INFECTION AND IMMUNITY, Mar. 1979, p. 618-625
0019-9567/79/03-0618/08$02.00/0

Vol. 23, No. 3

Erythrocyte Binding Properties of Streptococcal Lipoteichoic Acids

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Received for publication 4 December 1978

The lipoteichoic acids (LTA) of gram-positive bacteria are known to bind spontaneously to a variety of animal cell membranes. We investigated the biological and biochemical characteristics of the binding of LTA of *Streptococcus pyogenes* and *S. faecalis* to human and sheep erythrocytes. The kinetics of the binding of the radiolabeled LTA ([^{3}H]LTA) from each of these organisms to erythrocytes was similar. The dissociation constants for sheep and adult human erythrocytes were 1.6 μM and 4.5 μM, respectively, whereas that of human cord blood erythrocytes was approximately 10-fold higher, 31 μM. The number of binding sites for sheep erythrocytes was calculated to be $7.2 \times \times 10^6$ per cell, and that of human erythrocytes, 29×10^6 per cell. Binding was reversible. More than 50% of bound [^{3}H]LTA was displaced from erythrocytes by a 50-fold excess of unlabeled LTA. LTA prepared from heterologous species of gram-positive bacteria were all inhibitory to the binding of [^{3}H]LTA whether derived from *S. pyogenes* or from *S. faecalis*. Among a number of potential receptor analogues and other inhibitors tested, including serum albumin, gangliosides Gm_2 and Gm_3, lipopolysaccharide of gram-negative bacteria, and various sugars, only albumin and the gangliosides significantly inhibited LTA binding. Trypsin or neuraminidase treatment of erythrocytes had no effect on LTA binding. Deacylation of [^{3}H]LTA abolished binding ability and binding was restored by esterification of the deacylated material with stearoyl chloride, indicating that ester-linked lipids are necessary for membrane binding.

Because of its cell membrane binding affinity, lipoteichoic acid (LTA) has been suggested to play a central role in the pathogenesis of streptococcal infections (9, 27) and possibly postinfection complications such as arthritis (13) and nephritis (24). Recent studies have shown that the integrity of the ester bonds between the lipid and polyglycerophosphate components of the LTA molecules are essential for the molecule's ability to bind to cell membranes (15, 27), to mediate the adherence of streptococci to animal cells (2, 4, 15), and to suppress the immune response in laboratory animals (12). It was of interest, therefore, to determine whether or not the interaction of LTA with animal cells involves specific mechanisms of recognition between the lipid complex of LTA and a specific binding site (receptor) on the cell membrane.

The present investigation was undertaken to determine the biochemical and biological characteristics of the membrane binding of LTA, focusing in particular on studies of the specificity, affinity, and number of LTA binding sites. Erythrocytes were used since the cells should serve as a readily available source of pure membranes that could be used in subsequent studies to isolate and identify the putative membrane receptors for LTA.

In this paper we present evidence that intact erythrocytes possess a single population of binding sites for LTA and that binding is reversible and specific. Among a number of substances tested, including serum albumin, gangliosides, lipopolysaccharides (LPS) of gram-negative bacteria, and various sugars, only albumin and the gangliosides significantly inhibited LTA binding. Deacylation of the LTA molecule by use of mild conditions of hydrolysis abolished binding. Binding activity was restored by esterification of the deacylated LTA molecule with stearoyl chloride, suggesting that LTA is recognized by membrane receptors for ester-linked lipids.

