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Influence of Lipoteichoic Acid Structure on Recognition by the Macrophage Scavenger Receptor

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Lipoteichoic acids (LTAs) belong to the immunostimulatory class of molecules of gram-positive bacteria (GPB). Previous investigations showed that the macrophage scavenger receptor (SR), a glycosylated trimeric transmembrane protein, binds directly to many GPB, possibly via LTA. SR binding to other ligands is dependent upon the spatial characteristics of the repeating negative charge of the ligand. We therefore investigated SR recognition of LTA species with various charge densities and distributions by determining the capacity of these LTAs to compete with the binding of metabolically labeled SR to beads coated with the known SR ligand polyguanylic acid. *Staphylococcus aureus* LTA, a classical LTA type (unbranched 1,3-linked polyglycerophosphate chain covalently bound to a membrane diacylglyceroglycolipid), had a 50% inhibitory concentration (IC_{50}) for inhibition of SR binding of 0.84 μg/ml. When the *S. aureus* LTA was rendered more negatively charged by removal of ester-linked alanine from the polyglycerophosphate backbone, the IC_{50} dropped to 0.23 μg/ml. Other polyglycerophosphate LTAs from *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Listeria monocytogenes*, *Listeria welshimeri*, and *Streptococcus sanguis* showed IC_{50}s of 0.5 to 2.1 μg/ml, supporting the role of negative charge in binding to SR. Accordingly, the zwitterionic LTA of *Streptococcus pneumoniae* and *Clostridium innocuum* LTA substituted with positively charged sugar residues had no binding capacity. Monoglycerophosphate branches, but not succinyl ester, affected binding of lipoglycans. The data presented above parallel the previous findings for whole organisms and support the role of surface-associated LTA as a major ligand of GPB for binding to SR. Whether binding of LTA and whole GPB to macrophages initiates uptake and degradation or results in signal transduction remains to be determined.

Sepsis and septic shock due to gram-positive bacteria (GPB) have become increasingly important clinical problems over the last decade (4). Whereas septic shock due to gram-negative bacteria is generally believed to be initiated by lipopolysaccharide (LPS), the bacterial constituents responsible for induction of septic shock by GPB are less clear. Among the candidates so far considered are the surface components lipoteichoic acid (LTA) and peptidoglycan. LTAs are spontaneously released from the cell surface, and release can be augmented by penicillin and other cell-wall-active antibiotics (13). A number of LTAs are recognized by blood monocytes and macrophages, which they stimulate to secrete cytokines and other inflammatory signals in the same pattern as do LPSs (3, 8, 22, 34–36). Peptidoglycan preparations from various GPB (19, 32, 37) can also induce cytokine and mediator release. In spite of these qualitatively similar effects, neither LTA nor peptidoglycan, at immunostimulatory doses, leads to the septic shock syndrome evoked by LPS (9, 20, 36). The reason for this difference may lie in the concentration necessary to stimulate macrophages and monocytes, which is approximately 2 to 3 orders of magnitude higher for LTAs and peptidoglycan than for LPS (19, 35). However, as shown recently, LTA and peptidoglycan may act in synergy to cause shock and multiple organ failure (9).

LTAs represent a group of structurally related lipid macroamphiphiles which are hydrophobically anchored to the cytoplasmic membrane (11a, 13). Because of their intrachain phosphodiester groups, LTAs, together with teichoic acids (TAs), create a negatively charged or zwitterionic network which extends from the membrane to the surface of the bacterium. Structurally, LTAs in the low-G+C subdivision of GPB, which have DNA containing less than 50% guanine plus cytosine (11a, 13), consist of two fatty acid tails hydrophobically anchored in the cell membrane and a long charged hydrophilic chain, with various spatial distributions and densities of charges, depending upon the LTA species.

In the high-G+C subdivision of GPB, LTAs are largely replaced by lipoglycans. They are also anchored to the membrane by a diacylglycerol moiety but contain a linear or branched polysaccharide as the hydrophilic moiety, which may become charged by substitution with monoglycerophosphate branches or succinyl esters (13).

The macrophage scavenger receptor (SR) is a trimeric transmembrane protein found on macrophages. The type I SR binds to a wide range of polyanionic ligands, including modified proteins, acidic phospholipids, endotoxin, and polynucleotides (5). Previous investigations have implicated the collagen-like domain (CLD) as the ligand binding region (1). While the SR is capable of binding to a broad array of ligands, the spatial arrangements of charges within the ligand and within the CLD of the receptor appear to be critical in determining avidity.

