*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 10716–10720, November 1992
Microbiology

# Cloning and surface expression of *Pseudomonas aeruginosa* O antigen in *Escherichia coli*

(lipopolysaccharide/mucosal immunity/oral vaccines)

JOANNA B. GOLDBERG*, KAZUE HATANO, GLORIA SMALL MELULENI, AND GERALD B. PIER

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115-5899

*Communicated by Bernard D. Davis, July 27, 1992 (received for review May 17, 1992)*

ABSTRACT **As a step toward developing recombinant oral vaccines, we have explored the feasibility of expression of O polysaccharide antigens from *Pseudomonas aeruginosa* by *Escherichia coli*. We cloned in *E. coli* HB101 a 26.2-kilobase DNA fragment from *P. aeruginosa* strain PA103 that specifies the production of the O polysaccharide of Fisher immunotype 2 (IT-2) strains. The recombinant organism incorporated the *P. aeruginosa* IT-2 O polysaccharide onto the core of the *E. coli* lipopolysaccharide (LPS). Transfer of the recombinant plasmid to three LPS-rough strains of *P. aeruginosa* resulted in synthesis of IT-2 O antigen, and two of these transconjugant strains also synthesized a second O polysaccharide, presumably representing expression of a repressed, or an incomplete, set of genes for an endogenous O polysaccharide. Rabbits injected with the purified recombinant LPS made antibody specific for *P. aeruginosa* IT-2 O side chains, as did mice fed the recombinant *E. coli* strain. Expression of *P. aeruginosa* O antigens by enteric bacteria makes it possible to study these recombinant strains as oral vaccines to prevent *P. aeruginosa* infections.**

Lipopolysaccharide (LPS) is an important virulence factor for *Pseudomonas aeruginosa* and is a major target antigen for protective immunity against infection caused by LPS-smooth strains (1–6). Variation in the structure of the O side chains differentiates the serotypes among LPS-smooth strains of *P. aeruginosa* (7, 8). The smooth form of LPS is also responsible for resistance to the bactericidal activity of normal serum (9, 10): LPS-rough strains making either short O-side-chain antigens or low levels of longer O side chains are often killed by serum concentrations of <10%.

*P. aeruginosa* is responsible for acute infections in compromised hosts and for chronic pulmonary infections in patients with cystic fibrosis (CF) (5). Isolates from non-CF patients elaborate a smooth LPS (4, 11). Among CF patients, the initial colonization of the respiratory tract usually involves LPS-smooth strains; as colonization becomes chronic, these strains tend to convert to production of a rough LPS (9, 12, 13). Phenotypic conversion from an LPS-smooth to an LPS-rough strain is accompanied by a second transition involving elaboration of an extracellular polysaccharide, called mucoid exopolysaccharide or alginate (9, 12–14).

In patients at risk for *P. aeruginosa* infections, the organism probably initially colonizes a mucosal surface. Thus one potential approach towards the prevention of *P. aeruginosa* infections is to inhibit the LPS-smooth strain's initial colonization of the mucosal surface. Recently, attenuated enteric bacteria that express protective antigens have been developed that are still capable of colonizing the gastrointestinal tract and provoking an immune response from a mucosal surface (15–17). These strains can potentially be used as vehicles for the delivery of protective antigens, such as O polysaccharides, derived from a variety of bacteria (18–20). Here we report the isolation and characterization of cloned DNA that encodes production of the LPS O side chain from the Fisher immunotype-2 (IT-2) strain of *P. aeruginosa*. When this DNA fragment was present in *Escherichia coli* HB101, the IT-2 serotype antigen was expressed and attached to the lipid A core of the LPS. Injection of the recombinant LPS in rabbits induced serum antibodies to the IT-2 antigens. In addition, oral immunization with *E. coli* expressing this recombinant LPS elicited serum and mucosal antibodies reactive with *P. aeruginosa* IT-2 O antigens.

## MATERIALS AND METHODS

**Bacterial Strains and Plasmid Mobilization.** *P. aeruginosa* LPS-rough strains 2192, 2344, and FRD1 were isolated from the sputa of CF patients. *P. aeruginosa* strain PA103 is a Fisher IT-2 strain (ATTC 29260) initially described by P. V. Liu (21). The strains of *P. aeruginosa* used for the isolation of control LPS were clinical isolates representative of the seven Fisher immunotypes (22) and were obtained from the microbiology laboratory of Brigham and Women's Hospital. A gene bank from *P. aeruginosa* strain PA103 was constructed from partially *Eco*RI-digested DNA fragments ligated into the broad-host-range plasmid pLAFR1 (23) as previously described (24) and was provided by D. E. Ohman (University of Tennessee, Memphis). Recombinant plasmids were introduced into recipient strains of *P. aeruginosa* via triparental matings as previously described (24). Transformation of plasmid DNA into *E. coli* was accomplished by treatment with $CaCl_2$ (25).