MATERIALS AND METHODS

Extraction of LTA from streptococci. The

1RP41 strain of group A streptococci was kindly furnished by Rebecca Lancefield, The Rockefeller University, New York, N.Y. The organisms were grown in 60-liter batches in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) at 37°C for 18 to 24 h, collected by continuous-flow centrifugation, and washed three times in distilled water. LTA was extracted as described previously (15). A 10% suspension (wet packed wt/vol) was made in 0.05 N HCl and heated at 95°C in a boiling-water bath for 10 min. The suspension was cooled, neutralized with 1 N NaOH, washed three times with ice-cold distilled water, and finally suspended in 10 volumes of distilled water. An equal volume of 95% phenol was added, and, after gentle mixing for 1 h at 4°C, the suspension was centrifuged at 18,000 × *g* for 30 min. The aqueous phase, which contained the LTA, was removed with a pipette, dialyzed for 3 days against six changes of distilled water, lyophilized, and further purified on a column (1.6 by 90 cm) of Sepharose 6B (Pharmacia, Uppsala, Sweden) with 0.2 M ammonium acetate as eluant (1, 2a). Chemical analyses performed as previously described (15) revealed the presence only of glycerol, alanine, and a range of fatty acids.

LTA was also prepared from *Streptococcus faecalis* ATCC 9790, *Lactobacillus fermentum* NCTC 6991, *Streptococcus lactis*, and *Lactobacillus casei* ATCC 6375 by methods described previously (7, 8, 25, 26). LPS of *Serratia marcescens* was obtained from Difco. *Salmonella enteritidis* LPS was the kind gift of G. D. F. Jackson.

Preparation of radiolabeled LTA. LTA was radiolabeled by growing *S. pyogenes* 1RP41 in 60-liter batches of Todd-Hewitt broth to which had been added 2.5 mCi of [2-^{3}H]glycerol (specific activity, 200 mCi/mmol; New England Nuclear Corp., Boston, Mass.) per liter. After 18 to 24 h of incubation at 37°C, the organisms were harvested and the [^{3}H]LTA was extracted as described above. [^{3}H]LTA was prepared from *S. faecalis* ATCC 9790 by growing the organisms in a defined medium (20) with the addition of [2-^{3}H]-glycerol as previously described (15). Radiolabeled LTA was isolated from late-log-phase cells by extraction with hot phenol and was purified by column chromatography on AcA22 molecular sieve gel (LKB Produkter, Stockholm, Sweden) with 0.2 M ammonium acetate, pH 6.9, as eluant (5). LTA having specific activities of the order of 10^6 to 2×10^6 cpm/μmol was routinely obtained by this procedure.

Deacylation and esterification of LTA. Deacylation of LTA was accomplished by ammonia hydrolysis: 3 mg of LTA was dissolved in 0.5 ml of distilled water, mixed with an equal volume of 30% NH_4-OH (Fisher Scientific Co., Fairlawn, N.J.), incubated at 25°C for 18 h, and then flash evaporated to dryness. In some experiments LTA was hydrolyzed with 0.2 M KOH in methanol. Ten milligrams of LTA was dissolved in 0.5 ml of distilled water mixed with 10 volumes of KOH-methanol and incubated at 37°C for 15 min. The reaction was terminated by dilution with 20 volumes of distilled water and chilling in an ice-water bath. The mixture was then poured through a column (1 by 10 cm) of Dowex 50 (hydrogen form at 23°C). The material was eluted with 7 column volumes of water, lyophilized, redissolved in 1 ml of distilled water, and extracted with chloroform-methanol (2:1). The aqueous phase was then flash evaporated to dryness.

Esterification of the resultant polyglycerophosphate was performed as previously reported (15) by a modification of the method described by Hammerling and Westphal (6). The dried material was dissolved in 3 ml of *N,N*-dimethyl formamide (Fisher Scientific Co.), and the solution was divided into three equal parts. To one part 0.08 ml of stearoyl chloride (Sigma Chemical Co., St. Louis, Mo.) and 0.08 ml of pyridine were added. Pyridine only was added to the second tube, and no additions were made to the third. After being mixed for 1 h at 24°C, the mixtures were extracted with 3 ml of chloroform-methanol (2:1). The aqueous phase was removed with a pipette, and the solutions were extracted three times with an equal volume of chloroform-methanol (2:1). The aqueous phase from each was flash evaporated and redissolved in 1 ml of 0.15 M NaCl-0.02 M phosphate, pH 7.4 (PBS), and a 10-μl sample was counted in a Packard TriCarb scintillation counter (Packard Instrument Co., Downer's Grove, Ill.) to determine the amount of radioactivity remaining.

