitudinal study of concentrations in serum of antibodies to *Pseudomonas* LPSs during the course of chronic lung infection in CF patients, we found a good positive correlation between immunoglobulin (IgG) antibodies to LPS from a clinical isolate of *P. aeruginosa* O:3/9 and the number of precipitins to *Pseudomonas* sonic extracts (8). An increase in the number of precipitins, in turn, reflects the severity of the disease (8, 15). In the study reported here, we have further characterized the immune response to LPS of a polyagglutinable *P. aeruginosa* strain involving IgG, IgG subclasses 1 to 4, IgM, and IgA at the onset of chronic *P. aeruginosa* lung infection in CF patients and evaluated the diagnostic usefulness of these antibodies.

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MATeRIALS AND METHODS

NORMAL VALUES. To establish normal values for concentrations of anti-LPS antibodies, serum samples from 25 infants (0 to 1 year of age), 15 children (2 to 14 years), and 60 adults (15 to 40 years) with no history of P. aeruginosa infection were investigated.

CF patients. CF patients were monitored regularly by clinical and bacteriological examinations at the Danish CF Center at Rigshospitalet. The diagnosis of CF was based on accepted criteria, including abnormal sweat electrolyte levels, exocrine pancreatic insufficiency, and altered pulmonary function (28). Noncolonized CF patients were defined as CF patients with no growth of P. aeruginosa in sputum at monthly examinations and with no or one precipitin in crossed immunoelectrophoresis (CIE). The onset of chronic P. aeruginosa lung infection was defined as the point at which the bacteria had been found in every sputum sample for at least 6 months and serum contained two or more CIE precipitins to P. aeruginosa (15). Serum samples obtained while the patients were noncolonized (CF0) were available from 64 patients. These were paired with the first serum samples taken after the onset of chronic P. aeruginosa lung infection (CF+) for enzyme-linked immunosorbent assay (ELISA) determination of IgM, IgG, and IgA anti-Pseudomonas LPS antibody concentrations. Serum samples from 10 CF patients with lung infection for 8 to 16 years who showed consistent growth of P. aeruginosa in sputum were examined for precipitins and anti-LPS antibodies and used as examples of samples from patients with late chronic infection (CF+).

LPS antigens. A polyagglutinating mucoid P. aeruginosa 170 O-group 3/9 bacteriophage type 21,48,68,109,352,1214, F3,M4,col11+ was isolated from sputum of a CF patient with chronic pulmonary infection. LPS was extracted by the phenol-chloroform-petroleum ether method (10). The LPS was purified by successive ultracentrifugation steps, purged of associated inorganic cations and low-molecular-weight basic amines by electrodialysis (9), and converted to its uniform triethylamine salt form. The detailed chemical composition and immunochemical characterization of this O-poly saccharide-deficient LPS are reported elsewhere (5).

Pseudomonas standard antigens. Water-soluble antigens from P. aeruginosa strains representing the 17 serotypes of the International Antigen Typing Scheme were prepared as previously described (15) and used as a standard antigen in CIE.

Anti-LPS ELISAs. For ELISAs, 96-well polystyrene microtiter plates (Immunoplate I, Nunc A/S, Roskilde, Denmark) were coated with 1 μg of P. aeruginosa O:3/9 LPS per 100 μl in phosphate-buffered saline containing 0.02 M MgCl2. The coated plates were covered and stored at 4°C, with the coating solution remaining in the wells for at least 72 h, and were usable for at least 6 months. Before use and between subsequent incubation steps, the plates were washed with 0.15 M NaCl-0.1% Tween 20 (washing solution). The test sera were diluted 1:50 and 1:500 in phosphate-buffered saline-0.1% Tween 20 and incubated in the LPS-coated wells (100 μl per well) overnight at 22°C. Patient sera containing high concentrations of anti-LPS antibodies were diluted further (1:5,000 and 1:50,000) and reasayed. Although the serum incubation was sufficiently complete with these concentrations, incubation was repeated for practical reasons. After washing, incubation proceeded for 1 h, using 100 μl of peroxidase-conjugated rabbit anti-human IgG (1:15,000; Dakopatts, Glostrup, Denmark), rabbit anti-human IgM (1:2,000; Dakopatts), or rabbit anti-human IgA (1:5,000; Dakopatts) diluted in phosphate-buffered saline-0.1% Tween 20 per well. Color was developed by addition to each well of 100 μl of enzyme substrate consisting of 14 mg of o-phenylenediamine and 7.5 μl of hydrogen peroxide (30%, vol/vol) in 18 ml of distilled water. The reaction was terminated after 30 min by addition of 150 μl of 2.5 M sulfuric acid per well. A492 was measured with a spectrophotometer (model SLT-210; Kontron, Zurich, Switzerland). Quantitative results of IgG, IgM, and IgA anti-LPS antibodies were obtained by comparison with results for a standard CF serum pool. Concentrations of specific anti-LPS antibodies in the standard CF serum pool were determined by standard-free quantitation (6). Briefly, double-antibody sandwich ELISAs for total IgG, IgM, and IgA were performed simultaneously on a separate part of the ELISA plate, using the same conjugates, buffers, and assay conditions. The concentrations of specific IgG, IgM, and IgA anti-Pseudomonas LPS antibodies in the CF serum pool were then read on the respective standard curves for total IgG, IgM, and IgA. Results were corrected for differences in antibody binding as described previously (6).