**Selection for Serum-Resistant Transconjugants.** Transconjugants of LPS-rough *P. aeruginosa* strains from triparental matings were initially grown on cetrimide agar (to select for *P. aeruginosa*) containing 100 μg of tetracycline per ml (to select for the plasmid-encoded antibiotic resistance). Growth from these plates was then suspended at approximately $10^7$ colony-forming units (cfu)/ml in 25% fresh human serum in 1% proteose peptone (9) to enrich for LPS-smooth, serum-resistant transconjugants. After overnight incubation at 37°C, survivors were transferred to the same medium for a second round of enrichment under the same conditions. Cultures were then diluted and plated onto cetrimide agar containing 100 μg of tetracycline per ml for the isolation of single colonies of *P. aeruginosa*.

**Serotype Analysis.** Serum-resistant transconjugants were screened for elaboration of smooth LPS by agglutination with O-side-chain-specific polyclonal rabbit antibodies raised to purified LPS (26), by immunodiffusion with autoclave extracts of broth-grown bacteria (9) and rabbit antisera raised to whole *P. aeruginosa* cells, and by colony blots (27) with the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LPS, lipopolysaccharide; CF, cystic fibrosis; IT, immunotype; cfu, colony-forming units.

*To whom reprint requests should be addressed at: Channing Laboratory, 180 Longwood Avenue, Boston, MA 02115-5899.

O-side-chain-specific polyclonal sera. The elaboration of a smooth LPS was confirmed by using SDS/PAGE analysis of LPS preparations (see below).

**DNA Isolation and Characterization.** Plasmid DNA was isolated as described from *P. aeruginosa* (24) and *E. coli* (28). Restriction endonuclease digestions were performed according to the supplier. Southern blot hybridizations were performed as previously described (24). Plasmid DNA was labeled with [$^{32}$P]dCTP by using a nick translation kit (Boehringer-Mannheim).

**LPS Isolation.** LPS was extracted from LPS-smooth *P. aeruginosa* and *E. coli* by the phenol/water procedure (29). Material in both the aqueous phase and the phenol phase was recovered by precipitation with ethanol (4 vol of 95% ethanol added to each phase) and was further purified as previously described (30). LPS from rough strains was extracted with chloroform/methanol/ether (31) and further purified as described (30). Crude extracts containing serologically active LPS were prepared by autoclaving $10^9$ cfu of *P. aeruginosa* for 20 min in saline and removal of the bacterial cells by centrifugation. Whole-cell extracts were depleted of proteins by treatment with proteinase K, as described by Hitchcock and Brown (32).

**Antisera.** Polyclonal rabbit antibodies to purified LPS of *P. aeruginosa* were prepared as described elsewhere (26). These sera react in ELISA only with homologous LPS, indicating their specificity for the O-side-chain-serotype antigens. Antibodies to LPS isolated from *E. coli* HB101(pLAFR1) and *E. coli* HB101(pLPS2) (see below) were raised in rabbits by an initial subcutaneous injection into different sites of five 0.1-ml vol of complete Freund's adjuvant containing 0.1 mg of LPS. One week later, 0.1 mg of LPS in 0.5 ml of saline was injected intravenously three times weekly for 2 more weeks. One week after the final injection blood was obtained from the ear artery and serum was collected.

**Serologic Analysis.** Sera from immunized animals were tested by ELISA for antibody to the various LPS antigens. Each LPS was dissolved to a concentration of 10 μg/ml in 0.04 M sodium phosphate buffer, pH 7.2, and 100 μl was used to sensitize ELISA plates (Immulon II, Dynatech) for 2 hr at 37°C. The remainder of the procedure utilized standard ELISA protocols.

**SDS/PAGE and Immunoblots.** Isolated LPS was subjected to SDS/PAGE analysis on 12.5% polyacrylamide gels and visualized by silver staining (27). Immunoblots were obtained by electroblot transfer of LPS from SDS/PAGE to nitrocellulose membranes followed by visualization as described (33).

**Chemical Analysis.** LPS isolated from *P. aeruginosa, E. coli* HB101(pLAFR1), and *E. coli* HB101(pLPS2) was analyzed for lipid content after hydrolysis in 4 M HCl in methanol for 18 hr at 95°C. Free fatty acid methyl esters were analyzed by gas–liquid chromatography on a 0.53-mm inside diameter, 25-m-long Nukol column (Supelco) with hydrogen as a carrier gas (30 ml/min), and flame ionization detection in a Hewlett-Packard 5880 gas–liquid chromatograph. The oven temperature was maintained at 190°C.