We have recently demonstrated that the SR binds directly to many GPB via negative charges and possibly also recognizes polyanionic hydrophilic chains of LTAs (10). In the present study, we therefore examined a number of different LTA species and negatively charged lipoglycans to determine their capacities to bind to the SR. Since little is known of the fate of these macroamphiphiles in the mammalian host (13), our experiments provide insight into LTA binding by an innate pathogen recognition system of the host.

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MATERIALS AND METHODS

Buffers and reagents. Buffers used in experiments were as follows: buffer A, 20 mM Tris base (pH 8.0)–150 mM NaCl–1 mM $CaCl_2$; and buffer A + BSA, buffer A with 2 mg of bovine serum albumin (BSA) per ml and 0.05% NaN_3. Phenylmethylsulfonyl fluoride, leupeptin, pepstatin, poly(G), poly(G) coupled to agarose beads, and geneticin disulfate were purchased from Sigma (St. Louis, Mo.). Ham's F-12 media, L-glutamine, and penicillin-streptomycin were purchased from GIBCO BRL (Gaithersburg, Md.). Fetal bovine serum was from Gemini Bioproducts (Calabasas, Calif.). [^{35}S]methionine (Tran^{35}S-label with 70% methionine) was from ICN Biomedical (Irvine, Calif.).

Bacteria. *Listeria monocytogenes* NCTC 7973 and *Listeria welshimeri* SLCC 5334 were from H. Hof, Institut für Medizinische Mikrobiogie und Hygiene, Mannheim, Germany; *Enterococcus faecalis* Kiel 27738 was from G. Hahn, Institut für Hygiene der Bundesanstalt für Milchforschung, Kiel, Germany; *Enterococcus hirae* ATCC 9790 was from G. Shockman, Temple University, Philadelphia, Pa.; *Staphylococcus aureus* DSM 20233 and *Staphylococcus aureus* Hgol$^-$ϕR were from L. J. Douglas, Glasgow, United Kingdom; *Enterococcus faecium* DSM 2918 and *Bifidobacterium bifidum* DSM 20239 were from Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; *Clostridium innocuum* ATCC 14501 and *Micrococcus luteus* ATCC 4698 were from the American Type Culture Collection, Rockville, Md.; *Lactococcus garvieae* NCDO 2155 was from the National Collection of Dairy Organisms, Reading, United Kingdom; and *Streptococcus pneumoniae* R6 was from R. Hakenbeck, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany.

LTA and lipoglycan preparations. For preparation of LTAs and lipoglycans, bacteria were grown to the late logarithmic phase as described previously (17). The lipid macroamphiphiles were extracted from mechanically disrupted bacteria with hot phenol-water and purified by hydrophobic-interaction chromatography (11, 12, 15). The purified LTAs and lipoglycans were free of detectable amounts of nucleic acids, proteins, polysaccharides, and cell wall material. The structures of the polyglycerophosphate LTAs (11a, 13), polyglycosylglycerophosphate LTA (25), the LTA from *S. pneumoniae* (2), the lipoglucogalactofuranan from *B. bifidum* (11), and the lipomannan from *M. luteus* (12, 31) used in these experiments are shown in Fig. 1. The extent of substitution at position 2 of the polyglycerophosphate LTAs is provided in Table 1 (11a). Alanine esters were removed from *S. aureus* and *Streptococcus sanguis* by mild alkaline treatment (16) to obtain the dealanylated forms.

The tested species of LTAs and lipoglycans contained two fatty acids on their glycolipid anchors. As outlined recently (22, 30), two patterns of fatty acids were observed. One contained branched and straight-chain saturated fatty acids, while the other contained straight-chain saturated and monounsaturated fatty acids. The mean lengths of the fatty acids were between 15 and 17 carbon atoms.

Radiolabeled LTA. *S. aureus* was grown for eight generations in antibiotic medium 3 containing 125 kBq of [2-^{3}H]glycerol per ml (7.4 GBq/mmol). LTA was extracted and purified as described above. The specific ^{3}H radioactivity of purified LTA was 310 kBq/μmol of phosphorus.

Teichoic acid and cell wall preparations. For cell wall preparations, freshly harvested bacteria (*S. aureus* DSM 20233 and *S. aureus* Hgol$^-$ϕR) were suspended in 0.05 M sodium citrate buffer, pH 3, and broken with glass beads in a Braun disintegrator. Extraction with boiling 4% sodium dodecyl sulfate (SDS) and successive digestion with nucleases and pronase were essentially as described previously (18). Teichoic acids were released from cell walls and purified by established procedures (14).

Cells. Chinese hamster ovary cells stably transfected with a truncated form of the cDNA encoding the type I bovine macrophage SR, CHO[s-bSR-I], were kindly provided by M. Krieger (Massachusetts Institute of Technology, Boston). Descriptions of these constructs and details of the transfection have been previously published (33). One positive colony was cloned to yield the cell line CHO[s-bSR-I]-A2, which was used for all experiments involving the type I SR. The secreted receptor retained the binding specificities and affinities of the native form.

