INFECTION AND IMMUNITY, Oct. 1985, p. 107–115
0019-9567/85/100107-09$02.00/0

Vol. 50, No. 1

Kinetic and Chemical Analyses of the Biologic Significance of Lipoteichoic Acids in Mediating Adherence of Serotype III Group B Streptococci

TIMOTHY J. NEALON† AND STEPHEN J. MATTINGLY*

Department of Microbiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Received 25 March 1985/Accepted 1 July 1985

The mechanism(s) involved in the binding of lipoteichoic acid (LTA), isolated from virulent, asymptomatic, or avirulent serotype III strains of group B streptococci, to human embryonic epithelial cells (HEC), human fetal epithelial cells (HFC), and human adult buccal epithelial cells was investigated. It was determined that the binding of purified [^{3}H]LTA to human adult buccal epithelial cells differed from the binding to HEC and HFC. LTA from all group B streptococcus strains bound to human adult buccal epithelial cells in a similar manner and was enhanced by the lipid portion of the polymer; in contrast, [^{3}H]LTA binding to HEC and HFC was mediated by hydrophobic as well as specific interactions due to the glycerolphosphate backbone of LTA. Binding avidity of the LTAs to HEC and HFC varied depending on the bacterial strain. Polymers from asymptomatic and avirulent strains were easily dissociated from cell surfaces with unlabeled virulent LTA through competitive interactions; however, 10-fold greater levels of the same material were required to displace virulent [^{3}H]LTA from HEC and HFC surfaces. These observed differences in binding avidity were shown to be due to longer LTA chains (30 to 35 glycerolphosphate units) in virulent strains when compared with LTA chains (10 to 12 glycerolphosphate units) of asymptomatic and avirulent strains. Thus, LTA appears to enhance the ability of virulent group B streptococci to bind to HEC and HFC with stronger avidity by virtue of the increased length of the cell-associated polymers synthesized by these strains. Mild enzymatic treatment of HEC and HFC with trypsin or periodate abolished LTA binding, which suggests the presence of a certain glycoprotein receptor(s) for LTA which does not appear to be present on human adult buccal epithelial cells. These data may therefore partially explain the increased susceptibility of newborn infants to group B streptococcal infections.

Lipoteichoic acids (LTA) have been shown to function as mediators of adherence of type III group B streptococci (GBS) to human embryonic epithelial cells (HEC), human fetal epithelial cells (HFC), and human adult buccal epithelial cells (HBEC) (17). However, GBS isolated from infected infants were demonstrated to have significantly higher quantities of cell-associated LTA (16) and adhered to HEC and HFC with greater binding avidity and in greater number than to HBEC (17). These results suggest that two major factors may influence the adherence of GBS to neonatal epithelial cell surfaces: (i) strain variability in the level of LTA synthesized, and (ii) the nature of the receptors for LTA on neonatal cells compared with the nature of those on adult cells. Additional support for the specificity of attachment of GBS was provided by Goldschmidt and Panos (12), who demonstrated greater affinity of *Streptococcus agalactiae* for amnion cells than for HeLa cells. The present study was undertaken to elucidate the mechanism of binding of purified LTA obtained from GBS isolated from infected and asymptomatically colonized infants and to determine whether biochemical differences in the LTA molecules could account for the adherence characteristics of the GBS strains.

MATERIALS AND METHODS

Bacterial strains and growth medium. GBS serotype III strains 110 and 122, (isolated from infected infants), 177 and 179 (isolated from asymptomatically colonized infants), and D136C (Lancefield immunizing strain) have been described previously (16). Organisms were stored in Todd-Hewitt broth at −70°C until needed. Thawed cultures were streaked onto 5% sheep blood agar plates and incubated at 37°C for 14 to 18 h.

All growth experiments were performed in a chemically defined medium (FMC) (22) modified for growth of GBS (15). For radioisotopic studies, 15 μg of carrier glycerol per ml and 0.5 μCi of [^{3}H]glycerol (specific activity, 10 mCi/mmol) per ml were included.

Isolation and purification of cell-associated LTA and deacylated LTA (dLTA) from GBS isolated from infected and asymptomatically colonized infants. GBS strains were grown and treated as previously described (16). Cell pellets were extracted with a solution containing 45% aqueous phenol and chloroform-methanol (2:1, vol/vol) and purified by DEAE-Sephacel anion-exchange chromatography as previously described (16). Type III and group B-specific polysaccharides were isolated and purified as described earlier (24).

Human epithelial cells and other cell lines used in binding assays. HEC, human fetal brain epithelial cells (HFB), and human fetal lung epithelial cells (HFL) used in these studies were secondary passaged cells obtained from the primary culture established by Kendall O. Smith, Department of Microbiology, The University of Texas Health Science Center at San Antonio. Ampoules containing respective cell types were removed from liquid nitrogen and immediately placed into a 37°C water bath to avoid cell death. The thawed cells were immediately transferred to 150-cm^2 tissue culture flasks (Costar, Cambridge, Mass.), each containing 50 ml of

* Corresponding author.
† Present address: Research Immunology, San Antonio State Chest Hospital, San Antonio, TX 78223.

basal modified Eagle medium (BME) (Flow Laboratories, Inc., Rockville, Md.) supplemented with 20% fetal bovine serum (Sterile Systems, Inc., Logan, Utah), 2 mM L-glutamine, 50 μg of gentamicin sulfate per ml, and 0.056% $NaHCO_3$. This mixture was designated as complete BME. The flasks then were incubated for 90 min at 37°C with a 5% CO_2 tension to remove contaminating fibroblasts. After incubation, the supernatant fluids were transferred to clean sterile tissue culture flasks. All cell types were passed once in tissue culture and then removed from the flasks by incubation with 0.4% EDTA (in BME) for 10 to 15 min at 37°C. The isolated cells then were washed twice with complete BME to remove the EDTA, and respective cell types were adjusted to approximately 10^6/ml in complete BME containing 10% sterile glycerol and then aliquoted (2 ml) into freezing vials (Nunc Products, Santa Ana, Calif.). The vials were allowed to stand in a prechilled ethanol bath for 18 to 24 h at −70°C for a slow freezing process and finally stored in liquid N_2 until needed. Before each experiment, the vials containing the desired cell types were quickly thawed as described above and immediately washed twice with prewarmed phosphate-buffered saline (PBS; 37°C) to remove the 10% glycerol–BME used for cell storage.

HBEC were obtained from human volunteers immediately before each experiment. To obtain HBEC, the buccal mucosa was scraped with pairs of sterile cotton-tipped swabs. The cells were immediately suspended in separate 5-ml volumes of prechilled PBS and gently washed twice. HeLa human epithelial carcinoma (ATCC CCL2) cells were obtained from Charles J. Gauntt, Department of Microbiology, University of Texas Health Science Center at San Antonio. Cell viabilities of respective cell lines were determined by trypan blue dye exclusion and adjusted to a cell concentration of approximately 10^5/0.25 ml for use in binding experiments.