Monosaccharide constituents of LPS antigens were released by hydrolysis in 2 M methanolic HCl at 95°C for 48 hr. The samples were dried under $N_2$ and converted to the trimethylsilyl derivatives with Sil-Prep (Alltech Associates). Monosaccharide constituents were analyzed on a 0.25-mm inside diameter, 25-m-long RSL 310 column (Alltech Associates) in the gas–liquid chromatograph. Hydrogen (1 ml/min) was the carrier gas, nitrogen was the make-up gas (30 ml/min), and detection was by flame ionization. The initial oven temperature of 130°C was maintained for 3 min, then the temperature was increased to 150°C at a rate of 5°C/min and finally increased to 210°C at a rate of 30°C/min. This final temperature was maintained for 9 min.

**Mouse Colonization.** Outbred Swiss-Webster female mice, 6–8 weeks of age, were housed in groups of five and except just prior to bacterial feeding were given food and water ad lib. For depletion of the endogenous facultative flora from the gastrointestinal tract, mice were given 1 mg of streptomycin per ml in their drinking water for 5 days. Cultures of fecal homogenates (0.1 g/ml) obtained before administration of streptomycin and after 5 days of treatment showed a decrease in bacterial numbers from approximately $10^4$ cfu/g of feces to <10 cfu/g of feces (lower limit of detection) in all samples examined. The plasmid-containing strains of *E. coli* were grown overnight in L broth containing 10 μg of tetracycline per ml, washed twice in 2% sucrose/2% sodium bicarbonate, and resuspended in this buffer to a concentration of $10^{10}$ cfu/ml. Water was removed from the mouse cages for 2 hr, and the mice were then fed 100 μl of the bacterial suspension from the tip of a micropipette. This was repeated once per week for 3 additional weeks. Blood was obtained before the initial feeding and at weekly intervals thereafter. Fecal homogenates were also prepared before each administration of *E. coli* and tested for the presence of endogenous or plasmid-containing *E. coli*; these cultures were uniformly negative for these strains. Sera were analyzed for the presence of IgG, IgM, and IgA by ELISA, as were filter-sterilized fecal homogenates analyzed for IgA. ELISA plates were coated with *P. aeruginosa* IT-2 LPS for these analyses.

## RESULTS

**Isolation of *P. aeruginosa* DNA That Confers a Serum-Resistance Phenotype.** Nonmucoid derivatives of the mucoid, LPS-rough, serum-sensitive *P. aeruginosa* CF isolates 2192, 2344, and FRD1 (designated 2192 nmr, 2344 nmr, and FRD2, respectively) were derived by laboratory passage. A gene bank of DNA from the LPS-smooth (Fisher IT-2) *P. aeruginosa* strain PA103 was transferred to these strains. Selection for survival in 25% human serum yielded serum-resistant transconjugants. Plasmid DNA isolated from two serum-resistant transconjugants of each *P. aeruginosa* strain was used to transform *E. coli*. Restriction endonuclease digestion of these plasmids showed that all contained the same seven *Eco*RI fragments, with a total insert size of 26.2 kilobases (kb), in the cloning vector pLAFR1. The recombinant plasmid is referred to as pLPS2. Southern blot analysis of *Eco*RI-digested chromosomal DNA from *P. aeruginosa* strain PA103, with pLPS2 as a probe, showed a pattern identical to that of the cloned DNA present in pLPS2, thus confirming that the DNA was derived from strain PA103.

When plasmid pLPS2 was transferred from *E. coli* back to the LPS-rough CF isolates of *P. aeruginosa*, all transconjugants obtained grew in 25% serum, while control transconjugants containing the cloning vector alone were readily killed. This result indicated that the cloned DNA in pLPS2 could confer a serum-resistant phenotype in these transconjugants.