Binding experiments. Heparinized (10 U/ml) peripheral blood was obtained from healthy adult donors. The whole blood was centrifuged at 500 × *g* for 10 min, and the plasma and buffy coat were removed. The erythrocytes were washed three times in PBS. The washed cells were resuspended in PBS, counted with a Coulter-B cell counter (Coulter Electronics, Hialeah, Fla.), and diluted to the appropriate concentrations. Sheep erythrocytes were prepared in a similar manner.

Binding assays were performed at 37°C in 1.5-ml Eppendorf microtest tubes (Brinkman Instruments, Westbury, N.Y.). The assay mixtures were composed of 100 μl of PBS containing various amounts of [^{3}H]-LTA and erythrocytes as stated in the text. After a specified time of incubation, the assay was terminated by adding a 10-fold excess of PBS to each tube and centrifuging in an Eppendorf tabletop centrifuge for 2.5 min. The supernatant fluid was drawn off with a pipette, and any free [^{3}H]LTA remaining was removed by two additional washes with 1 ml of PBS. The red-cell pellet was lysed with 100 μl of distilled water and pipetted into a scintillation vial. The tubes were rinsed with an additional 100 μl of 1 N NaOH. After 15 min of NaOH digestion, the mixture was bleached for 15 min with 0.3 ml of 30% H_2O_2. Standard controls were composed of identical amounts of erythrocytes, water, NaOH, and H_2O_2 with a known quantity of [^{3}H]LTA. Aquasol (10 ml) was added to each vial, and after refrigeration overnight samples were counted in a Packard TriCarb liquid scintillation counter.

Competitive binding assays were performed in 100 μl of PBS containing 5% erythrocytes (packed volume/milliliter), 50 μg of [^{3}H]LTA, and the appropriate inhibitor. All were added simultaneously and incubated for 2 h at 37°C. The following carbohydrates were tested at a final concentration of 2.5% (wt/vol): D-galactose, D-glucose, D-arabinose, L-fucose, D-mannose, *N*-aceytl-D-glucosamine, D-xylose, D-galactosamine, and methyl-α-D-mannoside (these sugars were all obtained from Pfanstiehl, Waukegan, Ill.). Gm_2 and Gm_3 gangliosides (50 μg/ml, Pharmacia Inc., Piscataway, N.J.), bovine serum albumin (Cohn fraction V,

Sigma), and LPS derived from *S. marcescens* or *S. enteritidis* were also tested. At the end of the 2-h incubation period, the reactions were terminated by centrifugation and washing as stated above. The cell pellet was lysed, digested, and decolorized, as described above, and the amount of radiolabeled LTA bound to the erythrocytes in the presence of the inhibitor was compared to control values obtained with no inhibitor added.

Enzyme treatment. Erythrocytes at the same concentration as used in the binding assays were treated with 10 U of neuraminidase (Calbiochem, La Jolla, Calif.) per ml at pH 7.2 or with 1 mg of trypsin (Sigma) per ml at pH 8.0 for 60 min at 37°C. The cells were then washed and assayed for their ability to bind [^{3}H]LTA.

RESULTS

Binding of LTA to erythrocytes. The binding of radiolabeled LTA prepared from *S. pyogenes* or *S. faecalis* to human or sheep erythrocytes was dependent on cell concentration (Fig. 1) and on time and temperature (Fig. 2). The binding approached a maximum after 2 h of incubation, a significantly longer period than that reported previously for the binding of LTA to platelets (3) and peripheral blood lymphocytes (2a). Over 50% of the erythrocyte-bound [^{3}H]LTA was displaced within 2 h after addition of a 50-fold excess of unlabeled LTA (Fig. 3). In other experiments, when the excess unlabeled LTA was added simultaneously with [^{3}H]LTA, the radioactivity associated with erythrocytes was reduced to less than 10% of that of a duplicate incubation performed in the absence of unlabeled LTA. The residual radioactivity represents nonspecific binding, and in subsequent experiments each value of binding was corrected by subtracting the radioactivity bound to erythrocytes incubated with excess unlabeled LTA.