Collection of cell supernatant containing labeled SR. CHO[s-bSR-I]-A2 cells were plated at a density of 9×10^5/100 cm^3 in 75-ml Falcon flasks and placed in a 5% CO_2 atmosphere at 37°C. Twenty-four hours after the cells were plated, a 1:100 dilution of a 100 mM solution of sodium butyrate was added. Cells were then grown to 80% confluence (approximately 48 h) and, after removal of spent medium and washing, were metabolically labeled by addition of 4.5 ml of serum-free labeling medium (GIBCO) containing 2 mM glutamine, 100 U of penicillin-streptomycin per ml, 1 mM sodium butyrate, and 80 μCi of [^{35}S]methionine per ml. After a 5-hour incubation at 37°C in a 5% CO_2 atmosphere, supernatant was collected and treated with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μM [each] leupeptin and pepstatin). This labeled supernatant was added to Falcon 2099 tubes that had been precoated with and contained buffer A + BSA (2 volumes of supernatant to 1 volume of buffer A + BSA) and was stored at −80°C.

Competition assay using lipoteichoic acids. Microcentrifuge tubes (1.6 ml; National Scientific Supply Co., Inc., San Rafael, Calif.) were precoated with BSA in water at a concentration of 6 mg/ml for 5 min. The BSA solution was then removed, and the tubes were placed on ice. Twofold serial dilutions of LTA species suspended in sodium acetate buffer (pH 4.7) were added to buffer A to a total volume of 875 μl. Labeled type I bovine macrophage SR (sBSRI) (100 μl) was then added, and the tubes were incubated on ice for 30 min. Poly(G) agarose suspension (25 μl) was added, and the tubes were rotated at 4°C for 4 h. The beads were then allowed to settle, washed with 1 ml of ice-cold buffer A, allowed to settle again, resuspended in 1 ml of buffer A, and transferred to a new microcentrifuge tube. The beads were again allowed to settle, and the supernatant was removed. The pellet was resuspended in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and subjected to SDS–10% PAGE under reducing conditions. The gel was subsequently incubated in 0.125 M sodium salicylate with 30% methanol, dried, and autoradiographed using Kodak X-AR film. The intensity of the band at 77 kDa (the molecular mass of monomeric SR) was measured by densitometry scanning, and the LTA concentration required to inhibit SR binding to poly(G) beads by 50% (IC_{50}) was determined.

Competition assay using cell wall preparations. The competition assay utilized to test for SR binding to cell walls was the same as that performed on the purified LTA preparations, except that the cell wall preparations were sonicated for 10 s three times in a horn sonicator to obtain a homogeneous suspension. An additional control assay was also performed in the absence of poly(G) beads. This assay was done to ensure that SR was not binding to cell wall fractions which were then settling out of suspension, thereby preventing detection of a binding interaction.

Cell binding assay. J774 macrophages (2×10^5) were plated and grown to confluence on coverslips in a 24-well plate containing 1 ml of RPMI 1640 with 5% fetal bovine serum. The cells were washed three times with 500 ml of cold RPMI 1640 lacking fetal bovine serum and then incubated in the same medium with 10-fold dilutions of poly(G) at 4°C for 30 min. The medium was then replaced with medium containing the same concentration of poly(G) and 5 μg of ^{3}H-labeled dealanylated *S. aureus* LTA per ml. The incubation was continued for 4 h. The cells were then washed three times with 500 ml of cold RPMI 1640 without fetal bovine serum. The coverslips were subsequently placed in scintillation cocktail and vortexed to solubilize the cells, and the radioactivity was then counted with a liquid scintillation counter.