Binding of LTA and dLTA to embryonic, fetal, adult, and continuous cell lines. Assays examining binding of [^{3}H]glycerol-labeled LTA and [^{3}H]dLTA isolated from virulent, asymptomatic, or avirulent serotype III strains of GBS to HEC, HFB, HFL, HBEC, and continuous cell lines were performed in triplicate by incubating 10^5 cells per 0.25 ml with 50 μg of [^{3}H]LTA (or [^{3}H]dLTA)–0.25 ml of Ca^{2+}-Mg^{2+}-free PBS (pH 7.2) in sterile polystyrene culture tubes (12 by 75 mm; Fisher Scientific Co., Houston, Tex.). The tubes were incubated at 4, 25, or 37°C for 1, 5, 10, 15, 30, or 60 min, and the reaction was terminated at respective times by filtration of the LTA- or dLTA-cell mixtures through membrane filters with a pore size of 3 μM (Millipore Corp., Bedford, Mass.) under reduced pressure. The complexes collected on the filters were washed three times with PBS and air dried at room temperature. The filters then were placed in glass scintillation vials, and 10 ml of Econofluor containing 3% Protosol (New England Nuclear Corp., Boston, Mass.) was added. The vials then were mixed on a rotary shaker (200 rpm) for 16 to 18 h at 25°C, dark adapted at 4°C, and counted in a Searle Mark III liquid scintillation spectrophotometer with computer conversion of counts per minute to disintegrations per minute.

To determine the binding affinity of virulent, asymptomatic, and avirulent LTA to HEC, HFB, and HFL, 50 μg of [^{3}H]LTA was incubated with 10^5 of the respective cell type for 10 min at 37°C. After this incubation, a range of 0 to 1,000 μg of purified unlabeled LTA then was added to the mixtures. The [^{3}H]LTA-cell complexes then were harvested at 15 min after the addition of unlabeled material by filtration, and the filters were washed and analyzed by scintillation spectrometry as described above. Data are expressed as micrograms of [^{3}H]LTA bound to a given cell type.

Determination of the nature of LTA or dLTA binding sites on HEC, HFB, HFL, and HBEC surfaces. To better understand the biochemical nature of LTA or dLTA binding sites, HEC, HFB, HFL, and HBEC were incubated with 0.05 or 0.25% trypsin (Sigma Chemical Co., St. Louis, Mo.) for 15 min at 37°C or with sodium periodate (10 or 100 mM) (Sigma) for 60 min at 37°C in complete darkness. After respective incubation periods, the periodate reactions were terminated by the addition of 0.5 mg of glucose per ml, and the cells then were washed twice with PBS and adjusted to a cell concentration of 10^5/0.25 ml. To each cell type was added 50 μg of either [^{3}H]LTA or [^{3}H]dLTA (GBS strain 110), and the mixtures were further incubated for 10 or 60 min, respectively, at 37°C. In addition, cell types not treated with trypsin or sodium periodate were incubated with radiolabeled LTA or dLTA in a similar manner. All cells then were washed twice with PBS to remove unbound material and harvested by filtration, and the filters were processed and subjected to scintillation spectrometry as previously described. Data are expressed as percent binding of LTA or dLTA to a given cell type, either treated or untreated with trypsin or sodium periodate.

Inhibition of binding of [^{3}H]LTA or [^{3}H]dLTA to HFL preincubated with specific and nonspecific ligands. HFL (10^5) were preincubated with 400 μg of specific or nonspecific ligands analogous to LTA and dLTA per 0.20 ml in PBS. The ligands included the following components: β-glycerolphosphate; gangliosides III and V; *N*-acetylglucosamine; galactose; α- and β-D-glucose; human and bovine serum albumin; and bovine serum albumin fraction V. All materials were obtained from Sigma Chemical Co. Negative controls consisted of HFL preincubated with PBS or 400 μg of purified group-specific antigen (GSA) or type-specific antigen (TSA), both of which were described above. HFL were incubated with or without one of the above components for 60 min at 37°C on a rotating shaker and then washed twice with PBS to remove all unbound material. The cells then were incubated with 50 μg of [^{3}H]LTA or [^{3}H]dLTA (GBS strain 110) per 0.20 ml of PBS for 15 and 60 min, respectively. After this incubation period, HFL again were washed twice with PBS, then harvested by filtration, and analyzed for binding as previously described. Data are expressed as percent binding of LTA or dLTA to HFL cells preincubated with or without the ligand.

Inhibition of binding of [^{3}H]LTA to HFL cells by anti-LTA or monoclonal anti-poly(glycerolphosphate) antibody or by both. To demonstrate specific inhibition of [^{3}H]LTA binding to HFL and HBEC by rabbit anti-LTA (provided by K. Knox, Institute of Dental Research, Sydney, Australia) or monoclonal anti-poly(glycerolphosphate) (provided by G. D. Shockman, Temple University School of Medicine, Philadelphia, Pa.), antibody or by both, purified [^{3}H]LTA (GBS strain 110) (1 mg/ml) was incubated with the respective antibody (1:10 dilution) for 30 min at 37°C. The antibody-[^{3}H]LTA mixtures then were added to 10^5 HFL or HBEC and incubated for an additional 30 min at 37°C with shaking. The cells were harvested by filtration, and the filters were washed, air dried, and placed in glass scintillation vials with Econofluor containing 3% Protosol. Binding of [^{3}H]LTA to HFL or HBEC then was analyzed by scintillation spectrometry, and data are expressed as percent binding. Controls in these experiments included a given cell type incubated with [^{3}H]LTA (positive control) and cells incubated in PBS as well as in respective antibody dilutions. In addition, HFL or

HBEC were preincubated with antibody before the addition of [^{3}H]LTA to reduce nonspecific inhibition of LTA binding. Furthermore, monoclonal antibody to TSA (serotype III; Bethesda Research Laboratories, Rockville, Md.) also was used to demonstrate specific inhibition of [^{3}H]LTA binding by monoclonal anti-poly(glycerolphosphate).

Determination of the effects of high concentrations of LTA and dLTA on HFL and HeLa cell viability. Cellular viabilities of HFL or HeLa cells were determined by trypan blue dye exclusion initially, and each cell type was adjusted to approximately 10^7 (in complete BME) in 15-ml glass conical centrifuge tubes. The cells were centrifuged at 1,000 × *g* for 15 min, and the supernatant fluid was discarded. BME medium (3 drops) then was added to each cell pellet, followed by 3 drops of fetal bovine serum (Sterile Systems, Inc.), and the cells were gently suspended. Chromium-51 (375 mCi of chromium per mg; 100 μCi) (Amersham Corp., Arlington Heights, Ill.) was added to each tube, gently mixed, and incubated for 1 h at 37°C in a circulating water bath to label the given cell type. After this incubation period, the cells were washed twice with complete BME, and the cell pellets then were suspended in complete BME to a concentration of 10^5 radiolabeled HFL or HeLa cells per 0.20 ml. The cells (10^5) were added to tissue culture tubes (12 by 75 mm) in triplicate and incubated with a range of 0 to 2,000 μg of purified unlabeled LTA or dLTA for 1.5 h at 37°C. In addition, triplicate samples of spontaneous and complete release controls were incubated simultaneously. After this incubation, 0.5 ml of 1 N HCl was added to the complete release controls, and all tubes were centrifuged at 1,000 × *g* for 15 min. Supernatants then were carefully removed and placed in clean tissue culture tubes (12 by 75 mm) and counted for 1 min in a Beckman Gamma 310 counter (Beckman Instruments, Inc., Irvine, Calif.). All data are expressed as percent release of chromium-51 when compared with complete release controls.