Determination of dissociation constant and number of LTA binding sites. To determine the average number of LTA receptor sites per adult human erythrocyte as well as their binding affinity, we plotted the amount of [^{3}H]LTA bound to erythrocytes as a function of free LTA added to the incubation mixture. The data were than analyzed by the method of Scatchard (19) (Fig. 4A). Assuming a molecular weight of 10,000 for *S. pyogenes* LTA (18), we calculated the number of molecules bound to erythrocytes at saturation from the intercept with the abscissa. It was determined that erythrocytes from adults possess a single population of approximately 29×10^6 LTA binding sites per cell, with a dissociation constant of 4.5 μM.

Similar studies of the binding of LTA to cord blood erythrocytes demonstrated a single population of a similar number (30×10^6) of binding sites per cell but a higher dissociation constant of 31 μM (Fig. 4B). The binding characteristics

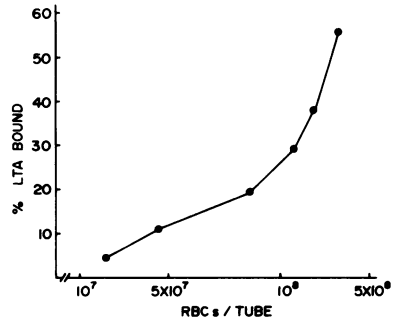

FIG. 1. *Relationship of the number of human erythrocytes (RBCs) and the binding of S. pyogenes [^{3}H]lipoteichoic acid (LTA).*

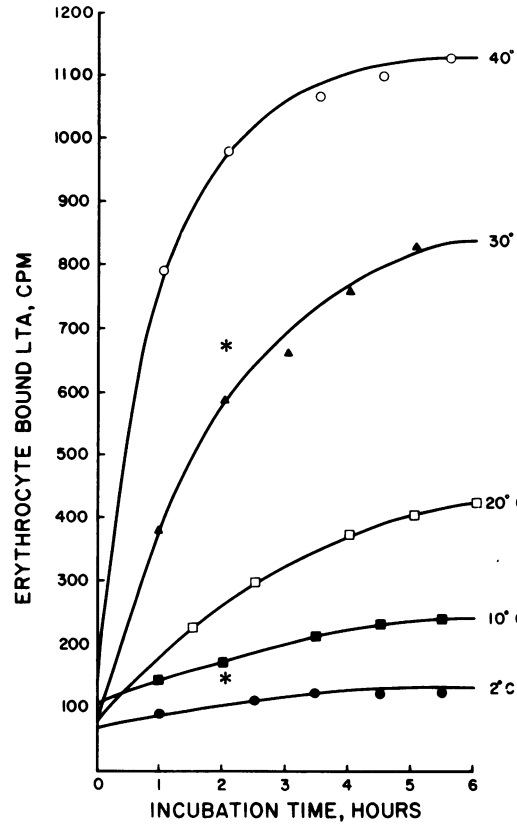

FIG. 2. *Time and temperature dependence of the binding of S. faecalis [^{3}H]lipoteichoic acid (LTA) to sheep erythrocytes. Cells were incubated with [^{3}H]-LTA at 40, 30, 20, 10, and 2°C for the times indicated. The binding of S. pyogenes [^{3}H]LTA to these erythrocytes after 2 h of incubation at 4 and 37°C is shown by the asterisks.*

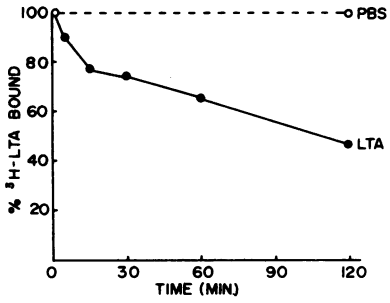

FIG. 3. *Dissociation of cell-bound S. pyogenes [³H]lipoteichoic acid ([³H]LTA) by unlabeled LTA. Cells were incubated with 50 μg of [³H]LTA for 2 h at 37°C. At zero time 2.5 mg of unlabeled LTA in 200 μl of PBS was added and allowed to incubate further (●). Control cells (○) were incubated with 200 μl of PBS.*