RESULTS

Types I and II lipoteichoic acid and a glycerophosphate-substituted lipoglycan bind to bSRI. We first examined the ability of typical polyglycerophosphate LTAs (type I in Fig. 1) to bind to the SR. These LTAs consist of an unbranched 1,3-linked polyglycerophosphate chain covalently bound to a membrane diacylglyceroglycolipid. The polyglycerophosphate chain varies in length from 18 to 40 glycerophosphate residues and is partially substituted at position 2 of the glycerophosphate residue with D-alanine ester (which confers a positive charge, leading to charge compensation) or with a combination of D-alanine ester and glycosyl residues (11a). LTAs, serially diluted twofold, were used to compete for binding of radiolabeled SR to poly(G) beads, a known ligand of the SR. As an example, the results of the experiment with *L. welshimeri* LTA are shown in Fig. 2. The IC_{50}s of LTAs from seven different GPB are listed in Table 1. All LTAs showed effective binding. The strongest binding (IC_{50} = 0.23 μg/ml) was observed with purely anionic *S. aureus* LTA which was prepared by removal of the positively charged alanine ester from the polyglycerophosphate chain by gentle alkaline hydrolysis. Native *S. aureus* LTA, 59% substituted with alanine, bound to SR with an IC_{50} of 0.84 μg/ml, suggesting that partial compensation of the negative charges decreases affinity for SR. Other alanine-free (native or dealanylated) type I LTAs tested contained variable amounts of glycosyl substituents comprising monogalactosyl residues in *Listeria* species and mono-, di-, and oligoglucosyl residues in enterococci and *S. sanguis*. The IC_{50}s of these compounds ranged from 0.5 to 2.1 μg/ml. These results probably reflect the role of the sugar residues in steric hindrance to SR binding, although an exact correlation between substitution and avidity is difficult to determine.

The LTA from *L. garvieae* (type II in Fig. 1) differs from type I in that it has digalactosyl residues intercalated between the glycerophosphate residues. At the C-2 position they are consistently substituted with monogalactosyl residues (25). The IC_{50} of this anionic LTA was 2.15 μg/ml (Table 2). The higher IC_{50} in comparison to that of anionic dealanylated *S. aureus* type I LTA is likely explained by the increased distance be-