The double-sandwich ELISA for quantitation of IgG and IgM has been described elsewhere (3, 7). A description of the ELISA for total IgA is given below.

ELISA for total IgA in serum. Each well of polystyrene microtiter plates was coated with 100 μl of rabbit anti-human IgA (Dakopatts) diluted 1:5,000 in 0.1 M carbonate buffer (pH 9.8). Plates were stored at 4°C for at least 72 h and used within 3 months. After four washes with washing solution, the plates were incubated overnight at 22°C with serial dilutions of 5- to 200-μg/liter concentrations of an IgA standard human serum (Seronom 103; Nycomed, Oslo, Norway) in phosphate-buffered saline-0.1% Tween 20-0.5% bovine serum albumin (incubation buffer). After washing, the wells were incubated for 1 h with peroxidase-conjugated rabbit anti-human IgA (α-chain specific; Dakopatts) diluted 1:10,000 in the incubation buffer. Color was developed as described above. Quantitative results were obtained from a calibration graph (3, 6, 7). Measurement of IgA in serum and colostomy by this ELISA was compared with results obtained by conventional rocket immunoelectrophoresis (17).

ELISA for anti-P. aeruginosa LPS IgG subclasses. ELISAs were developed to investigate the distribution and development of specific anti-P. aeruginosa O:3/9 LPS IgG subclasses. Serum samples from healthy donors were used to optimize the incubation conditions. Use of sera from healthy persons ensured the development of an assay sensitive enough to measure anti-LPS antibodies in healthy controls and in noncolonized CF patients. The optimal concentrations for the conjugates were determined as previously described (3). For assay of anti-LPS IgG subclasses, peroxidase-conjugated mouse monoclonal antibodies specific for human IgG1 (1:5,000), IgG2 (1:1,000), IgG3 (1:500), or IgG4 (1:1,000) in PBS-Tween 20 (Janssen Biochemica, Beerse, Belgium) were used. Coating of microtiter plates, incubation times, and experimental conditions were as described above. Anti-LPS IgG1 to -4 titers were expressed as the reciprocal dilutions of samples corresponding to an A492 of 0.500.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. LPS preparations from P. aeruginosa strains 70 (6) and clinical isolates 1118 (O3), 174 (O9), 170 (O3:9), 1570/483 (O3:6/9), and from Shigella sonnei, Klebsiella pneumoniae, Salmonella minnesota R60 (Ra), Escherichia coli Ra, E. coli
O111:B4, and *E. coli* O55:B5 were treated for 5 min at 100°C in 0.05 M Tris hydrochloride (pH 6.8)–2% sodium dodecyl sulfate–10% sucrose–0.01% bromphenol blue and separated on a sodium dodecyl sulfate-polyacrylamide gel containing 5 and 14% acrylamide in the stacking and separating gels, respectively. Gels were silver stained (26) or the samples were transferred to nitrocellulose paper as previously described (8). After blocking in 1% gelatin–Tris-buffered saline (TSG), the nitrocellulose paper was incubated for 2 h with CF serum diluted 1:500 in TSG. The nitrocellulose paper was then washed three times in Tris-buffered saline and incubated for 1 h with peroxidase-conjugated rabbit antibodies to human IgG, IgM, or IgA (Dakopatts) diluted 1:500 in TSG. Color was developed in 0.003% (wt/vol) 4-chloro-1-naphthol-0.05% (vol/vol) *H*<sub>2</sub>*O*.<sup>2</sup>

Precipitating antibodies against *P. aeruginosa*. *P. aeruginosa* precipitins were determined by CIE as described earlier (15). Briefly, 2 µl of the standard antigen preparation (22 µg/ml of protein) was applied to a well and separated in the first dimension (10 V/cm, 1 h) in 1% agarose gels. Second-dimension separation was performed for 18 h at 1 to 2 V/cm into 1% agarose containing 15 µl of patient serum per cm<sup>2</sup>. The immunoprecipitates were stained with Coomassie brilliant blue, and the number of precipitin peaks was counted.