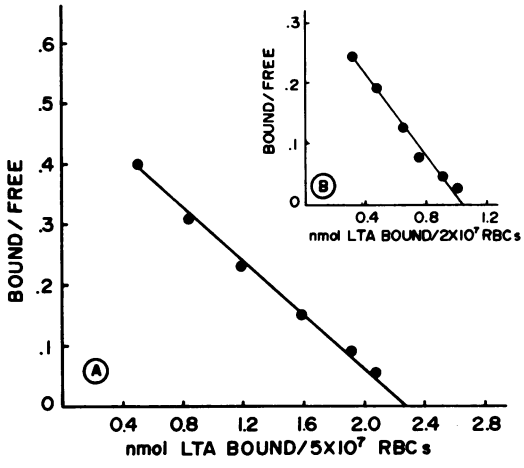

FIG. 4. *Scatchard plot of the binding of S. pyogenes [³H]lipoteichoic acid (LTA) to human adult (A) and cord blood (B) erythrocytes (RBCs). The cells were incubated with various concentrations of added (free) [³H]LTA, and the amount of bound LTA was determined after 2 h at 37°C.*

of LTA prepared from *S. faecalis* were similar to those of LTA prepared from *S. pyogenes*. Scatchard plot analysis (Fig. 5) of the binding of increasing concentrations of *S. faecalis* LTA to a constant number of sheep erythrocytes indicated a single population of 7.2×10^6 binding sites per cell, with a dissociation constant of 1.6 μM, which is in the same order of magnitude as that found for the binding of *S. pyogenes* LTA to adult human erythrocytes (see above).

Specificity of the binding of various LTA preparations. Cold-chase experiments were performed to study the specificity of erythrocyte binding of LTA derived from different species of gram-positive bacteria. Sheep erythrocytes were sensitized with [³H]LTA prepared from *S. faecalis*. When washed and resuspended in a fivefold excess of LTA derived from three heterologous species, the bound radioactivity was displaced from the erythrocytes by each of the heterologous LTAs (Fig. 6). At 50-fold excess concentrations, unlabeled LTA from heterologous *L. fermentum* and homologous *S. faecalis* displaced 32.5% and 49% of the labeled LTA, respectively (Fig. 7). In another experiment the binding of *S. pyogenes* LTA to human erythrocytes was blocked by 25-fold excesses of heterologous LTA preparations (Table 1). These results suggest that the various species of LTA bind to a similar receptor site(s) on intact erythrocytes. In contrast, up to a 25-fold excess (by weight) of *S. marcescens* LPS, another amphipathic molecule derived from gram-negative bacteria, failed to inhibit binding of radiolabeled

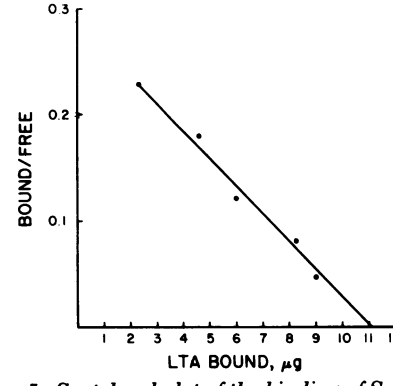

FIG. 5. *Scatchard plot of the binding of S. faecalis [³H]lipoteichoic acid (LTA) to sheep erythrocytes.*

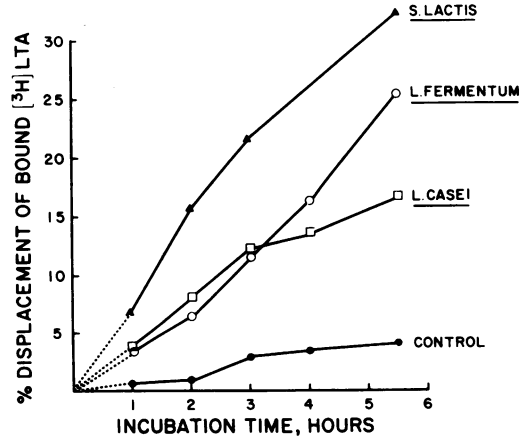

FIG. 6. *Kinetics of displacement of S. faecalis [³H]lipoteichoic acid (LTA) from sheep erythrocytes by a fivefold excess prepared from heterologous species.*