**Expression of Smooth LPS by Isolates of *P. aeruginosa* Containing pLPS2.** *P. aeruginosa* strains 2192 nmr, 2344 nmr, and FRD2, along with these same strains containing either pLAFR1 or pLPS2, were tested in agglutination assays against serum raised to purified LPS from the seven Fisher immunotypes of *P. aeruginosa*. The parental strains and those containing pLAFR1 reacted either with none of the antisera (nonagglutinable) or with multiple antisera (polyagglutinable). Strains 2192 nmr(pLPS2) and 2344 nmr(pLPS2) each agglutinated in antisera to the IT-2 and IT-4 serotypes, while strain FRD2(pLPS2) agglutinated only in antiserum to the IT-2 serotype. The expression of O antigens in the pLPS2-bearing strains was confirmed by Ouchterlony immunodiffusion and by immuno-dot-blot with LPS extracted from these strains by autoclaving. Extracts of strains 2192 nmr(pLPS2) and 2344 nmr(pLPS2) precipitated with antisera to both IT-2 and IT-4 *P. aeruginosa* cells and gave lines of

identity with authentic LPS isolated from these strains. Extracts of strain FRD2(pLPS2) precipitated with antisera to IT-2 LPS. The same pattern of reactivity was apparent with immuno-dot-blots of autoclave extracts of *P. aeruginosa* strains carrying pLPS2 (Fig. 1).

SDS/PAGE analysis confirmed that pLPS2 conferred expression of O-side-chain antigens upon strains of *P. aeruginosa* normally deficient in the production of these structures. LPS extracts from *P. aeruginosa* strains 2192 nmr, 2344 nmr, and FRD2 containing no plasmid, the cloning vector pLAFR1, or the recombinant plasmid pLPS2 were separated by SDS/PAGE and silver-stained. LPS from the parental strains containing either no plasmid or the cloning vector showed only low molecular weight, fast-migrating species typical of rough LPS. *P. aeruginosa* strains containing pLPS2 showed a ladder-like pattern typical of smooth LPS and similar to that seen with authentic LPS isolated from an IT-2 strain. When the material separated by SDS/PAGE was electroblotted to nylon membranes and exposed to immunotype-specific antisera, a ladder-like pattern typical of smooth LPS was evident. Strains 2192 nmr(pLPS2) and 2344 nmr-(pLPS2) reacted with antisera specific to the IT-2 O side chain, as did the IT-2 LPS (Fig. 2*A*). As expected, IT-4 LPS did not react with this serum. A similar ladder-like pattern was seen in LPS from strains 2192 nmr(pLPS2), 2344 nmr-(pLPS2), and IT-4 LPS when an IT-4-specific antiserum was used in the immunoblot (Fig. 2*B*). *P. aeruginosa* strain FRD2(pLPS2) showed a ladder-like pattern that reacted only with IT-2 serum and not with IT-4 serum.

**Expression of *P. aeruginosa* IT-2 O Antigens by *E. coli* Containing pLPS2.** Normally >99% of *E. coli* HB101 cells are killed in serum concentrations as low as 5%. In our studies we found that >70% of *E. coli* HB101 containing pLPS2 survived in 10% serum, suggesting that the recombinant plasmid might be expressing genes responsible for serum resistance. When allowed to react in colony blots with antiserum specific to *P. aeruginosa* IT-2 antigens, *E. coli* HB101(pLPS2) gave a strong reaction, while *E. coli* HB101(pLAFR1) gave none. When LPS was extracted from these strains and subjected to SDS/PAGE, a ladder-like pattern typical of smooth LPS was seen for *E. coli* HB101(pLPS2) but not for *E. coli* HB101(pLAFR1). Similarly, when LPS was electroblotted to nylon membranes and allowed to react with antiserum to *P. aeruginosa* IT-2 O side chains, the laddering pattern was observed for the LPS from the strain carrying pLPS2 but not for the LPS from the strain carrying pLAFR1 (Fig. 3). These LPS antigens did not react with antisera specific to *P. aeruginosa* IT-4 O antigens.

To exclude the possibility that the phenotype of *E. coli* HB101(pLPS2) was due to mutations arising from the selection for serum-resistant *P. aeruginosa* transconjugants, the *P. aeruginosa* PA103 gene bank in *E. coli* HB101 was screened directly for the expression of *P. aeruginosa* IT-2 antigens by colony blot. One positive colony was obtained from >2000 colonies screened. Plasmid DNA isolated from

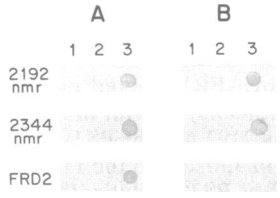


FIG. 1. Immuno-dot-blots of extracts from *P. aeruginosa* strains 2192 nmr, 2344 nmr, and FRD2. Extracts were allowed to react with antisera specific to either the IT-2 O antigen (*A*) or the IT-4 O antigen (*B*). These strains contained no plasmid (column 1), the cloning vector pLAFR1 (column 2), or the recombinant plasmid pLPS2 (column 3).

