INFECTION AND IMMUNITY, Nov. 1986, p. 494–499
0019-9567/86/110494-06$02.00/0

Vol. 54, No. 2

The Ability to Sensitize Host Cells for Destruction by Autologous Complement Is a General Property of Lipoteichoic Acid

BARRY D. WEINREB,[1]* GERALD D. SHOCKMAN,[2] EDWIN H. BEACHEY,[3] ANDREA J. SWIFT,[1,4] AND JERRY A. WINKELSTEIN[1,4]

The Department of Pediatrics[1] and the Department of Molecular Biology and Genetics,[4] The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; the Department of Microbiology, The Temple University School of Medicine, Philadelphia, Pennsylvania 19140[2]; and the Departments of Medicine and Microbiology, Veterans Administration Medical Center, Memphis, Tennessee 38104[3]

Received 7 July 1986/Accepted 12 August 1986

Previous studies have demonstrated that lipoteichoic acid (LTA) from *Streptococcus pneumoniae* binds to erythrocytes and renders them susceptible to lysis by autologous complement. The present study was performed to determine whether LTA from two other gram-positive bacterial species had the ability to render mammalian cells susceptible to lysis by autologous complement. Human erythrocytes were sensitized with LTA from *S. pneumoniae*, *Streptococcus pyogenes*, or *Lactobacillus fermentum*. Under incubation in normal autologous serum, lysis was observed with each of the LTA-sensitized erythrocyte preparations. When erythrocytes from a C2-deficient patient were sensitized with the LTA preparations and then incubated in autologous, C2-deficient serum, the erythrocytes sensitized with *S. pyogenes* or *L. fermentum* LTA demonstrated relatively little lysis, whereas the erythrocytes sensitized with *S. pneumoniae* LTA yielded near-total lysis. After reconstitution of the C2-deficient serum with purified human C2, lysis was observed with all three LTA preparations. When erythrocytes from an agammaglobulinemic patient were sensitized with either the *S. pyogenes* or the *L. fermentum* LTA, they were not lysed in the presence of autologous agammaglobulinemic serum, whereas the erythrocytes sensitized with *S. pneumoniae* LTA were completely lysed. Serum obtained from the agammaglobulinemic patient after reconstitution with intravenous pooled gamma globulin was able to lyse autologous erythrocytes sensitized with each of the three LTA preparations. These results demonstrate that the ability to render host cells susceptible to lysis by autologous complement is a general property of LTA. Whether activation of the autologous complement occurs by the classical or alternative pathways and whether it is antibody dependent depends on the nature of the bacterial LTA.

Many gram-positive bacterial species have been shown to possess cell surface amphiphiles (27, 28). A variety of gram-positive bacteria, including many *Streptococcus*, *Lactobacillus*, *Staphylococcus*, and *Bacillus* species, contain amphiphilic lipoteichoic acids (LTA) which consist of a substituted or unsubstituted polyglycerophosphate chain covalently linked to a hydrophobic lipid moiety (see references 16, 27, and 28 for reviews). The Forssman antigen of *Streptococcus pneumoniae* (4, 8), termed pneumococcal LTA (4), is a complex amphiphile which contains ribitol phosphate, choline, glucose, galactosamine, and fatty acids (4, 6, 8).

LTA of gram-positive bacteria are considered to be closely associated with the bacterial cytoplasmic membrane via hydrophobic interactions. However, a variety of data indicate that these molecules extend through the cell wall to the bacterial surface (28). In addition, bacteria are known to secrete these amphipathic molecules in both the fully acylated and unacylated forms (15, 22); these events are greatly influenced by environmental factors (28), including enhancement of release by exposure to antibiotics (1, 5). Once released from bacteria, LTA can bind to a variety of mammalian cells (2, 19, 24), including erythrocytes (3), and retain their antigenic activity (9, 16, 21, 27). In addition, cell-bound LTA is capable of activating the complement system in heterologous serum (7, 11, 21).