Effect of temperature change on binding efficiency of virulent GBS strains to HEC, HFB, and HFL. To better understand the nature of the forces involved in adherence of GBS to HEC, HFB, and HFL, virulent serotype III GBS strains 110 and 122 were grown to late-exponential–early-stationary phase in FMC medium containing 0.10 μCi of [^{14}C]thymidine (10 μg of carrier thymidine per ml) per ml. Radiolabeled thymidine incorporated into GBS was used as an intracellular marker for cellular DNA, and the data are based on the correlation between incorporation of [^{14}C]thymidine and cellular dry weight or CFU (17). The radiolabeled strains (10^8 CFU per 0.25 ml of PBS) were incubated with HEC, HFB, or HFL (10^5 cells per 0.25 ml of PBS) for 10, 30, or 60 min at 37°C. After these incubation periods, the cell-bacterium mixtures were either incubated at 37°C for an additional 15 min or placed in an ice bath for 15 min. The samples then were harvested by filtration, and the filters were washed, air dried, and analyzed by scintillation spectrometry. Data obtained from these experiments were expressed as percent binding of GBS to HEC, HFB, or HFL.

Inhibition of virulent GBS binding to HEC, HFB, and HFL by monoclonal anti-poly(glycerolphosphate) antibody. To demonstrate that the binding of serotype III GBS strains to HEC, HFB, and HFL requires the glycerolphosphate backbone of cell-associated LTA, approximately 10^8 CFU of GBS strain 110 or 122 per 0.25 ml of PBS were incubated with 0.25 ml of monoclonal anti-poly(glycerolphosphate) or anti-TSA (1:10 dilution) for 30 min at 37°C. The antibody-bacterium mixtures were washed twice with PBS to remove unbound antibody and incubated with 10^5 HEC, HFB, or HFL per 0.25 ml of PBS for 10, 30, or 60 min at 37°C with shaking as previously described. After the initial incubation period, the cell-bacterium mixtures were either incubated for an additional 15 min at 37°C or placed in an ice bath for 15 min. Controls in these experiments included a given type of cell incubated with GBS strain 110 or 122 without pretreatment with antibody (positive control), cells pretreated with antibody before incubation with the GBS strains, and the cells or GBS incubated in PBS. The binding efficiency is expressed as percent binding of GBS to HEC, HFB, and HFL.

Chemical analysis of LTA and dLTA isolated from virulent, asymptomatic, and avirulent strains of type III GBS. Triplicate samples (200 to 300 μg) of purified material were hydrolyzed with 2 N HCl and trimethylsilyl derivatives prepared as previously described (8). The derivatized hydrolysates (4 to 10 μl) then were injected into a Shimadzu GC-4B gas chromatograph (Seisa Kusho, Ltd., Kyoto, Japan) and eluted on a 1.7 M glass column packed with 3% GE-SE-30 on Gas-Chrom Q with a 100/120 mesh (Alltech Associates, Inc., Applied Science Div., State College, Pa.). The samples were analyzed over a temperature range of 60 to 180°C programmed at 4°C/min. The detector oven was set at 280°C, and the nitrogen flow rate was 25 cm^3/min. Glycerol, β-glycerolphosphate, and standard sugars were prepared in the same manner and subjected to chromatographic analysis in various concentrations for the development of standard curves.

The presence of fatty acids in the sample preparations also was qualitatively and quantitatively analyzed by gas-liquid chromatography. Each sample (20 mg) was dried under N_2 gas and treated with 3 ml of methanolic HCl reagent. This solution was composed of 5 ml of acetylcholine (Alltech) which was added dropwise to 100 ml of Lipo-pure methanol (Alltech) and allowed to stand for 15 min at room temperature before use. The sample mixtures were subjected to methanolysis by heating at 90°C for 2 h and then neutralized with 10% Na_2CO_3. The hydrolysates were initially extracted by the addition of 3 ml of deionized water–3 ml of petroleum ether (high grade), followed by two more extractions with petroleum ether for quantitative recovery of fatty acid methyl esters. The ether extracts were pooled, evaporated to dryness with N_2 gas, and suspended in 100 to 200 μl of isooctane. Samples (4 to 10 μl) then were injected into a Shimadzu GC-4B gas chromatograph and eluted on a 1.7 M glass column packed with 10% SP-2330 Chromosorb W AW I-185 (Supelco, Inc., Bellefonte, Pa.). The fatty acid esters were analyzed over a temperature range of 160 to 180°C programmed at 3°C/min.

The chain length of LTA and dLTA polymers was determined by alkaline phosphatase hydrolysis. Samples (1.0 mg) were treated with 0.5 mg of alkaline phosphatase (Sigma) in 0.5 ml of 0.02 M $(NH_4)_2CO_3$ buffer (pH 9.5) at 37°C for 18 h to release terminal phosphoric acid groups. Organic and inorganic phosphorus was determined by the method of Chen et al. (7), and the ratio of total to inorganic phosphorus established the chain length.

RESULTS

Effect of temperature on the binding of purified LTA and dLTA to HFL. Minimal binding of LTA (obtained from virulent GBS strain 110) to HFL occurred at 4°C, and binding at 25°C was less efficient when compared with that at 37°C; at 37°C, binding was determined to be approximately 95% (~48 μg) after 60 min of incubation (Fig. 1A). In addition, the binding of LTA to HFL at 37°C was rapid, and

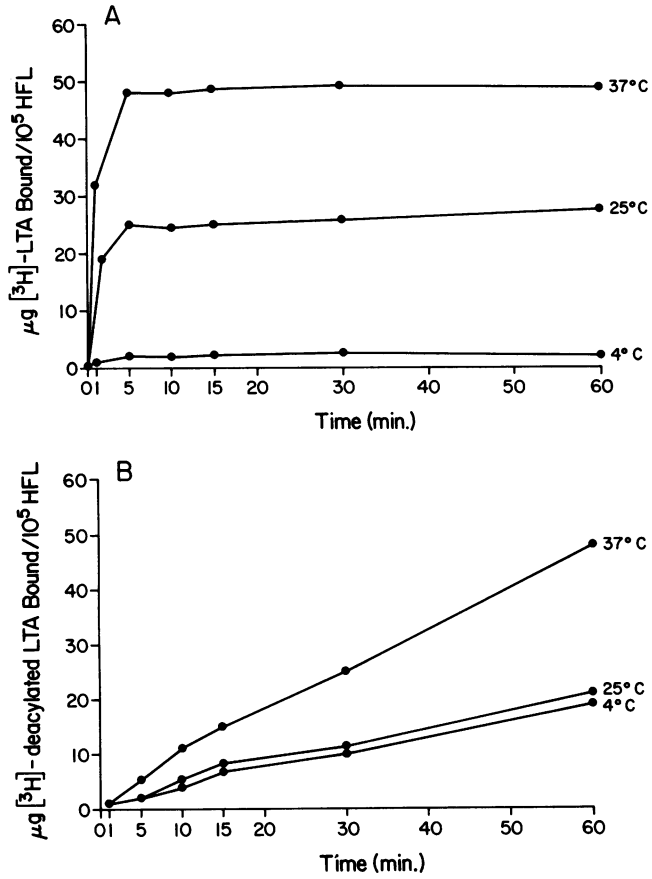

FIG. 1. Binding of [^{3}H]LTA (A) and [^{3}H]dLTA (B) of GBS strain 110 (virulent) to HFL at 4, 25, and 37°C. Purified radiolabeled LTA or dLTA (50 μg) was incubated with 10^5 HFL at the various temperatures, harvested at indicated time periods, washed, and analyzed for binding as described in Materials and Methods.