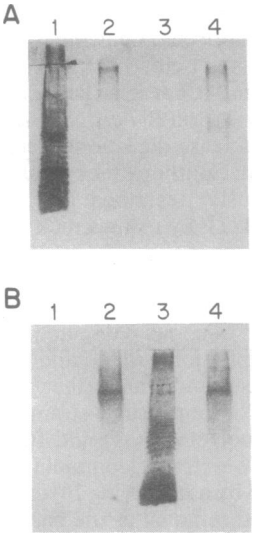


FIG. 2. Expression of *P. aeruginosa* IT-2 and IT-4 O antigens on LPS isolated from *P. aeruginosa* strains 2192 nmr(pLPS2) and 2344 nmr(pLPS2). LPS extracts were applied to SDS/PAGE, electroblotted onto nitrocellulose, and allowed to react with antisera specific to IT-2 (*A*) or IT-4 (*B*) O antigens. Lanes 1, LPS from *P. aeruginosa* IT-2 strain; lanes 2, LPS from *P. aeruginosa* 2192 nmr(pLPS2); lane 3, LPS from *P. aeruginosa* IT-4 strain; lanes 4, LPS from *P. aeruginosa* 2344 nmr(pLPS2).

this clone revealed seven *Eco*RI fragments in pLAFR1, which were of the same size as, and homologous to, pLPS2, by Southern hybridization. This confirmed that the cloned DNA obtained without selection could express *P. aeruginosa* IT-2 antigens in *E. coli*.

**Immunogenicity of LPS from *E. coli* HB101(pLAFR1) and *E. coli* HB101(pLPS2).** Immunization of rabbits by injection of the LPS from *E. coli* HB101(pLPS2) elicited high-titered antiserum to the homologous antigen and to authentic IT-2 LPS from *P. aeruginosa* (Table 1). Immunization with LPS from *E. coli* HB101(pLAFR1) resulted in the production of antibody to the immunizing LPS and to the related LPS from *E. coli* HB101(pLPS2), but not to *P. aeruginosa* IT-2 LPS. LPS from *E. coli* HB101(pLPS2) did not elicit antibody to LPS from *E. coli* HB101(pLAFR1), though they likely share cross-reactive antigenic determinants associated with the shared lipid-A core (see below). This result is probably due to the immunodominance of the IT-2 O antigens.

**Chemical Analysis of *E. coli* Recombinant LPS.** Analysis by gas–liquid chromatography of LPS from *P. aeruginosa* IT-2, *E. coli* HB101(pLAFR1), and *E. coli* HB101(pLPS2) indicated the presence of the IT-2 O-side-chain sugars *N*-acetylfucosamine and glucose in the *P. aeruginosa* and recombi-

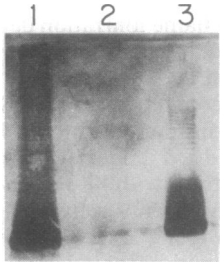


FIG. 3. Expression of *P. aeruginosa* IT-2 antigen on *E. coli* HB101(pLPS2). Isolated LPS was subjected to SDS/PAGE, electroblotted onto nitrocellulose, and allowed to react with antiserum raised to *P. aeruginosa* IT-2 LPS. Lane 1, LPS from *P. aeruginosa* IT-2 strain; lane 2, LPS from *E. coli* HB101(pLAFR1); lane 3, LPS from *E. coli* HB101(pLPS2).

Table 1. Immune response of rabbits immunized with LPS isolated from *E. coli* HB101(pLAFR1) or *E. coli* HB101(pLPS2)

| LPS immunogen source | Titer for LPS antigen | | |
|---|---|---|---|
| | *E. coli* HB101-(pLAFR1) | *E. coli* HB101-(pLPS2) | *P. aeruginosa* IT-2 |
| *E. coli* HB101(pLAFR1) | 1600 | 1600 | <100 |
| *E. coli* HB101(pLPS2) | <100 | 1600 | 12,800 |

The numbers are titer (reciprocal of highest serum dilution) of immune serum giving ≥3-fold increased OD reading compared with corresponding dilution of preimmune serum. The lowest serum titer tested was 100.

nant LPS (Table 2). In addition, galactose, which is part of the core of *E. coli* LPS, was detected only in LPS isolated from the *E. coli* strains and not in the *P. aeruginosa* LPS (Table 2). LPS from both *E. coli* strains contained fatty acids typical of enteric bacteria, consisting of straight-chain fatty acids 12 and 14 carbons in length, and 3-hydroxy fatty acids 14 carbons in length. *P. aeruginosa* LPS is known to be acylated by straight-chain and 2- and 3-hydroxy fatty acids 10 and 12 carbons in length (34); this information was confirmed for the preparation analyzed here (Table 2). Therefore, the LPS from *E. coli* HB101(pLPS2) is most probably a hybrid molecule containing *P. aeruginosa* O side chain attached to an *E. coli* core-lipid-A structure. The *P. aeruginosa* O side chain likely attaches to the terminal glucose acceptor on the outer core of the *E. coli* LPS (35), which is the same acceptor residue used in *P. aeruginosa* to attach O side chains to the LPS core (36).