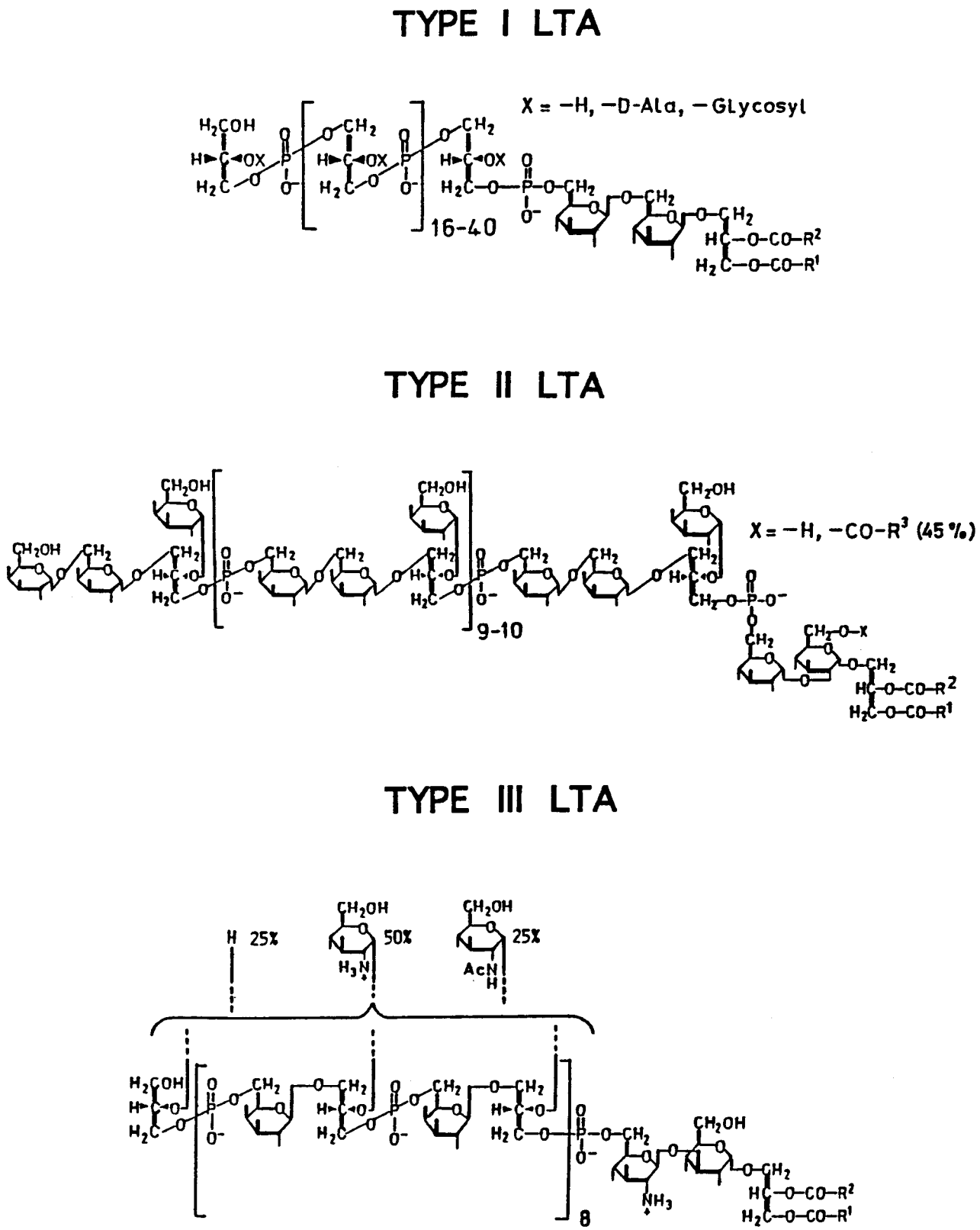
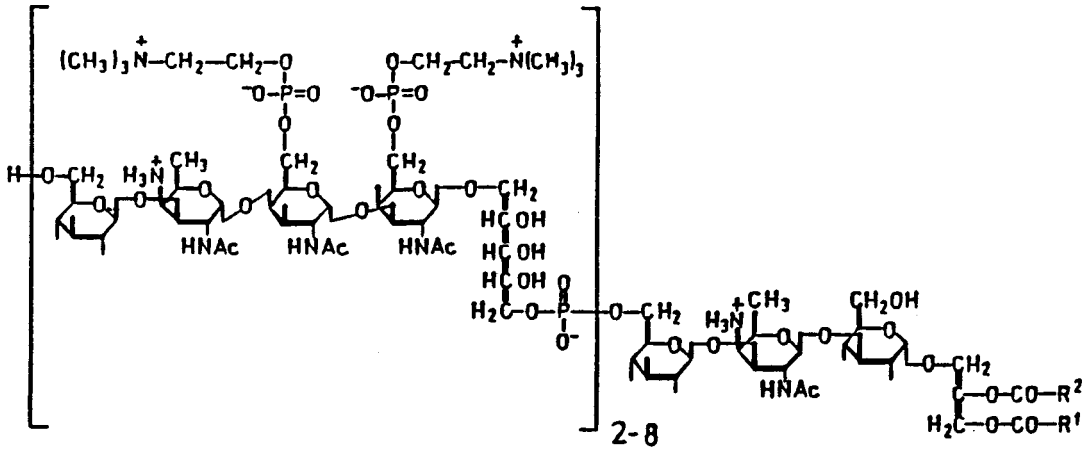
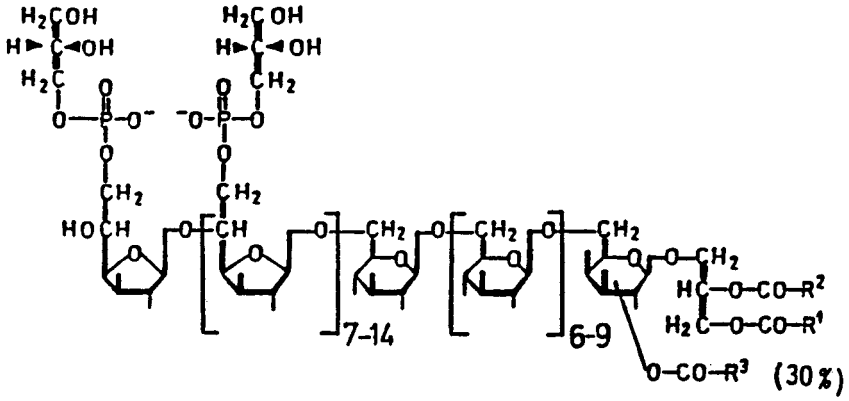


FIG. 1. Structures of the LTAs and the lipoglycans (lipoglucogalactofuranan and lipomannan) used in these experiments. The top structure under lipoglycans is from *B. bifidum*. The bottom structure under lipoglycans is from *M. luteus*. See the text and tables for details.