**RESULTS**

**ELISA for total IgA and IgA anti-LPS.** A comparison of IgA concentrations in serum as measured by conventional rocket immunoelectrophoresis and the IgA ELISA is shown in Fig. 1. The correlation of the two assays (excluding the two extreme values [rocket, ELISA] = [<=0.01, 0.002] and [10.9, 14.2]) can be expressed as ELISA = 0.06 + 0.93 × rocket (n, 97, r, 0.96). The dose-response curves of IgA in serum (predominantly monomer IgA) and colostrum (predominantly secretory dimer IgA) as measured by the IgA ELISA are shown in Fig. 2. Identical maximal bound enzyme activity as well as parallel dilution curves of serum and colostrum IgA indicate that the α-chain was detected with equal efficiency regardless of molecular configuration.

The dose-response curve of IgA anti-*P. aeruginosa* LPS in the standard CF serum pool was assayed simultaneously to confirm parallelism with the extended IgA standard curve (●) used for quantification of specific IgA anti-LPS.

**Anti-Pseudomonas LPS antibody concentrations.** Table 1

**FIG. 2.** Dose-response curves of IgA in serum and colostrum as measured by the IgA ELISA. The dose-response curve of IgA anti-*P. aeruginosa* LPS antibodies in the CF serum pool was assayed simultaneously to confirm parallelism with the extended IgA standard curve (●) used for quantification of specific IgA anti-LPS.
shows the number of precipitins in CIE and the concentrations of anti-LPS antibodies in healthy subjects and CF patients. The number of precipitins in normal subjects was zero to one. In contrast, the IgM, IgG, and IgA anti- *P. aeruginosa* LPS antibody concentrations in normal subjects were age related. The IgG and IgA anti-LPS antibody concentrations reached the highest levels in adults, whereas IgM anti-*P. aeruginosa* LPS concentrations reached the highest levels in children. The median IgA anti-*P. aeruginosa* LPS concentrations were the same for healthy subjects and CF patients before the onset of chronic lung infection (CF0) but increased significantly (*P < 0.001*, paired *t* test) in CF patients at the onset of chronic infection (CF+). IgG and IgA anti-LPS concentrations and the number of precipitins continued to rise to very high levels in later stages of the infection (Table 1). In contrast, the median concentrations of IgM anti-*P. aeruginosa* LPS antibodies increased from normals, via CF0 to CF+ patients, after which no further increase was found. In fact, there was a small decrease in median IgM anti-*P. aeruginosa* LPS in early (CF+) and late (CF++) chronically infected patients (Table 1). Figure 3 shows the distribution of IgG, IgA, and IgM anti-*P. aeruginosa* LPS antibodies among healthy subjects and 64 CF patients before and immediately after diagnosis of chronic *P. aeruginosa* lung infection. Significant differences were observed in the distribution of IgG, IgA, and IgM anti-LPS antibodies among the three groups (normal subjects, CF0 patients, and CF+ patients; *P < 0.001* in all three cases, chi-square test). There was, however, some overlap in IgG and IgA anti-LPS concentrations between CF0 and CF+ patients (Fig. 3).

The nosographic sensitivities and specificities of the anti-*P. aeruginosa* LPS ELISAs were evaluated in CF+ and CF0 samples defined as infected and noninfected, respectively, by the CIE test (Table 2). The predictive value of a positive anti-LPS ELISA (PVpos), used to detect early *P. aeruginosa* infection, was the diagnostic specificity, and the predictive