maximum attachment occurred within a period of 5 min, similar to that observed with the binding of whole cells of type III GBS to HFL (17). The binding of dLTA (virulent GBS strain 110) to HFL was approximately 40% more efficient at 37°C, when compared with binding at 4 or 25°C (Fig. 1B). However, in contrast to LTA, binding of dLTA was gradual and increased in a linear manner at 37°C, with maximum attachment of the material (~50 μg) occurring at 60 min. These data suggest that the binding mechanism of purified LTA to HFL may require both the lipid portion and the glycerolphosphate backbone of the polymer.

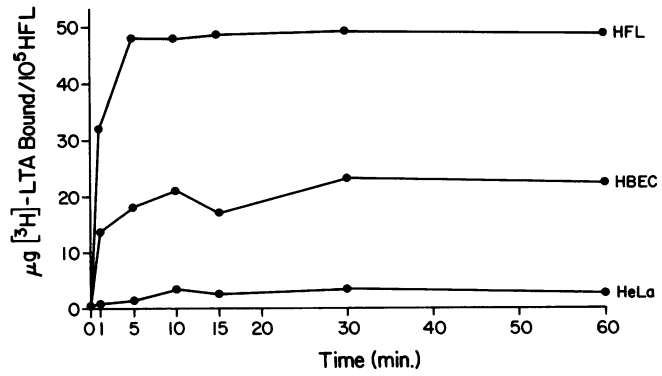

FIG. 2. Specificity of binding of [^{3}H]LTA (GBS strain 110) to HFL, HBEC, and HeLa cells at 37°C. Experimental conditions are described in the legend for Fig. 1.

Specificity of the binding of LTA and dLTA to various cell lines. To examine binding specificity of LTA, 50 μg of [^{3}H]LTA or [^{3}H]dLTA (obtained from virulent GBS strain 110) was incubated with 10^5 HFL, HBEC, and human carcinoma cells (HeLa) at 37°C for 0 to 60 min. Figure 2 demonstrates the specificity of binding of LTA to HFL (95% efficient) in contrast to the binding to HBEC (40%) and HeLa (<5%). Binding of dLTA to HFL was similar to that observed in Fig. 1B; however, binding to HBEC or HeLa cells did not occur under these time and temperature conditions (data not shown). These results suggest that the binding mechanism of LTA to HFL differs from that of HBEC, with both the lipid and the glycerolphosphate backbone participating in the binding to HFL, while LTA binding to HBEC appears to require only the lipid portion of the polymer. Interestingly, minimal binding of LTA and dLTA to HeLa cells was observed, and these data were found to agree with a previous study (12).

Determination of virulent and avirulent LTA binding affinity and avidity to HFL. The binding affinity and avidity of LTA isolated from virulent, asymtomatic, and avirulent GBS strains were examined by incubating 60 μg of [^{3}H]LTA with 10^5 cells of a respective cell type for 10 min at 37°C. At this time, various amounts (0 to 1,000 μg) of purified unlabeled LTA (virulent GBS strain 110) then were added to the [^{3}H]LTA-cell mixtures and incubated for an additional 15 min at 37°C. Data indicate that the binding affinity of virulent and avirulent [^{3}H]LTA was similar, as demonstrated by the ability of these respective polymers to bind rapidly within the designated incubation period (Fig. 3). However, binding avidity of virulent LTA appeared to be increased when compared with that of avirulent LTA. These conclusions were derived from data which indicated that low concentrations of exogenous unlabeled LTA (~100 μg) were capable of displacing avirulent material from HFL surfaces. In contrast, increased levels of unlabeled material (~800 μg)

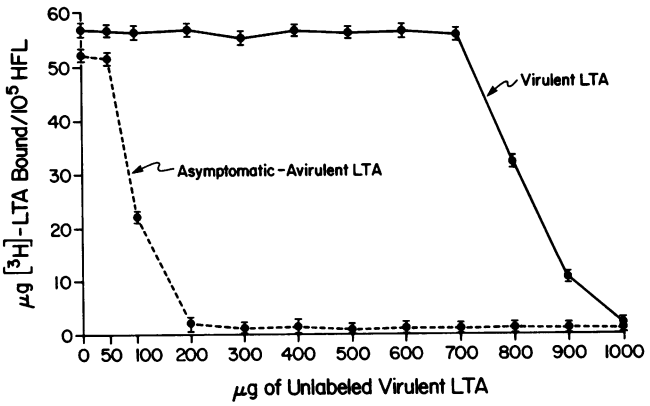

FIG. 3. Displacement of virulent, avirulent, and asymptomatic [^{3}H]LTA from HFL at 37°C by the addition of unlabeled virulent LTA. [^{3}H]LTA (60 μg) from each bacterial strain was incubated with 10^5 HFL for 10 min, and the cells were washed to remove unbound material. Unlabeled virulent LTA (0 to 1,000 μg) then was added in an attempt to displace the bound [^{3}H]LTA. The [^{3}H]LTA-cell complexes then were incubated for an additional 15 min at 37°C and washed, and the level of cell-bound [^{3}H]LTA was determined.

were required to displace virulent [^{3}H]LTA from the cell surface. These data suggest that the binding affinity of virulent and avirulent LTAs for human embryonic and fetal epithelial cells is similar, whereas the degree of avidity of virulent LTA is significantly increased when compared with that of avirulent material.

Nature of the LTA binding sites on HFL and HBEC surfaces. In view of the different binding efficiencies of LTA isolated from virulent GBS for HFL and HBEC, the chemical nature of the LTA binding receptor on surfaces of the respective cell types was examined by modifying epithelial cell surfaces chemically or enzymatically before [^{3}H]LTA binding. Data indicate that pretreatment of HFL with various concentrations of trypsin or sodium periodate significantly altered the binding efficiency of LTA and dLTA (Table 1). Pretreatment of HBEC with trypsin or sodium periodate did not affect binding of either LTA or dLTA (Table 2), suggesting that possible receptor sites for the glycerolphosphate backbone of LTA on human embryonic and fetal epithelial cells might be composed of a glycoprotein. These results suggest that receptor sites for the glycerolphosphate backbone do not appear to be present on adult cells and that their presence on HFL may partially explain the increased binding efficiency as well as the difference in the mechanism of LTA binding to these cells.