TYPE IV LTA



LIPOGLYCANS



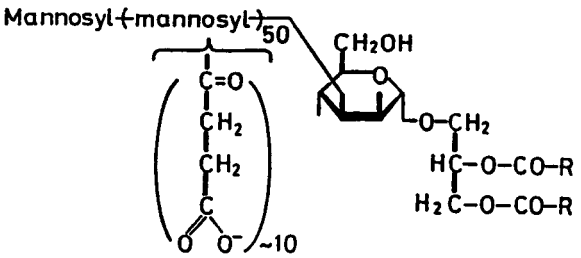


FIG. 1—*Continued.*

TABLE 1. Inhibition of SR binding to poly(G) beads by classical (type I) LTAs

LTA source	LTA IC_{50}[a]		Fraction substituted with[b]:	
	μg/ml	mol/liter	D-Alanine	Glycosyl residues
S. aureus	0.84	1.2×10^{-7}	0.59	GlcNAc, <0.01
S. aureus, dealanylated	0.23	3.8×10^{-8}	NA[c]	GlcNAc, <0.01
S. aureus, deacylated	>>100	$>>10^{-5}$	NA	GlcNAc, <0.01
E. faecalis	0.65	1.0×10^{-7}	0.32	Glc_2, 0.36
E. faecalis zym., deacylated	>>100	$>>10^{-5}$	NA	Glc_2, 0.49
E. faecium	1.8	2.5×10^{-7}	NA	Glc, 0.23; Glc_2, 0.31
E. hirae	2.1	2.3×10^{-7}	NA	Glc, 0.32; Glc_2, 0.17; Glc_3, 0.02; Glc_4, 0.12
L. monocytogenes	1.68	2.5×10^{-7}	NA	Gal, 0.21
L. welshimeri	2.00	2.7×10^{-7}	NA	Gal, 0.37
S. sanguis	0.51	7.4×10^{-8}	0.28	Glc, 0.06; Glc_2, 0.21; Glc_3, 0.08
S. sanguis, dealanylated	0.33	4.8×10^{-8}	NA	Glc, 0.06; Glc_2, 0.21; Glc_3, 0.08

[a] The IC_{50} for inhibition of SR binding to poly(G) beads was determined as described in Materials and Methods.
[b] Fraction of glycerophosphate residues substituted at the 2′ position with the indicated residue.
[c] NA, not applicable.

tween the negative charges and possibly the high glycosyl content. *B. bifidum* produces a lipoglycan instead of an LTA (Fig. 1). The amphiphilic chain is a linear lipoglucogalactofuranan with glycerophosphate residues attached as monomeric side branches to the galactofuranosyl residues. This lipoglycan exhibited strong binding to the SR, with an IC_{50} of 0.65. This relatively strong inhibition of SR binding may be due to the particular location of the phosphate, which is extended away from the backbone.

Since the mean lengths of the LTAs and lipoglycans were known (30) and the solutions used in the present experiments were defined by phosphorus or sugar determinations, the IC_{50}s are also expressed in terms of molar concentrations. As shown in Tables 1 and 2, the molar IC_{50}s lie in the range between 4×10^{-8} and $>> 10^{-5}$.

LTA types III and IV and micrococcal lipomannan do not bind to bSRI. In place of a typical polyglycerophosphate-type LTA, *S. pneumoniae* contains a unique zwitterionic macroamphiphile known as pneumococcal Forssman antigen or, more correctly, pneumococcal lipoteichoic acid (2). In this LTA (type IV in Fig. 1), the negative charges of the side chain phosphate groups are compensated by the adjacent positively charged choline residues and the negative charge of the intrachain phosphate group is compensated by the positive charge of the 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose residue (23, 24). With concentrations as high as 100 μg/ml there was no inhibition of SR binding. This finding demonstrates the necessity of the net negative charge for the binding of these macroamphiphiles to the SR, particularly if one compares the different responses to the choline phosphate residues of *S. pneumoniae* LTA and the glycerophosphate residues of the *B. bifidum* lipoglycan.

Type III LTA from *C. innocuum* also contains a number of positively charged residues. This charge is provided by the $GlcN^+$ residues which are randomly distributed along the polygalactosylglycerophosphate chain (13a). Similar to the *S. pneumoniae* LTA results, there was no inhibition of SR binding by this LTA at concentrations as high as 100 μg/ml. Although in this LTA only 50 to 65% of the negative chains are compensated, the positively charged $GlcN^+$ residues, protruding from the chain, may act by electrostatic repulsion.

M. luteus was the first GPB identified that lacks LTA (31). When phenol extraction is performed, an acidic lipomannan is obtained that consists of 50 to 70 mannose residues and is anchored at its reducing terminus by diacylglycerol (Fig. 1). The acidic nature of the molecule is due to the esterification of approximately 20% of the mannose residues with succinyl groups (12, 31). When tested at concentrations of up to 100 μg/ml, there was no inhibition of SR binding. This is probably attributable to the scattered arrangement of negative charges over the molecule resulting in insufficient charge density.

Deacylated LTAs and TA do not bind to bSRI. LTAs and lipoglycans form micelles in an aqueous dispersion which contain approximately 150 molecules in a spherical arrangement with a high density of negative charges on their surface (26). This supramolecular structure is dependent upon the presence of the hydrophobic fatty acid residues. Deacylation and concomitant loss of micellar formation have been shown to result in a loss of cellular biological activities. Accordingly, deacylated LTAs from *S. aureus* and *E. faecalis* did not inhibit SR binding at concentrations of up to 100 μg/ml. Polyribitol phosphate TA, which like deacylated LTAs forms monomeric solutions, also failed to inhibit SR binding.