The studies cited above suggest that LTA and other bacterial amphiphiles bound to host cells could interact with the complement system of the host to cause complement-mediated damage during the course of a natural bacterial infection. However, numerous studies have reported that mammalian cells sensitized with antibody are relatively resistant to complement-mediated lysis when the complement source is autologous or homologous serum (10, 20). Despite the relative resistance of cells to lysis by autologous complement, recent studies have shown that *S. pneumoniae* LTA can render human erythrocytes susceptible to lysis by autologous complement via activation of either the alternative or the classical pathway (12).

The unique and complex chemical structure of pneumococcal LTA (4, 6) raises the question of whether its ability to render mammalian cells susceptible to autologous complement damage is common to LTA from other bacterial species. The present study examined the ability of LTA from two other gram-positive bacterial species, *Streptococcus pyogenes* and *Lactobacillus fermentum*, to endow human erythrocytes with the ability to activate autologous complement and cause complement-mediated lysis. Additional studies were performed to determine whether complement activation occurred via the classical or alternative pathway and whether it was antibody dependent.

MATERIALS AND METHODS

Buffers. Veronal-buffered saline, pH 7.4, with an ionic strength of 0.147 and containing 0.15 mM Ca^{2+} and 1.0 mM Mg^{2+} (VBS^{2+}) was prepared as described previously (18). Veronal-buffered saline, pH 7.4, ionic strength 0.147, containing 0.15 mM Ca^{2+}, 1.0 mM Mg^{2+}, and 0.1% gelatin

* Corresponding author.

(GVB^{2+}), was also prepared. Veronal-buffered saline, pH 7.4, ionic strength 0.147, containing 0.01 M EDTA, was also prepared with 0.1% gelatin (GVB/EDTA) and without gelatin (VBS/EDTA).

Sera. Sera were obtained from a normal volunteer, from a patient with a genetically determined complete deficiency of C2 (13), and from a patient with newly diagnosed agammaglobulinemia (immunoglobulin G [IgG], 22 mg/dl; IgA, 7 mg/dl; and IgM, 14 mg/dl). The sera were stored in small volumes at −70°C until use. To evaluate the role of immunoglobulin in complement activation by LTA, serum samples were obtained from the agammaglobulinemic patient before (IgG, 22 mg/dl) and after (951 mg/dl) the infusion of intravenous IgG (Gamimmune; Cutter Biologicals, Emeryville, Calif.).

Complement components. Purified human C2 was purchased from Cordis Laboratories, Miami, Fla.

Erythrocytes. Erythrocytes were obtained from the normal volunteer, the C2-deficient patient, and the agammaglobulinemic patient as noted above. Whole blood was drawn into sterile tubes containing sufficient EDTA to yield a final concentration of 0.01 M and then stored at 4°C for no longer than 4 days before use. All erythrocyte preparations were washed three times in VBS/EDTA and twice in VBS^{2+} and then standardized spectrophotometrically to 5.4×10^8 cells per ml in VBS^{2+} immediately before use.

***S. pneumoniae* LTA.** Purified LTA was prepared from an unencapsulated pneumococcal strain, R36A, as described previously (4, 11). Briefly, log-phase organisms were harvested, washed, and allowed to undergo autolysis. The resulting pellet was treated with trypsin and defatted sequentially with 95% acetone, ethanol-ether (3:1), and chloroform-methanol (1:1). The residue was extracted with H_2O, and the extract was treated with DNase and RNase and then precipitated with absolute ethanol. The precipitate was extracted with H_2O, precipitated with acetone, redissolved in H_2O at a concentration of 1.0 mg/ml, and stored at −70°C.

***L. fermentum* LTA.** Purified LTA isolated from *L. fermentum* NCTC 6991, prepared as described previously (25, 26), was the generous gift of A. J. Wicken, University of New South Wales, Sydney, Australia. The LTA was reconstituted in H_2O at a concentration of 1.0 mg/ml and stored at −70°C.

***Streptococcus pyogenes* LTA.** Purified LTA isolated from *S. pyogenes* 1-RP41 was prepared as described previously (23). Briefly, the streptococci were grown for 24 h in 60-liter batches in Todd-Hewitt broth at 37°C. The cells were collected by centrifugation, washed twice in saline, and then extracted with cold phenol at pH 4.7. The aqueous phase, after extensive dialysis against distilled water, was purified by molecular sieve chromatography on Sepharose 6B by using 0.2 M ammonium acetate as eluent and lyophilized. Purity was determined by chemical analysis (23). The LTA was reconstituted in H_2O at a concentration of 1.0 mg/ml and stored at −70°C.