Effect of specific and nonspecific ligands on binding of LTA and dLTA to HFL. To further investigate the nature of the binding of LTA and dLTA to HFL, a study was undertaken to examine the ability of gangliosides, bovine serum albumin (BSA), human serum albumin (HSA), sugars, purified glycerolphosphate, and other GBS cell wall polysaccharides (TSA and GSA) to inhibit binding of LTA or dLTA, or both, to HFL. The cells were preincubated with the respective ligand, washed to remove all unbound material, and then incubated further with 50 μg of either [^{3}H]LTA or [^{3}H]dLTA. Data demonstrate the ability of glycerolphosphate to significantly inhibit binding of both LTA and dLTA, with a greater inhibitory effect on dLTA (Table 3). These data suggest that the glycerol phosphate backbone of LTA is partially required for binding to HFL. The albumins and gangliosides inhibit the binding of LTA only, suggesting an interaction of the fatty acid-binding sites present on these components with cell receptors for the lipid portion of the LTA (21). In addition, the various sugars, including GBS cell wall polysaccharides, the type III and group-specific antigens, failed to inhibit the binding of either polymer.

Inhibition of LTA and dLTA binding to HEC, HFB, HFL, and HBEC by rabbit anti-LTA antiserum or anti-poly(glycerolphosphate) monoclonal antibody. Data obtained from the studies described above indicate that the mechanism of LTA binding to human embryonic and fetal epithelial cells differs from that of binding to human adult cells. Thus, an attempt was made to inhibit the binding of these polymers to the various cell types with rabbit anti-LTA or monoclonal antibody specific for the poly(glycerolphosphate) backbone of LTA. [^{3}H]LTA obtained from virulent GBS strain 110 was incubated with either antibody, the respective LTA-antibody mixtures then were incubated wtih HEC, HFB, HFL, or HBEC, and LTA binding was analyzed as previously described. Results (Table 4) indicate that the binding of LTA to any cell type is significantly inhibited when the polymers are preincubated with rabbit anti-LTA. However, only LTA binding to HEC, HFB, or HFL is markedly inhibited by monoclonal anti-poly(glycerolphosphate) antibody in contrast to binding to HBEC, where binding remains relatively unaffected. When the respective cell types were preincubated with either antibody before the addition of LTA, the polymers bound in a similar manner comparable to controls. In addition, monoclonal antibody specific for TSA did not inhibit binding to any of the cell types examined.

TABLE 1. Effect of trypsin and sodium periodate treatment of HFL on binding of [^{3}H]LTA and [^{3}H]dLTA

GBS strain	% Binding to HFL[a]				
	No treatment	+T (0.05%)	+T (0.25%)	+P (10 mM)	+P (100 mM)
[^{3}H]LTA					
110	98	89	75	62	44
122	96	87	78	60	43
D136C	90	81	72	69	40
177	94	83	70	68	43
179	92	81	69	65	38
[^{3}H]dLTA					
110	95	61	38	34	26
122	91	58	35	39	25
D136C	67	41	29	28	17
177	64	43	28	23	12
179	67	40	22	24	18

[a] +T, Trypsin added at indicated levels; +P, sodium periodate added at indicated levels.

TABLE 2. Effect of trypsin and sodium periodate treatment on HBEC on binding of [^{3}H]LTA

GBS strain	% Binding to HBEC[a]				
	No treatment	+T (0.05%)	+T (0.25%)	+P (10 mM)	+P (100 mM)
[^{3}H]LTA[b]					
110	35	31	39	38	41
122	39	38	36	39	36
D136C	32	34	41	47	41
177	35	30	31	42	45
179	37	35	36	39	43

[a] +T, Trypsin added at indicated levels; +P, sodium periodate added at indicated levels.

[b] The percent binding of [^{3}H]dLTA was less than 1% under identical conditions with the same five strains.

TABLE 3. Effect of glycerolphosphate, various albumins, sugars, and gangliosides on binding of [^{3}H]LTA and [^{3}H]dLTA to HFL

Substance tested (400 μg/0.2 ml)	% Binding ± SD[a]	
	LTA	dLTA
None (control)	97 ± 1.5	95 ± 1.8
Glycerolphosphate	63 ± 2.1	34 ± 0.8
Bovine serum albumin	46 ± 3.0	89 ± 4.3
Bovine serum albumin (fraction V)	44 ± 1.4	86 ± 2.8
Human serum albumin	55 ± 1.5	94 ± 1.5
Alpha-D-glucose	90 ± 0.9	99 ± 0.2
Beta-D-glucose	93 ± 1.3	98 ± 0.1
Galactose	92 ± 1.4	98 ± 0.8
N-Acetylglucosamine	93 ± 2.1	97 ± 0.8
Ganglioside III	53 ± 3.7	94 ± 2.5
Ganglioside V	38 ± 4.1	99 ± 0.1
Group B antigen (serotype III GBS strain 110)	94 ± 2.3	99 ± 0.9
Type-specific antigen (serotype III GBS strain 110)	99 ± 0.3	97 ± 0.8

[a] Values are the averages of three independent experiments.

TABLE 4. Effect of preincubating virulent [^{3}H]LTA with antibody on binding to HEC, HFB, HFL, and HBEC[a]

[^{3}H]LTA + cell type[b]	% Binding ± SD			
	No preincubation with anti-LTA, anti-P-G or anti-TSA antibody	Preincubation with:		
		Anti-LTA	Anti-P-G	Anti-TSA
HEC	95 ± 2.5	50 ± 1.3	32 ± 2.7	96 ± 1.5
HFB	92 ± 3.6	49 ± 3.1	33 ± 0.8	95 ± 2.5
HFL	95 ± 1.8	52 ± 2.3	30 ± 1.8	93 ± 2.0
HBEC	42 ± 0.7	21 ± 1.4	43 ± 3.5	40 ± 4.2

[a] Virulent [^{3}H]LTA (60 μg; GBS strain 110) was preincubated with or without a 1:10 dilution of rabbit anti-LTA, monoclonal anti-poly(glycerolphosphate) (anti-P-G), or anti-TSA antibody, and percent binding to HEC, HFB, HFL, and HBEC was measured after 15 min of incubation. Values are averages of three independent experiments.

[b] [^{3}H]LTA was incubated with 10^5 HEC, HFB, HFL, or HBEC.

Therefore, these data further indicate that the binding mechanism of LTA to human embryonic and fetal epithelial cells differs when compared with that to human adult cells.