Whole cell wall binds minimally to bSRI. Whole cell walls from two strains of *S. aureus* were tested for their ability to compete for binding of SR to poly(G) beads. The cell wall preparations contained covalently attached polyribitol phosphate TA (1.1 to 1.2 mmol of phosphorus per g [dry weight] of material) which in one strain (*S. aureus* DSM 20233) was substituted with acetyl GlcN (GlcNAc) residues (GlcNAc/phosphorus = 0.62) while that of the other strain (*S. aureus*$Hgol^-$

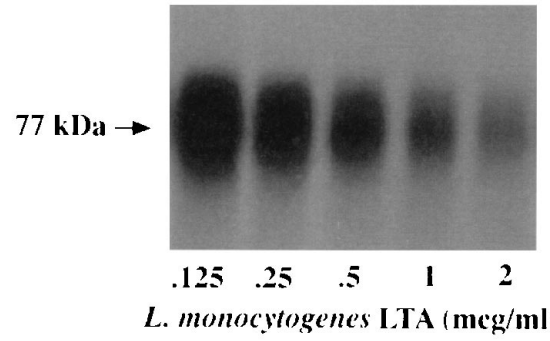


FIG. 2. Inhibition of bSRI binding to poly(G) beads by *L. monocytogenes* LTA. Labeled bSRI was incubated with poly(G) beads in the absence (data not shown) or presence of increasing concentrations of *L. monocytogenes* LTA, as described in Materials and Methods. bSRI binding to the beads was analyzed by SDS-PAGE autoradiography. The IC_{50} for inhibition of bSRI binding was determined by densitometry scanning.

TABLE 2. Inhibition of SR binding to poly(G) beads by nonclassical LTAs and lipoglycans

Macroamphiphile	Source	IC_{50}[a]	
		μg/ml	mol/liter
LTA			
Type II	*L. garvieae*	2.15	2.4×10^{-7}
Type III	*C. innocuum*	>>100	$>>10^{-5}$
Type IV	*S. pneumoniae*	>>100	$>>10^{-5}$
Lipoglycans			
GroP-lipoglucogalacto-furanan	*B. bifidum*	0.65	1.2×10^{-7}
Succinyl lipomannan	*M. luteus*	>>100	$>>10^{-5}$

[a] The IC_{50} for inhibition of SR binding to poly(G) beads was determined as described in Materials and Methods.

ϕR) was GlcNAc free. We tested these walls both with native alanylated (Ala/phosphorus = 0.65) and dealanylated-TA. Even with dealanylated and nonglycosylated forms, at concentrations as high as 25 μg/ml, we detected only minimal evidence of competition for binding of SR.

Poly(G) competes for binding of radiolabeled LTA to J774 macrophages. Finally, we tested whether LTA binds to the SR in macrophages. The association of [^{3}H]LTA with J774 cells at 4°C was inhibited in a dose-dependent fashion by poly(G) at concentrations ranging from 0.2 to 20 μg/ml (Fig. 3). Further increases in concentration did not further affect binding. This result suggests that binding of LTA to macrophages is occurring at least in part via interaction with the SR.

DISCUSSION

By using a number of highly purified LTA and lipoglycan preparations, we have shown that many, but not all, species of LTA inhibit binding of SR to a known ligand. As with other SR ligands, binding occurs through the CLD of the receptor. The extent of binding appears to depend upon the presence of a repeating negative charge and the degree to which that charge is accessible to interact with the cationic residues of the CLD of SR. LTA species with the most highly accessible negative charge, such as dealanylated *S. aureus* LTA, provided the strongest binding. Those with partial or complete compensation with positive residues, such as native *S. aureus* and *S. pneumoniae* LTAs, respectively, showed diminished or a complete absence of binding. Steric hindrance by glycosyl substituents or spacing of negative charges also appeared to affect the binding. As shown with the lipoglycan of *B. bifidum*, glycerophosphate side chains attached to neighboring sugar residues bound to SR as effectively as the intrachain phosphate groups of LTAs. The lipomannan of *M. luteus*, which is also negatively charged but via substitution with succinyl ester, showed no binding, which may be attributed to insufficient arrangement and density of negative charges. This may also be relevant for the lipoarabinomannans of *Mycobacterium tuberculosis* and *Mycobacterium leprae*, which are likewise anionic, mostly because of their succinyl ester substituents (21). A complete loss of binding resulted from deacylation of LTAs. This may indicate that the high density of negative charges, as it occurs on the surface of micelles (26), is required or that the acyl chains themselves participate in binding. Reported critical micelle concentrations of LTAs range from 40 to 60 μg/ml (6) and from 1 to 10 μg/ml (38). The IC_{50}s reported here (0.25 to 2 μg/ml) are near the lower limit, but technical problems in critical micelle concentration measurements of macroamphiphiles do not allow the definitive definition of the biologically active form. The fatty acid composition has no obvious influence on binding to SR, because the equally effective *S. aureus* and *S. sanguis* LTAs are representatives of the two different fatty acid patterns (22, 30) observed among the macroamphiphiles used in this study.