**Elicitation of Antibodies to *P. aeruginosa* IT-2 LPS by Oral Immunization of Mice with *E. coli* HB101(pLPS2).** To test whether *E. coli* expressing *P. aeruginosa* O antigen elicits an immune response to this antigen after oral immunization, mice were fed *E. coli* HB101(pLPS2), or the control strain, *E. coli* HB101(pLAFR1), once a week for 4 weeks. Analysis of serum and of sterilized fecal homogenates, obtained each week just before feeding with the *E. coli* strains, revealed that mice fed *E. coli* HB101(pLPS2), but not those fed *E. coli* HB101(pLAFR1), produced serum IgG and IgA and mucosal IgA antibodies to *P. aeruginosa* IT-2 antigens (Fig. 4). No increase in serum IgM antibodies was noted.

## DISCUSSION

We have isolated a large DNA fragment from *P. aeruginosa* strain PA103 that encodes synthesis of this strain's O polysaccharide and expresses an endogenous O side chain on two of three LPS-rough clinical isolates of *P. aeruginosa*. Upon transfer to *E. coli* HB101, the recombinant plasmid, pLPS2, led to the expression of *P. aeruginosa* IT-2 O antigen on the cell surface of *E. coli*. The results of chemical characterizations indicate that the *P. aeruginosa* O antigen is attached to the rough core of *E. coli* HB101 LPS.

Table 2. Chemical components in LPS isolated from *P. aeruginosa* IT-2, *E. coli* HB101(pLAFR1), and *E. coli* HB101(pLPS2)

| Component | Composition, % | | |
|---|---|---|---|
| | *P. aeruginosa* IT-2 | *E. coli* HB101-(pLAFR1) | *E. coli* HB101-(pLPS2) |
| Lipids | | | |
| 3-Hydroxydecanoic acid | 21 | ND | ND |
| Dodecanoic acid | 25 | 13 | 15 |
| 2-Hydroxydodecanoic acid | 12 | ND | ND |
| 3-Hydroxydodecanoic acid | 40 | ND | ND |
| 3-Hydroxytetradecanoic acid | ND | 73 | 66 |
| Tetradecanoic acid | ND | 14 | 19 |
| Octodecanoic acid | Trace | ND | ND |
| Monosaccharides | | | |
| Galactose | ND | 26 | 8 |
| Glucose | 28 | 47 | 34 |
| *N*-Acetylfucosamine | 59 | ND | 55 |
| Hexosamine | 12 | 27 | 3 |

Numbers are weight percent of total fatty acids or total monosaccharides identified. ND, none detected.

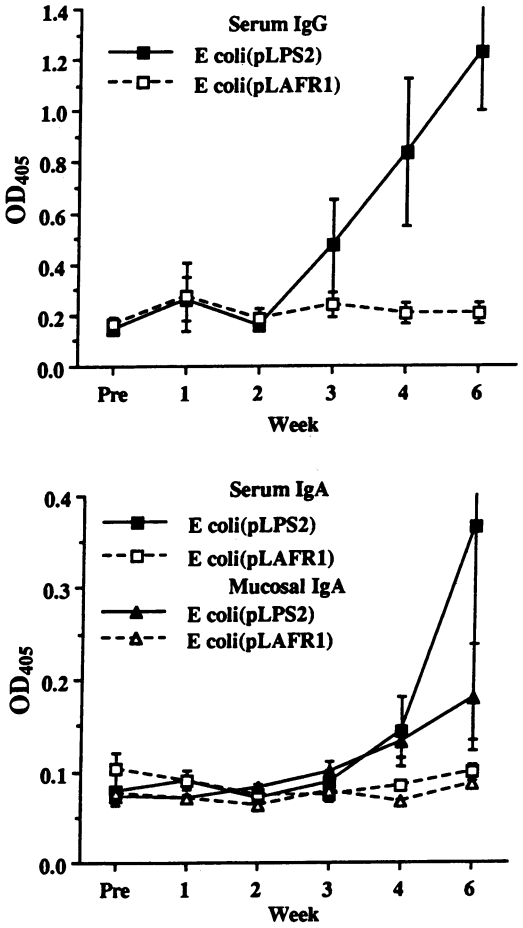


FIG. 4. IgG (*Upper*) and IgA (*Lower*) immune responses in serum or feces of mice fed the indicated *E. coli* strain in drinking water. Dilutions of serum (1:10) from individual mice were allowed to react with *P. aeruginosa* IT-2 LPS coated onto ELISA plates and probed with heavy chain-specific anti-mouse immunoglobulin conjugates. $OD_{405}$ was read after 60 min. Symbols indicate the mean value for five mice, and bars indicate the SEM.