### TABLE 2. Distribution of CFO and CF+ patients and diagnostic values of anti-LPS ELISAs

<table>
<thead>
<tr>
<th>Anti-LPS assay with given concn (μg/ml) of:</th>
<th>No. of patients</th>
</tr>
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<tr>
<td></td>
<td>CF+</td>
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<tr>
<td>IgG+</td>
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<tr>
<td>0-40</td>
<td>1</td>
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<tr>
<td>&gt;40</td>
<td>63</td>
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<td>IgA+</td>
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<td>0-8</td>
<td>2</td>
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<td>&gt;8</td>
<td>62</td>
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<td>IgM+</td>
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<tr>
<td>0-33</td>
<td>17</td>
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<tr>
<td>&gt;33</td>
<td>47</td>
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</table>

* Significant difference of CF+ versus CFO, *P < 0.0005* (chi-square test); nosographic specificity, 54/64 = 0.84; nosographic sensitivity, 63/64 = 0.98; PVpos, 63/73 = 0.86; PVneg, 54/55 = 0.98.
* Significant difference of CF+ versus CFO, *P < 0.0005* (chi-square test); nosographic specificity, 56/64 = 0.88; nosographic sensitivity, 62/64 = 0.97; PVpos, 62/70 = 0.89; PVneg, 56/58 = 0.97.
* Significant difference of CF+ versus CFO, *P < 0.0005* (chi-square test); nosographic specificity, 38/64 = 0.59; nosographic sensitivity, 47/64 = 0.73; PVpos, 47/73 = 0.64; PVneg, 38/55 = 0.69.
value of a negative test (PVneg), used to rule out chronic infection, was the diagnostic sensitivity (Table 2). PVpos and PVneg of the IgG and IgA anti- P. aeruginosa LPS ELISAs were high in both cases (Table 2). Higher values were obtained if a combination of elevated concentrations in both tests was used: PVpos and PVneg of IgG anti-LPS at >40 µg/ml and IgA anti-LPS at >8 µg/ml were 95% (61 of 64). However, PVpos and PVneg for elevated IgG or IgA anti-LPS concentrations or both were 81 (64/79) and 100% (49/49). In comparison, the sensitivity and specificity of the IgM anti-LPS ELISA were lower (Table 2).

The values of the IgG and IgA anti-LPS concentrations used to distinguish chronically infected CF patients from uninfected subjects may, however, be chosen differently and are a balance of false-positive and false-negative results. The successive overlap between CF+ and healthy subjects as well as between CF+ and CF0 patients can be illustrated for the IgG, IgA, and IgM anti-LPS ELISAs by means of receiver-operating characteristic curves (27), i.e., the relationship between the true-positive rate (rate of positives among patients with infection) and the false-positive rate (FPR; rate of positives among patients without infection) when the criterion of positive varies over the range of anti-LPS concentrations (Fig. 4). By using this method, the concentrations of anti-LPS antibodies resulting in the minimal overlap of CF+ and CF0 patients, as defined by CIE precipitins, was found and used to evaluate the diagnostic values presented above. Figure 4 shows equally good separation of CF+ and CF0 patients by the IgG and the IgA anti-P. aeruginosa LPS ELISAs. The IgM anti-LPS curve was always lower than the IgG and IgA anti-LPS curves, demonstrating less separation of patients by IgM anti-LPS regardless of the concentrations chosen. Figure 4 also demonstrates better separation of CF+ patients from normal subjects than from CF0 patients by IgG anti-LPS concentrations.

**Specificity of anti-LPS antibodies.** The specificities of the anti-P. aeruginosa LPS antibodies in sera with elevated anti-LPS concentrations were investigated further. The anti-P. aeruginosa LPS antibodies could in each case be absorbed by P. aeruginosa 0:3/9 LPS coated onto sheep erythrocytes (11). None of the 10 CF0 serum samples with elevated IgG anti-P. aeruginosa LPS antibody concentrations had elevated (>40 µg/ml) concentrations of antibodies to other LPSs (including strains of E. coli and of Salmonella and Serratia species) as measured by ELISA (6). In immunoblotting, IgG anti-LPS antibodies in the 10 CF0 serum samples with elevated IgG anti-P. aeruginosa LPS concentrations reacted to P. aeruginosa 0:3/9 LPS and also to LPSs from other clinical isolates of P. aeruginosa but only very weakly to LPSs of other gram-negative bacteria (Fig. 5).