Determination of the effect of LTA and dLTA on cell viability of HFL. To examine the binding kinetics of LTA and dLTA, the previously described studies required that high levels of polymers be included in the experimental protocols. Therefore, cell viability in all studies utilizing these purified materials was routinely analyzed by trypan blue dye exclusion. In addition, to ensure that the morphological integrity of the cell types was not deleteriously altered, the more sensitive chromium-51 release assay was also used to examine cells after exposure to high levels of LTA or dLTA. The cells examined were HFL and HeLa cells, the latter being used as a negative control since previous results presented indicated that neither LTA nor dLTA bound to this continuous cell line. Both cell types were radiolabeled by incubation with chromium-51 as previously described. The cells were washed and then incubated with 0 to 2,000 μg of purified unlabeled LTA or dLTA (virulent GBS strain 110). After incubation, the cells were removed by centrifugation, and supernatant fluids were analyzed for release of the isotope. Data (Table 5) indicate that levels up to 2,000 μg of either LTA or dLTA were nontoxic to HeLa cells. However, levels of LTA exceeding 1,500 μg were found to be toxic to HFL (Table 5); in contrast, dLTA failed to adversely affect HFL viability at these levels, suggesting that the lipid moiety of the LTA polymers is the toxic component for these cells.

TABLE 5. Effect of LTA and dLTA on HFL viability as determined by ^{51}Cr release

Mixture	cpm[a]	% Release
Complete release control[b]	348,618	100
Spontaneous release control[c]	3,634	1.0
HFL cells + LTA (μg)		
100	3,523	0.9
500	3,203	0.9
1,000	3,952	1.1
1,500	4,216	1.2
2,000	168,631	48
HFL cells + dLTA (μg)		
100	2,808	0.8
500	2,601	0.8
1,000	3,638	1.0
1,500	4,437	1.3
2,000	3,769	1.1

[a] Average of triplicate determinations.

[b] Determined by lysis of 0.5 ml of 1 N HCl after 1.5 h of incubation at 37°C.

[c] Medium control; no addition of LTA or dLTA.

In addition, these results demonstrate that the amounts of LTA or dLTA in the previous experiments did not affect cell viability.

Role of membrane fluidity on the binding efficiency of virulent GBS to HEC, HFB, and HFL. Data obtained from kinetic studies which examined the attachment of purified LTA and dLTA to HEC, HFB, and HFL indicated that the binding mechanism of the polymers to the various cell types was influenced by both the lipid moiety and the glycerolphosphate backbone of LTA. Thus, binding of LTA appeared to involve a hydrophobic interaction which is dependent on membrane fluidity of the human embryonic and fetal cells as well as a hydrophilic interaction of the glycerolphosphate backbone with these cell types. Support for these findings was demonstrated by previous temperature dependence experiments (17) which indicated that LTA-mediated attachment of GBS did not occur at 4°C. In view of these results, experiments were performed to examine the effects of temperature change on adherence of GBS. Virulent GBS strains 110 and 122 were incubated with HEC, HFB, or HFL for 10, 30, or 60 min at 37°C. Subsequently, incubation of the cell-bacterium mixtures was continued at either 37 or 4°C for an additional 15 min. The samples then were harvested by filtration, and the binding of GBS was analyzed as previously described. Data (Table 6) indicate that both GBS strains which were incubated at 37°C with respective cell types throughout the duration of the experiment attached in a similar manner as that previously described (17). In contrast, when GBS strain 110 or 122 was initially incubated with cells at 37°C for 10 min and then shifted to 4°C, the ability to bind was significantly decreased. However, when GBS were incubated for 30 min or longer and then transferred to 4°C, the binding efficiency of these organisms was unaffected. Therefore, these data suggest the involvement of two steps in the LTA-mediated attachment of GBS. The first appears to be hydrophobic and is influenced by membrane fluidity of the human embryonic and fetal cells. These initial interactions were time dependent, as indicated by the ability of GBS to become detached from cell surfaces when incubated at 4°C after 10 min at 37°C. However, when the adherence of GBS to cells was continued for 30 min or longer at 37°C and then was shifted to 4°C, attachment did not appreciably decrease. A possible explanation for the latter observation is that the second step in attachment of the organisms involves a specific interaction of the glycerolphosphate backbone of the LTA polymer with receptor sites present on human embryonic and fetal cell surfaces and would appear to be hydrophilic in nature. Therefore, it may be concluded from

TABLE 6. Percent binding of virulent serotype III GBS strains 110 and 122 to HFL with temperature change

Length (min) of initial incubation[a]	Temp (°C) after initial incubation	% Binding ± SD[b] of:	
		GBS 110	GBS 122
10	4	39 ± 2.5	36 ± 1.7
	37	95 ± 3.2	91 ± 4.7
30	4	87 ± 3.6	83 ± 3.1
	37	96 ± 1.4	94 ± 2.7
60	4	92 ± 4.2	95 ± 0.9
	37	94 ± 3.2	98 ± 1.6

[a] 10^8 CFU of GBS strain 110 or 122 were added to each cell culture system (10^5 HFL) and initially incubated at 37°C for the indicated times.

[b] Values are the averages of three independent experiments.

TABLE 7. Effect of preincubating virulent serotype III GBS strains 110 and 122 with antibody on binding to HFL with temperature change[a]

Length (min) of initial incubation[b]	Temp (°C) after initial incubation	% Binding ± SD[b]					
		Preinculation with:					
		No antibody		Anti-P-G		Anti-TSA	
		110	122	110	122	110	122
10	4	37 ± 1.2	32 ± 4.1	31 ± 4.2	34 ± 0.9	34 ± 2.2	36 ± 0.9
	37	92 ± 2.7	96 ± 0.9	39 ± 2.8	42 ± 1.4	93 ± 2.0	95 ± 1.7
30	4	82 ± 0.5	86 ± 2.2	42 ± 1.3	39 ± 2.7	86 ± 1.3	81 ± 1.6
	37	94 ± 2.3	98 ± 0.7	37 ± 2.4	43 ± 3.2	91 ± 0.8	89 ± 4.5
60	4	93 ± 3.5	97 ± 0.3	31 ± 4.3	36 ± 3.6	96 ± 2.2	91 ± 2.7
	37	95 ± 2.4	91 ± 1.4	43 ± 4.2	42 ± 3.3	94 ± 1.4	98 ± 0.3

[a] 10^8 CFU of GBS strain 110 or 122 were added to each cell culture system and preincubated with or without a 1 : 10 dilution of anti-poly(glycerolphosphate) (anti-P-G) or anti-TSA; percent binding to HFL with temperature change was measured.
[b] Values are averages of three independent experiments.

these data that the longer the initial interaction of GBS with HEC, HFB, or HFL at 37°C (allowing for hydrophobic forces to develop), the greater the opportunity for more specific hydrophilic forces to develop as a result of interaction of the glycerolphosphate backbone with cell surface receptors.

To test the hypothesis that the glycerolphosphate backbone of the LTA polymer is indeed responsible for the second step in the attachment of GBS to human embryonic and fetal epithelial cells, virulent GBS strain 110 or 122 was preincubated with monoclonal anti-poly(glycerolphosphate) or anti-TSA antibody for 30 min at 37°C. The organisms were washed to remove all unbound antibody and then subjected to temperature change experiments described above. The data (Table 7) indicate that after initial incubation periods of 10, 30, or 60 min, GBS were capable of binding to respective cell types as previously described. However, when the incubation temperature was decreased to 4°C, the adherence of GBS preincubated with anti-poly(glycerolphosphate) antibody to HEC, HFB, and HFL was significantly decreased when compared with that of controls. In addition, preincubation of GBS with anti-TSA monoclonal antibody failed to affect the binding efficiency of the organisms. These results confirm that two steps are required in the LTA-mediated attachment of GBS to human embryonic and fetal epithelial cells.