In *E. coli* and *Salmonella* species, many of the genes required for the O-side-chain-specific components are linked on the chromosome in a genetic region referred to as the *rfb* cluster (37, 38). These *rfb* regions include genes encoding enzymes involved in O-antigen synthesis. The cloned genes in pLPS2 may include the equivalent *rfb* region from *P. aeruginosa*.

When we transferred pLPS2 to three LPS-rough strains of *P. aeruginosa* isolated from CF patients we found that on two of the strains the IT-2 O antigen and the IT-4 O antigen were synthesized and expressed on isolated LPS. One strain [FRD2(pLPS2)] synthesized only the IT-2 O side chain, but it may synthesize an endogenous antigen that we failed to detect since we screened only for 7 of a possible 20 serotypes. We have subsequently found that pLPS2 can activate synthesis of endogenous O side chains in many LPS-rough CF isolates of *P. aeruginosa* (D. J. Evans, G.B.P., and J.B.G., unpublished results). Subcloning pLPS2 should allow us to identify the genes needed for activation of endogenous O

antigens and those needed for synthesis of the IT-2 O polysaccharide.

Large O antigens have been shown to be important for the virulence of *P. aeruginosa*. Mutants deficient in the production of long O-side-chain polysaccharides are known to be less virulent (1, 39). The presence of a sufficient quantity of long O side chains conveys resistance to serum, and it promotes induction of protective antibodies. For these reasons, the O antigen of *P. aeruginosa* is considered a good target for antibody-mediated immunotherapy.

The potential of oral or mucosal immunization to protect against *P. aeruginosa* infection is just beginning to be explored (40, 41). Most clinically important diseases due to *P. aeruginosa* infection emanate from a mucosal surface, including gastrointestinal, respiratory, and ocular surfaces. In CF patients, chronic colonization of the large airways and bronchi is associated with much of the morbidity resulting from *P. aeruginosa* infection. The ability to elicit expression of O antigens of *P. aeruginosa* on the surface of enteric organisms that can efficiently colonize the gastrointestinal tract represents an important tool for study of the induction of immunity by mucosal immunization and very possibly for its use in preventing *P. aeruginosa* infections. Indeed, the ability of *E. coli* HB101(pLPS2) to elicit IgG and IgA antibodies indicates that the evocation of specific immunity to *P. aeruginosa* O antigens by oral immunization will not be difficult. Since this strain of *E. coli* only poorly colonizes the gastrointestinal tract, the results will likely improve when the genes are transferred to enteric strains that are better able to evoke immune responses after oral introduction. In this connection, we have found that when pLPS2 is transduced into wild-type and attenuated strains of *Salmonella typhimurium* or into *Salmonella typhi* Ty21a, these strains also express the O antigen of *P. aeruginosa* IT-2 (unpublished results).

This work was supported by National Institute of Allergy and Infectious Diseases Grants AI 22535 and AI 30050.