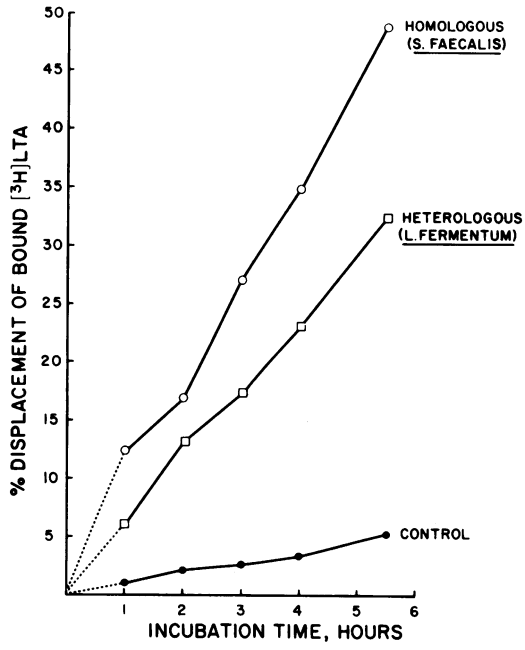

FIG. 7. *Kinetics of displacement of S. faecalis [^{3}H]lipoteichoic acid (LTA) from sheep erythrocytes by a 50-fold excess of homologous and heterologous LTA.*

TABLE 1. *Effect of homologous and heterologous lipoteichoic acid (LTA) preparations on binding of S. pyogenes radiolabeled LTA*

Source of unlabeled inhibitory LTA[a]	Percentage of control binding of [^{3}H]LTA
PBS control	100
S. pyogenes	47
S. lactis	20
S. faecalis	20
Lipopolysaccharide (*S. marcescens*)[b]	87

[a] Human erythrocytes (5×10^7) were incubated for 2 h at 37°C with 10 μg of [^{3}H]LTA prepared from *S. pyogenes* and 250 μg of the homologous or heterologous unlabeled LTA preparations in a final volume of 0.1 ml. Cells were washed as described in the text.

[b] Human erythrocytes were treated as above except that [^{3}H]LTA was increased to 40 μg and LPS to 1,000 μg in a final volume of 0.1 ml.

LTA (Table 1), indicating that the LPS and LTA binding sites are different. Similarly, the kinetics of binding of *S. faecalis* [^{3}H]LTA to sheep erythrocytes was not affected by the presence of a fourfold excess of *S. enteritidis* LPS. Binding of both amphipathic species to erythrocytes in these experiments was shown by hemagglutination with antisera specific to either LTA or LPS, and the titer was the same as with erythrocytes sensitized with either LTA or LPS alone.

Inhibition studies. In an attempt to identify the binding site(s) on intact erythrocytes for LTA, several potential receptor analogues were tested for their ability to inhibit erythrocyte binding of LTA. These included Gm_2 and Gm_3 gangliosides and each of eight sugars which have been shown to be represented in cell membranes (17). None of the sugars tested was capable of inhibiting LTA binding at concentrations as high as 25 mg/ml (Table 2). A ninth membrane sugar, *N*-acetyl-neuraminic acid, slightly enhanced the binding of LTA, but the result was difficult to assess as a result of marked erythrocyte agglutination in the presence of sugar and LTA. Subsequent experiments were performed on the binding of LTA to erythrocytes pretreated with neuraminidase or trypsin. The enzyme-treated cells retained full binding activity (Table 2), indicating that *N*-acetyl-neuraminic acid is probably not involved in membrane binding of LTA.