**Anti-P. aeruginosa IgG subclasses.** Figure 6 shows the distribution of the IgG subclass specificity of the anti-LPS antibodies of normal subjects and CF0 and CF+ patients. The increase in IgG anti-LPS from CF0 to CF+ involved all four subclasses. Highest anti-LPS titers were seen in IgG1 and IgG4. The largest relative increase within an anti-LPS IgG subclass was found in IgG3 and IgG2.
DISCUSSION

A sandwich ELISA for quantitation of total IgA was developed by using microtest plates and commercially available reagents. The quantitative results of IgA levels in sera obtained with this ELISA correlated well ($r$, 0.96) with those obtained by traditional rocket immunoelectrophoresis (Fig. 1). The high sensitivity and demonstrated detection of α-chains in both monomer and secretory dimer forms makes this ELISA convenient for measuring total IgA in sera and secretions. In comparison with rocket immunoelectrophoresis, the ELISA for total IgA has a higher capacity, is more sensitive, and is simpler, faster, and less expensive.

In this study, ELISA for total IgA was used together with an indirect ELISA for IgA anti-LPS antibodies to quantitate specific IgA anti- P. aeruginosa LPS antibodies in a combined design (Fig. 2). By reading the anti-LPS concentration on the simultaneously performed standard curve for total IgA, the amounts of specific anti-LPS antibodies could be conveniently estimated in the CF serum pool as described earlier (6). The same principle was used for quantitation of the IgG and IgM anti- P. aeruginosa antibodies in normal subjects and CF patients.

The results showed that there was no significant difference in the number of precipitins and the median IgA anti- P. aeruginosa LPS antibody concentrations between the healthy subjects and CF0 patients. In contrast, the median concentration of IgM anti- P. aeruginosa LPS antibodies and, to a lesser extent, IgG anti-LPS, increased from levels in age-matched normals, via CF0 to CF+ patients. No further increase in IgM anti-LPS was then found. In fact, there was a small decrease in median IgM anti- P. aeruginosa LPS from early (CF+) and late (CF+++) chronically infected patients (Table 2). These findings are in agreement with earlier results and are consistent with IgM being an early and IgG a later immune response to bacterial infections (4, 8). The less pronounced and persistent IgM anti-LPS response is also consistent with LPS being a T-cell-independent antigen (4).

Establishment of a diagnostic test defining the onset of chronic lung infection is complicated because the increase in concentrations of anti- P. aeruginosa LPS antibodies other than IgM is a continuum toward extremely high values in the later stages of infection (Table 1; 8). A useful definition of the onset of chronic P. aeruginosa lung infection is the demonstration of two or more precipitins against P. aeruginosa whole-cell sonic extracts in CIE (15). A comparison was therefore made of the diagnostic values of the anti-LPS ELISAs and CIE. By these criteria, the IgM anti- P. aeruginosa LPS concentrations in serum were of less diagnostic significance than the concentrations of anti-LPS IgG and IgA (Fig. 3). The nosographic specificities and sensitivities of the IgG and IgA anti- P. aeruginosa LPS ELISAs were higher and showed both high PVpos (86 and 89%) and high PVneg (98 and 97%) (Table 2). The concentrations of IgG and IgA anti- P. aeruginosa LPS antibodies chosen for evaluation of the anti-LPS ELISA as a diagnostic test are the points of minimal overlap between the groups tested (CF+ versus CF0) (Fig. 4). However, a different diagnostic value of anti-LPS concentrations could be chosen on the basis of other specific clinical and practical considerations. The lower nosographic specificity of the IgG anti-LPS ELISA in comparison with CIE was due to 10 of the 64 CF0 patients having elevated anti-LPS concentrations (Table 3). However, 7 of these 10 patients did not have elevated IgA anti- P. aeruginosa LPS antibody levels. Similarly, five of the eight CF0 patients with elevated IgA anti- P. aeruginosa LPS antibodies did not have elevated specific IgG anti-LPS antibody levels. Thus, the combination of both elevated IgG and IgA anti- P. aeruginosa LPS antibodies may be characteristic of chronically infected CF patients. The elevation of IgA or IgG anti- P. aeruginosa LPS concentrations alone may be an earlier sign of chronic lung infection.

The IgG anti- P. aeruginosa LPS antibodies in serum samples from the 10 CF0 patients with elevated antibody concentrations were found to be specific for P. aeruginosa LPS in a series of experiments, including immunoblotting. It is interesting that in immunoblotting (Fig. 5), most of the samples from CF patients reacted with LPS from clinical isolates of P. aeruginosa other than the O:3/9 strain. This result could be attributable to the similar chemical compositions of these LPSs, which differ only in the molar ratios of rhamnose, glucose, heptose, and alanine (5).