Analysis of the chemical composition of cell-associated LTA of virulent, asymptomatic, and avirulent serotype III GBS strains. It was previously demonstrated that virulent GBS adhered to human embryonic and fetal epithelial cells with greater avidity when compared with asymptomatic and avirulent strains and that these findings are attributed to increased quantities of cell-associated LTA on the surface of virulent GBS strains (17). To confirm these findings, purified LTAs isolated from virulent, asymptomatic, and avirulent GBS were analyzed quantitatively by gas-liquid chromatography. In addition, a qualitative analysis was performed to determine whether there were differences between these LTAs.

Table 8 represents an analysis of LTA isolated and purified from virulent, asymptomatic, and avirulent GBS strains. These data indicate substitution of glucose in the glycerolphosphate backbone for all LTAs examined. In addition, the presence of fatty acids was also confirmed; palmitic acid, 16:0; stearic acid, 18:0; monounsaturated oleic acid or *cis*-vaccenic acid, 18:1. The quantity of glycerolphosphate per microgram for all virulent LTAs was slightly higher when compared with that for asymptomatic and avirulent material. However, asymptomatic and avirulent LTAs per microgram contained approximately twice the amount of glucose and fatty acids when compared with that in virulent LTA. These data indicate there were no qualitative differences between the LTAs of the GBS strains examined, although quantitative differences were apparent in fatty acid and glucose contents.

Examination of the chain length of the various LTAs by alkaline phosphatase hydrolysis indicated that virulent LTAs ranged from 30 to 35 glycerolphosphate units in length compared with 10 to 12 units for asymptomatic and avirulent LTA (Table 9). However, the asymptomatic and avirulent

TABLE 8. Qualitative and quantitative analysis of LTA isolated from virulent, asymptomatic, and avirulent serotype III GBS strains

Component (nmol/μg)	Analysis of LTA from GBS strain[a]				
	110 (VIR)	122 (VIR)	D136C (AVIR)	177 (ASYM)	179 (ASYM)
Glycerolphosphate	6.480	5.910	4.200	4.460	4.590
Glucose	0.813	0.900	2.080	1.810	1.740
Palmitic acid (16:0)	0.028	0.032	0.065	0.080	0.082
Stearic acid (18:0)	0.011	0.013	0.030	0.033	0.034
Oleic acid (18:1)	0.012	0.013	0.032	0.037	0.037
Fatty acids (total)	0.051	0.058	0.127	0.150	0.153

[a] VIR, Virulent; AVIR, avirulent; ASYM, asymptomatic.

TABLE 9. Chain-length analysis of LTA isolated from virulent, asymptomatic, and avirulent serotype III GBS strains

Characteristic	GBS strains	
	Virulent	Avirulent or asymptomatic
Chain length (glycerolphosphate units)	30–35	10–12
No. of chains/μg	23–27	55–65
Fatty acids/chain (nmol)	0.0022	0.0025
Glucose/chain (nmol)	0.0350	0.0310

material contained approximately twice the number of chains per microgram when compared with the virulent LTA. These data explain why there was twice the amount of fatty acid and glucose material in asymptomatic and avirulent LTA (Table 8). When the amounts of fatty acids and glucose per chain were determined, it was found that the LTA of all GBS strains examined contained similar quantities. The results indicate that the cell-associated LTA of virulent GBS strains are approximately three times longer than are the LTA of asymptomatic and avirulent GBS strains. Therefore, these findings may partially explain the ability of virulent GBS to bind to human embryonic and fetal epithelial cells with greater avidity when compared with asymptomatic and avirulent strains.

DISCUSSION

LTA-mediated adherence of virulent, asymptomatic, and avirulent GBS to human embryonic and fetal cells appears to differ from that of adult cells, although similar interactions are required for initial binding of the organisms to the cell types. In binding to embryonic and fetal cells GBS appear to require more than one step in the attachment process. The initial interaction appears to be mediated by hydrophobic forces, suggested by the requirement for membrane fluidity of epithelial cells. After this initial contact, a subsequent interaction of the glycerolphosphate backbone of the polymers with eucaryotic cell surfaces then occurs. In contrast, GBS adherence to adult epithelial cells may involve one process only, that is, the hydrophobic interaction of the bacterial cell surface with cell plasma membranes. These results are in agreement with those observed in group A streptococcal adherence (3) as well as in attachment of *Staphylococcus aureus* (6). The inability of deacylated material, obtained by chemical deacylation of LTA and native dLTA, to bind and subsequently inhibit attachment also confirms these findings (17). Moreover, receptor sites on HBEC for the lipid moiety of LTA have been suggested by others (19). However, the factors mediating initial hydrophobic interactions of GBS with human embryonic, fetal, and adult cells at the present time remain obscure.

The present study demonstrates that after initial hydrophobic interactions by GBS, secondary binding possibly involves the interaction of the glycerolphosphate backbone of LTA with embryonic and fetal cell surfaces. However, unlike human adult cells, these cell types appear to possess receptor sites primarily composed of a glycoprotein(s), the nature of which has been demonstrated in previous studies (5, 11, 13, 23). In this study, these observations are based on data which suggest that binding of serotype III GBS strains, in addition to purified cell-associated LTA and dLTA, to HEC and HFC pretreated with sodium periodate or trypsin was significantly decreased. In contrast, similar treatment of adult cells failed to alter binding efficiency. Therefore, the present data lend support to the idea that during early development, receptor components on these cell surfaces may differ from those found on adult cells.

Chemical analysis of LTA from virulent, asymptomatic, and avirulent serotype III stationary phase GBS strains indicated that the only major difference between the LTA is the length of the glycerolphosphate backbone, virulent strains having an LTA of 30 to 35 glycerolphosphate units and asymptomatic strains having an LTA of 10 to 12 glycerolphosphate units. Of considerable interest was the finding that all GBS strains possessed chains of similar length at the mid-exponential-growth phase which correlated with extracted quantities of their cell-associated LTA (data not shown). In addition, quantitation of glucose substituted in the glycerolphosphate backbone of LTA indicated that similar levels also were present. Thus, virulence could be attributable to induction of enzymes responsible for additional synthesis of components that result in glycerolphosphate chain extension in virulent GBS strains.