Studies of the relation of LTA binding sites to blood group antigens. Sensitization of human erythrocytes with 10 μg of LTA per ml had no effect on the anti-A or anti-B titers of commercial typing antisera. However, at a sensitizing concentration of 1 mg of LTA per ml, the titers of anti-A and anti-B sera were reduced fourfold against group AB erythrocytes and that of anti-A was reduced twofold against group A

TABLE 2. *Effect of gangliosides, various sugars and enzymatic treatment on the binding of [^{3}H]-lipoteichoic acid to human erythrocytes*

Substance tested	Concn (mg/ml)	Percentage of control value
Albumin	5.0	47
Immunoglobulin (Cohn fraction II)	50.0	88
Ganglioside Gm_2	0.5	41
Ganglioside Gm_3	0.5	47
D-Galactose	25.0	102
β-D-Glucose	25.0	108
D-Arabinose	25.0	91
L-Fucose	25.0	107
D-Mannose	25.0	104
N-acetyl-D-glucosamine	25.0	100
D-Xylose	25.0	95
D-Galactosamine	25.0	90
Methyl-α-D-mannopyranoside	25.0	97
Neuraminidase	—[a]	123
Trypsin	10.0	104

[a] The concentration was 10 U/ml.

erythrocytes (Table 3). Thus, as occupation of LTA binding sites approached saturation, the A and B membrane antigens appeared to become less accessible to their respective antibodies.

Abolition and restoration of binding by deacylation and esterification of the LTA molecule with stearoyl chloride. It was previously shown that ester-linked lipids in the LTA molecule are necessary for the sensitization of erythrocytes for passive hemagglutination tests with anti-LTA antibody (9, 15, 27). Since anti-LTA might not be able to detect binding of deacylated LTA, the following experiment was performed to determine the role of ester-linked lipids in the binding of radiolabeled LTA to erythrocytes. [^{3}H]LTA was deacylated by limited alkaline hydrolysis (see Materials and Methods). The water-soluble phase of a chloroform-methanol extract, containing the deacylated polyglycerophosphate in which over 90% of the radioactivity was located, was then esterified with stearoyl chloride. Incubation mixtures containing the deacylated material and the various reagents in the absence of stearoyl chloride were included as controls. The deacylated [^{3}H]LTA lost virtually all of its binding activity, and esterification of the molecule with stearoyl chloride restored binding (Table 4). Treatment of the deacylated molecule with reagents alone had no effect. These results indicate that ester-linked lipids are involved in the binding of LTA to erythrocytes.

TABLE 4. *Restoration of erythrocyte binding activity of deacylated lipoteichoic acid (LTA) from S. pyogenes by esterification with stearoyl chloride*

Prepn	LTA bound (cpm)	Radioactivity of added LTA (cpm)	Percent bound
[^{3}H]LTA	468	2,804	16.7
Deacylated [^{3}H]LTA[a]	14	2,710	0.5
+ DMF[b]	17	2,030	0.8
+ DMF + pyridine	16	2,077	0.8
+ DMF + pyridine + stearoyl chloride	167	2,308	7.2

[a] Deacylated [^{3}H]LTA from *S. faecalis* similarly showed no binding to erythrocytes.
[b] DMF, *N,N*-dimethyl formamide.

DISCUSSION

The data reported in this paper indicate that intact erythrocytes possess specific binding sites for LTA. The dissociation constants for human and sheep erythrocytes were found to be in the same order of magnitude, whereas that of cord blood erythrocytes was 10-fold higher. The binding is consistent with our previous observation that buccal epithelial cells of newborn infants bound lower levels of LTA as judged in immunofluorescence tests (14). Recent studies (W. A. Simpson, I. Ofek, J. B. Dale, C. Sarasohn, J. Morrison, and E. H. Beachey, manuscript submitted for publication) using radiolabeled LTA confirmed the lower binding of LTA in newborn epithelial cells, and the reduced binding was found to be associated with a reduced binding of group A streptococci. There is evidence to suggest that the lower affinity of the neonatal cells for LTA is due to the partial masking of LTA binding sites; mechanical perturbation of the epithelial cell membranes increased LTA and streptococcal binding to adult levels (Simpson et al., submitted). It is possible that partial masking of LTA receptors in cord blood erythrocytes similarly may account for their reduced binding avidity.