The differences noted between the anti-LPS ELISA and the CIE precipitins may reflect differences in the sensitivity of the two tests and suggest an earlier onset of the anti- Pseudomonas antibody response than is currently recognized. The anti-LPS ELISA method may be more sensitive than the CIE precipitin method for detecting early lung infection and distinguishing early infection from colonization. The higher sensitivity of the anti-LPS ELISAs is very useful for early detection of rapidly developing lung infections.

The anti-LPS ELISA described here is a diagnostic tool comparable in sensitivity and specificity to CIE. Determination of precipitins is time consuming and requires a laboratory that routinely performs CIE, whereas the ELISA method has the advantage of easier handling of a large number of samples and rapid quantitation of results by an ELISA plate reader that can also be computerized. Another advantage of the anti-LPS ELISA is that it uses a single purified antigen (a chemically defined LPS molecule) in a quantitative method capable of distinguishing different antibody immunoglobulin classes and subclasses.

An increase in median titer of IgG anti-LPS CF+ was found in all four IgG subclasses (Fig. 5), as previously reported by Moss et al. (18). However, substantial individual variations were seen, and no single subclass was consistently elevated.
or depressed despite the overall increase in IgG response to *P. aeruginosa* LPS. The median titer of IgG2 anti-*P. aeruginosa* LPS in CF0 sera tended to be lower than in normal sera; however, this titer increased at the onset of chronic infection (Fig. 4). Similar results have been reported by Moss et al. (18) and suggest a relative deficiency in IgG2 antibody levels to polysaccharide antigens (25). Among the early chronically infected CF patients (CF+), the highest titers were seen in IgG1 and IgG4. The different concentrations of conjugated antibodies used for detection of the IgG subclasses makes direct comparison of the titers difficult. However, since the higher concentrations of conjugated antibodies were used to improve sensitivity in order to detect CF sera of low anti-LPS immunoactivity, it is reasonable to establish the range of immunoactivity of anti-*P. aeruginosa* IgG in early chronically infected CF patients as IgG1 > IgG4 > IgG2 > IgG3. The highest percentage increase in median titers was, however, observed in IgG3 and IgG2 anti-LPS, both known to be primarily antisaccharide antibodies (25). An increase in IgG3 and IgG4 anti-*P. aeruginosa* LPS was also found by Staryock et al. (24). However, these authors failed to demonstrate any IgG1 antibody response in CF sera. That finding is in contrast with the results of this study and the generally held opinion that opsonic IgG1 constitutes the majority of the total IgG in serum and that saccharide antigens and LPS primarily induce IgG1 antibodies (13). Moreover, the CF patients we examined at later stages of chronic lung infection had manyfold-higher titers of all anti-*P. aeruginosa* IgG subclasses (data not shown). The reason for this discrepancy is not clear but could be partly explained by individual variations in the 10 patients. Alternatively, there could be technical differences in the sensitivity of the anti-LPS ELISA, the LPS preparation, or the monoclonal anti-IgG1 conjugate used. Whether the distribution among specific IgG subclasses changes during the course of the chronic infection as suggested by Moss et al. (18) is currently being investigated in a longitudinal study.

The early increase in specific IgG and IgA anti-LPS antibodies and the extremely high concentrations in later stages of the infection suggest that *P. aeruginosa* LPS is a highly relevant antigen to investigate in CF patients. This phenomenon is probably due to the pronounced biological activity and toxicity of the LPS endotoxin molecule (22). IgG and IgM anti-LPS antibodies have been suggested to protect against the deleterious effects of endotoxinemia. However, continuous high concentrations of IgG and IgA anti-LPS antibodies may contribute to the formation of immune complexes and may activate complement and other mediators of inflammation locally in the lungs, thus contributing to lung tissue damage. Although IgA is principally an antibody of mucosal defense, the IgA anti-LPS levels in serum paralleled the increase in IgG anti-LPS and CIE precipitins. The high concentrations of IgA anti-*P. aeruginosa* LPS in the serum of CF patients would be expected because of the mucosal inflammation in the upper respiratory tract and could serve to eliminate LPS antigens by excretion via the bile.

We recommend the quantitation of specific IgG and IgA antibodies to *P. aeruginosa* LPS by ELISA as a diagnostic tool for CF to distinguish between superficial colonization and early or persistent infection caused by *P. aeruginosa*. The latter conditions would serve to initiate anti-*P. aeruginosa* therapy in our CF center.

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**LITERATURE CITED**