1. Cryz, S., Pitt, T., Furer, E. & Germanier, R. (1984) *Infect. Immun.* **44**, 508–513.
2. Pier, G. B. (1988) *Rev. Infect. Dis.* **10**, S337–S340.
3. Young, L. S. (1974) *J. Infect. Dis.* **130**, S111–S116.
4. Young, L. S. (1972) *J. Infect. Dis.* **126**, 277–284.
5. Pollack, M. (1990) in *Principles and Practice of Infectious Diseases*, eds. Mandell, G. L., Douglas, R. G. & Bennett, J. E. (Churchill Livingston, New York), pp. 1673–1691.
6. Pollack, M. & Young, L. S. (1979) *J. Clin. Invest.* **63**, 276–286.
7. Knirel, Y. A. (1990) *CRC Crit. Rev. Microbiol.* **17**, 273–304.
8. Knirel, Y. A., Vinogradov, E. V., Kocharova, N. A., Paramonov, N. A., Kochetkov, N. K., Dmitriev, B. A., Stanislavsky, E. S. & Lanyi, B. (1988) *Acta Microbiol. Hung.* **35**, 3–24.
9. Hancock, R. E. W., Mutharia, L. M., Chan, L., Darveau, R. P., Speert, D. P. & Pier, G. B. (1983) *Infect. Immun.* **42**, 170–177.
10. Pier, G. B. & Ames, P. (1984) *J. Infect. Dis.* **150**, 223–228.
11. Young, L. S., Yu, B. H. & Armstrong, D. (1970) *Infect. Immun.* **2**, 495–503.
12. Pitt, P. L., MacDougall, J., Penketh, A. R. L. & Cooke, E. M. (1986) *J. Med. Microbiol.* **21**, 179–186.
13. Penketh, A., Pitt, T., Roberts, D., Hodson, M. E. & Batten, J. C. (1983) *Am. Rev. Respir. Dis.* **172**, 605–608.
14. Fegan, M., Francis, P., Hayward, A. C., Davis, G. H. G. & Fuerst, J. A. (1990) *J. Clin. Microbiol.* **28**, 1143–1146.
15. Sanderson, K. E. & Stocker, B. A. D. (1987) in *Escherichia coli and Salmonella typhimurium*, eds. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington), pp. 1220–1224.
16. Tacket, C. O., Hone, D. M., Curtiss R., III, Kelly, S. M., Losonsky, G., Guers, L., Harris, A. M., Edelman, R. & Levine, M. M. (1992) *Infect. Immun.* **60**, 536–541.
17. Curtiss, R., III, & Kelly, S. M. (1987) *Infect. Immun.* **55**, 3035–3043.
18. Seid, R., Kopecko, D., Sadoff, J., Schneider, H., Baron, L. & Formal, S. (1984) *J. Biol. Chem.* **259**, 9028–9034.
19. Baron, L., Kopecko, D., Formal, S., Seid, R., Guerry, P. & Powell, E. (1987) *Infect. Immun.* **55**, 2797–2801.
20. Forrest, B. & LaBrooy, J. (1991) *Vaccine* **9**, 515–520.
21. Liu, P. (1973) *J. Infect. Dis.* **128**, 506–513.
22. Fisher, M. W., Devlin, H. B. & Gnabasik, F. (1969) *J. Bacteriol.* **98**, 835–836.
23. Friedman, A. M., Long, S. R., Brown, S. E., Buikema, W. J. & Ausubel, F. M. (1982) *Gene* **18**, 289–296.
24. Goldberg, J. B. & Ohman, D. E. (1984) *J. Bacteriol.* **158**, 1115–1121.
25. Boyer, H. W. & Roulland-Dussoix, D. (1969) *J. Mol. Biol.* **41**, 459–472.
26. Pier, G. B. & Thomas, D. M. (1982) *J. Infect. Dis.* **145**, 217–223.
27. Kocharova, N. A., Knirel, Y. A., Shashkov, A. S., Kochetkova, N. K. & Pier, G. B. (1988) *J. Biol. Chem.* **263**, 11291–11295.
28. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1525.
29. Westphal, O. & Jann, K. (1965) *Methods Carbohydr. Chem.* **5**, 83–91.
30. Pier, G. B., Sidberry, H. F., Zolyomi, S. & Sadoff, J. C. (1978) *Infect. Immun.* **22**, 908–918.
31. Keleti, G. & Lederer, W. H. (1974) *Handbook of Micromethods for the Biological Sciences* (Van Nostrand Reinhold, New York), p. 161.
32. Hitchcock, P. J. & Brown, T. M. (1983) *J. Bacteriol.* **154**, 269–277.
33. Blake, M. S., Johnston, K. H., Russel-Jones, G. J. & Gotschlich, E. C. (1984) *Anal. Biochem.* **136**, 175–179.
34. Bhat, R., Marx, A., Galanos, C. & Conrad, R. S. (1990) *J. Bacteriol.* **172**, 6631–6636.
35. Rick, P. D. (1987) in *Escherichia coli and Salmonella typhimurium*, eds. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington), pp. 648–662.
36. Rowe, P. S. & Meadow, P. M. (1983) *Eur. J. Biochem.* **132**, 329–337.
37. Bachmann, B. (1990) *Microbiol. Rev.* **54**, 130–197.
38. Sanderson, K. & Roth, J. (1989) *Microbiol. Rev.* **52**, 485–532.
39. Engels, W., Endert, J., Kamps, M. A. & van Boven, C. P. (1985) *Infect. Immun.* **49**, 182–189.
40. Hooke, A. M., Cerquetti, M. C., Wan, K. S., Wang, Z., Sordelli, D. O. & Bellanti, J. A. (1991) *Vaccine* **9**, 294–296.
41. Abraham, E. & Robinson, A. (1991) *Vaccine* **9**, 757–764.