The finding that sequential addition of glycerolphosphate units to the growing LTA chains in virulent GBS strains does not appear to include further glucose substitutions in the LTA backbone implies that an increased anionic charge may not be present on these polymers. This could result in increased hydrophilic interactions of the glycerolphosphate backbone of the cell-associated LTA with possible glycoprotein receptors on human embryonic and fetal epithelial cell surfaces and may account for the ability of virulent GBS strains to bind with greater avidity when compared with that of asymptomatic and avirulent strains (17). Furthermore, decreased binding efficiency of the latter strains may be attributed to weak negatively charged polymers that are shorter in length, and it would appear that the substituted glucose residues on the glycerolphosphate backbones of LTA from asymptomatic and avirulent strains may sterically interfere with possible hydrophilic bond formation between the shorter LTA polymers and host cell receptors (4). This would result in decreased binding avidity of these GBS strains as observed in this and a previous study (17). Therefore, the implication that LTA-mediated adherence of GBS to human embryonic and fetal epithelial cells is dependent on these types of forces for efficient LTA attachment may be similar to those hydrophilic interactions observed in binding of certain bacterial toxins to specific receptors on mammalian cells (9).

The results obtained in the present study may have clinical relevance in GBS disease of the newborn infant. Lim et al. (14) reported that the ability of newborns to become infected with GBS immediately after birth may be dependent on large numbers of organisms colonizing the maternal vaginal tract. They also demonstrated that those infants with early onset disease possessed significantly higher numbers of GBS on epithelial cell surfaces in contrast to those that were not infected. It appears then that the ability of GBS to adhere to neonatal tissue is dependent not only on quantities of teichoic acids on the cell surface of the organism but also on availability of specific receptor sites on neonatal cells, as described earlier in this discussion. Previous studies with monoclonal antibodies specific for defined carbohydrate sequences have shown that some of the changing antigenicities in the glycoproteins and glycolipids of cells during successive developmental stages may be brought about by the sequential addition or deletion of monosaccharide residues and that these components, which are present in linear forms on embryonic, fetal, and infant cells, are modified to branched forms approximately 12 months postnatally (10). These data suggest that specific receptor sites on adult cells may be different from those on newborn infants. Ofek et al. (18) previously demonstrated that LTA-mediated attachment of group A streptococci to adult epithelial cells is 10-fold higher than that to neonatal cells. This may partially explain the pathogenesis of group A streptococci in older infants, adolescents, and adults. Similarly, the ability of GBS to bind to human embryonic and fetal cells may describe just the reverse, that is, GBS infections are most frequent in neonates (1, 2, 20) in contrast to adults, who are rarely infected. Data obtained in the present study are compatible with this possibility.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant HD 19464 from the National Institutes of Health and by research grant AQ901 from the Robert A. Welch Foundation, Houston, Tex.

We thank Elizabeth Kay Eskew for her valuable technical assistance and Sally Skotvold for typing the manuscript.

LITERATURE CITED

1. **Anthony, B. F., and D. M. Ikada.** 1977. The emergence of group B streptococci in infections of the newborn infant. Annu. Rev. Med. **28**:355–369.
2. **Baker, C. J., F. F. Barrett, R. C. Gordon, and M. D. Yow.** 1973. Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. J. Pediatr. **82**:724–729.
3. **Beachey, E. H.** 1975. Binding of a group A streptococci to human oral mucosal cells by lipoteichoic acid (LTA). Trans. Assoc. Am. Physicians **88**:285–292.
4. **Bell, G. I.** 1978. Models for the specific adhesion of cells to cells. Science **200**:618–627.
5. **Brinton, C. C.** 1967. Contributions of pili to the specificity of the bacterial surface, p. 37–70. *In* B. D. David (ed.), The specificity of cell surfaces. Prentice-Hall, Inc., Englewood Cliffs, N.J.
6. **Carruthers, M. M., and W. J. Kabat.** 1983. Mediation of staphylococcal adherence to mucosal cells by lipoteichoic acid. Infect. Immun. **40**:444–446.
7. **Chen, P. S., Jr., T. Y. Toribara, and H. Warner.** 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1758.
8. **Doran, T. I., D. C. Straus, and S. J. Mattingly.** 1981. Factors influencing the release of type III antigens by group B streptococci. Infect. Immun. **31**:615–623.
9. **Eidels, L., R. L. Proia, and D. A. Hart.** 1983. Membrane receptors for bacterial toxins. Microbiol. Rev. **47**:596–620.
10. **Feizi, T.** 1981. Carbohydrate differentiation antigens. Trends Biochem. Sci. **6**:333–335.
11. **Gibbons, R. J., and I. Etherden.** 1982. Enzymatic modification of bacterial receptors on saliva-treated hydroxyapatite surfaces. Infect. Immun. **36**:52–58.
12. **Goldschmidt, J. C., Jr., and C. Panos.** 1984. Teichoic acids of *Streptococcus agalactiae*: chemistry, cytotoxicity, and effect on bacterial adherence to human cells in tissue culture. Infect. Immun. **43**:670–677.
13. **Kondo, W., M. Sata, and H. Ozawa.** 1976. Haemagglutinating activity of *Leptotrichia buccalis* cells and their adherence to saliva-coated enamel powder. Arch. Oral Biol. **21**:363–369.
14. **Lim, D. V., K. S. Kanarek, and M. E. Peterson.** 1982. Magnitude of colonization and sepsis by group B streptococci in newborn infants. Curr. Microbiol. **7**:99–102.
15. **Milligan, T. W., T. I. Doran, D. C. Straus, and S. J. Mattingly.** Growth and amino acid requirements of various strains of group B streptococci. J. Clin. Microbiol. **7**:28–33.
16. **Nealon, T. J., and S. J. Mattingly.** 1983. Association of elevated levels of cellular lipoteichoic acids of group B streptococci with human neonatal disease. Infect. Immun. **39**:1243–1251.
17. **Nealon, T. J., and S. J. Mattingly.** 1984. Role of cellular lipoteichoic acids in mediating adherence of serotype III strains of group B streptococci to human embryonic, fetal, and adult epithelial cells. Infect. Immun. **43**:523–530.
18. **Ofek, I., E. H. Beachey, F. Eyal, and J. Morrison.** 1977. Postnatal development of binding of group A streptococci to human oral epithelial cells. J. Infect. Dis. **135**:267–274.
19. **Ofek, I., W. A. Simpson, and E. H. Beachey.** 1982. Formation of molecular complexes between a structurally defined M protein and acylated or deacylated lipoteichoic acid of *Streptococcus pyogenes*. J. Bacteriol. **149**:426–433.
20. **Paredes, A., P. Wong, E. O. Mason, L. H. Taver, and F. F. Barrett.** 1977. Nosocomial transmission of group B streptococci in a newborn nursery. Pediatrics **59**:679–682.
21. **Simpson, W. A., I. Ofek, and E. H. Beachey.** 1980. Binding of streptococcal lipoteichoic acid to the fatty acid binding site on serum albumin. J. Biol. Chem. **255**:9092–9097.
22. **Terleckyj, B., N. P. Willet, and G. D. Shockman.** 1975. Growth of several cariogenic strains of oral streptococci in a chemically defined medium. Infect. Immun. **11**:649–655.
23. **Williams, R. L., and R. J. Gibbons.** 1975. Inhibition of streptococcal attachment to the receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun. **11**:711–718.
24. **Yeung, M. K., and S. J. Mattingly.** 1983. Biosynthesis of cell wall peptidoglycan and polysaccharide antigens by protoplasts of type III group B *Streptococcus*. J. Bacteriol. **154**:211–220.