TABLE 3. *Effect of sensitizing human groups A and AB erythrocytes with lipoteichoic acid (LTA) on anti-A and anti-B titers*

Erythrocyte typing antiserum[a]	Reciprocal titers of antisera against:			
	Group A		Group AB	
	Untreated	LTA sensitized[b]	Untreated	LTA sensitized[b]
Anti-A	256	128	1,024	256
Anti-B	0	2	512	128
Anti-D	2	2	2	2
Anti-LTA	<2	512	<2	512

[a] Commercial antisera obtained from Difco.
[b] Washed erythrocytes (10% suspension) were incubated with 1 mg of LTA per ml for 2 h at 37°C, washed three times, and resuspended to a final concentration of 2% in PBS.

Although low concentrations of LTA had no effect on the blood group antigens in serological assays, higher concentrations reduced the hemagglutination titers of both anti-A and anti-B antisera against their respective antigens. Since inhibition was not specific, it is probable that the bound LTA either sterically inhibited the binding of the antibodies to their antigens or interfered in some way with the agglutination reaction itself. The recent finding (10) that blood group antigens are composed of glycolipids may be relevant; the amphipathic LTA molecule may form complexes with these antigens by hydrophobic binding between the lipid portions of the respective molecules. The concept is supported by the apparent complexing of LTA with the gangliosides, glycolipids which share a common lipid moiety (23, 27). Unlike cholera toxin, which recognizes specific saccharide sequences on individual gangliosides (28), LTA binding is equally inhibited by Gm_2 and Gm_3, suggesting

that their common lipid moiety is interacting with LTA.

The concept that sugar moieties may not be involved as structural elements in the membrane receptor for LTA is supported by the inhibition studies with saccharides. None of the nine different saccharides present on mammalian cell membranes (24), or digestion with trypsin or neuraminidase, inhibited LTA binding. The slight increase in LTA binding after neuraminidase digestion suggests the possibility that sialic acid partially masks the receptor for LTA.

The inhibitory effect of serum albumin deserves further mention. Albumin, the major protein of serum, has the unique ability to bind a variety of substances, including a range of fatty acids (16). The binding of fatty acids has been shown to be highly specific and of high affinity (21). A not readily reversible binding of LTA to albumin conjugated with Sepharose 4B has been demonstrated in our laboratories (unpublished data). It is reasonable to assume that albumin similarly binds the LTA molecule through its fatty acid moieties and thereby neutralizes the ability of the molecule to attach to its receptor on the erythrocyte membrane.

Specifically what is the composition of the LTA binding site in erythrocyte membranes cannot be said at present. The data accumulated in this paper and previously (9, 15, 27) suggest that the lipid moieties of the LTA molecule are essential for binding, as indeed they are for the expression of most of the known in vitro and in vivo biological activities of LTA; many of these activities presumably require an initial binding to a eucaryotic cell membrane (5, 9, 27).

Lipid-dependent binding of another amphipathic molecule, LPS of gram-negative organisms, has been characterized. LPS binds via its lipid moiety to cell membrane (11), and the LPS receptor has been shown to consist of the peptide part of a lipoglycoprotein (22).

Now that we have shown that erythrocytes possess a single population of specific binding sites for LTA, the erythrocytes should serve as a readily available source of pure membranes for the isolation and identification of the LTA receptor itself. Such studies are the subject of another communication from our laboratories (M. L. Alkan, T. M. Chiang, and E. H. Beachey, submitted for publication).

ACKNOWLEDGMENTS

We are grateful to Robert Curtis for expert technical assistance and to Johnnie Smith for typing the manuscript.

These studies were supported by program-directed research funds from the U.S. Veterans Administration, by Public Health Service research grants AI-10085 and AI-13550 from the National Institute of Allergy and Infectious Diseases, and by funds from the Australian National Health and Medical Research Council. E.H.B. is the recipient of a Medical Investigatorship Award from the U.S. Veterans Administration, and W.A.S., of a postdoctoral Research Fellowship (F32-AI-05797) from the U.S. Public Health Service.

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