Free and cell wall-associated TAs displayed poor binding properties, even when alanine ester was removed and glycosyl substituents were absent. The reason for the inability of whole cell walls to efficiently compete with the binding of SR to poly(G) beads may relate to decreased accessibility of the polyribitol phosphate TA in the cell wall complex and/or to the larger distance between anionic chains compared with the interchain distance in LTA micelles.

The importance of the negative charge in LTA binding for the activation of the classical complement pathway has been described previously (27, 28). The degree of interaction with C1 and its subcomponent C1q, which also contains short collagenous domains (1), was shown to depend upon the presence of the negative charges of the phosphate groups of LTA. There was reduced interaction when there was alanine substitution or di- and trihexosyl residue substitution at the C-2 position, and deacylated LTA had minimal ability to activate complement. Our results generally matched this pattern, although there was minor variability among specific LTAs. This is not surprising since subtle changes in ligand conformation can greatly alter SR avidity. In the best-characterized situation, the polynucleotides poly(I) and poly(G) serve as ligands whereas poly(A) and poly(C) do not because of their inability to form a base-quartet-stabilized four-stranded helix structure (29). It is notable, however, that the pattern of LTA binding we obtained correlates with that which we previously demonstrated for binding of whole organisms to the SR (10).

By using *S. aureus* [^{3}H]LTA, we found that LTA binding to macrophages takes place, at least in part, through an SR-type interaction. Whether the observed competition for LTA binding by poly(G) reflects inhibition of binding to SR type I, SR type II, or another receptor with similar ligand specificity is not known. The inability of poly(G) to completely abolish the binding of labeled LTA to the macrophages is consistent either

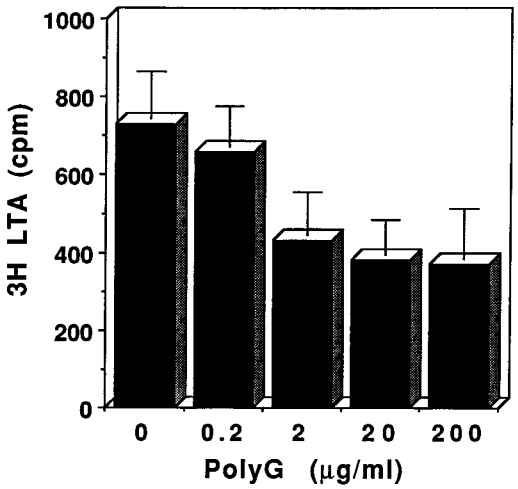


FIG. 3. Inhibition of [^{3}H]LTA (*S. aureus*) binding to J774 macrophages by poly(G). Binding of labeled *S. aureus* LTA to J774 macrophages was measured in the absence and in the presence of increasing concentrations of poly(G), as described in Materials and Methods. Bound [^{3}H]LTA was determined by scintillation counting. The data shown represent the mean results ± the standard deviations of three experiments. Significantly less binding occurred ($P < 0.05$) with poly(G) concentrations of 2.0 μg/ml or greater.

with redundancy of macrophage cell surface receptors for bacterial cell surface components or possibly with direct intercalation of LTA into the macrophage plasma membrane via acyl chains. Interestingly, CD14, a molecule which is known to participate in the inflammatory cascade by binding the LPS-LPS binding protein complex, has recently been shown to activate J774 cells when exposed to GPB cell wall components (32, 37).

GPB sepsis has become an increasingly important clinical entity, and LTA and peptidoglycan have been shown to have inflammatory properties similar to although less potent than those of LPS (3, 7, 8, 19, 34). Nevertheless, it has recently been demonstrated that LTA and peptidoglycan may act in synergy to cause shock and multiple organ failure (9). The means by which LTA is recognized and processed by mammalian cells is poorly understood. We have shown that SR, a receptor present on macrophages, avidly binds via a CLD to a wide range of LTAs, apparently by means of charge pattern recognition. Whether this pathway for LTA contributes to the derangements seen in GPB sepsis or is a means by which the macroamphiphile can be removed through a less harmful route is still unknown. Future studies examining the subcellular distribution of LTA following uptake by the SR and its role in activation of the macrophage should help to unravel this question.

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