Monoclonal antibody to polyglycerol phosphate. A murine monoclonal antibody of the IgG3 class, which recognizes the polyglycerol phosphate immunodeterminants of LTA of *Streptococcus mutans* Ingbritt 7B11, was prepared as described previously (14).

In vitro complement activation. Erythrocytes at a concentration of 5.4×10^8/ml in VBS^{2+} were incubated for 30 min at 37°C with the desired concentrations of LTA (LTA-E) or with VBS^{2+} (VBS-E). Treated erythrocytes were washed three times in GVB^{2+}. The LTA-E or VBS-E were then incubated at a concentration of 5.4×10^7/ml in the desired concentration of autologous serum for 30 min at 37°C. The percent lysis was measured spectrophotometrically, and specific lysis was calculated by subtracting the percent lysis of unsensitized erythrocytes (VBS-E) from that of erythrocytes sensitized with LTA (LTA-E). Unsensitized erythrocytes preincubated with buffer (VBS-E) consistently demonstrated less than 3% lysis over the range of serum concentrations used.

Agglutination of sensitized erythrocytes. Erythrocytes at a concentration of 5.0×10^8/ml in VBS^{2+} were incubated with each of the three LTA preparations (20 μg/ml) or with VBS^{2+} for 30 min at 37°C. The cells were then washed three times and resuspended in VBS^{2+} to their initial concentration. Samples of 50 μl of LTA-E or VBS-E were then incubated in a microtiter plate (Dynatech Laboratories, Inc., Alexandria, Va.), with 25 μl of the 7B11 monoclonal antibody diluted 1:10, 1:100, or 1:1,000 in GVB/EDTA or with 25 μl of buffer at 37°C. Wells were examined for gross and microscopic hemagglutination after 1 h.

RESULTS

Binding of LTA to human erythrocytes. Previous studies have shown that, under the conditions of the present experiments, pneumococcal LTA binds to human erythrocytes (11). To determine whether LTA isolated from *L. fermentum* and *S. pyogenes* would also bind to human erythrocytes under the conditions of the current experiments, we used the monoclonal IgG antibody to polyglycerol phosphate (7B11) to agglutinate erythrocytes preincubated with these two LTA preparations. Erythrocytes which had been preincubated with the *L. fermentum* or *S. pyogenes* LTA (20 μg/ml) were agglutinated by the anti-polyglycerol phosphate antibody at a 1:10, 1:100, and 1:1,000 dilution. In contrast, erythrocytes preincubated with 20 μg of pneumococcal LTA per ml, which does not contain a polyglycerol phosphate backbone, were not agglutinated by the anti-polyglycerol phosphate antibody at any dilution. Control erythrocytes which had not been preincubated with any LTA were not agglutinated by the monoclonal antibody.

Lysis of LTA-sensitized erythrocytes by autologous serum. Normal human erythrocytes were preincubated with LTA isolated from *L. fermentum*, *S. pyogenes*, or *S. pneumoniae* (50 μg/ml) and then incubated with various concentrations of normal autologous serum. Increasing lysis was seen for all three species of LTA with increasing concentrations of serum, with complete lysis occurring at serum concentrations of 10% and higher (Fig. 1). Normal human erythrocytes were also preincubated with various concentrations of the same preparations of LTA and then incubated with 20% autologous serum. A dose-dependent increase in lysis for all LTA species was observed, with complete lysis occurring at a concentration of 10 μg of LTA per ml and higher (Fig. 2). To demonstrate that the lysis was mediated by complement, erythrocytes presensitized with each of the three LTA preparations (20 μg/ml) were incubated with 20% autologous serum which had been either heated at 56°C for 1 h or treated with 0.01 M EDTA. Significant lysis was not observed in either case.

Role of the alternative pathway in lysis of LTA-sensitized erythrocytes by autologous complement. Erythrocytes from a patient with a genetically determined complete deficiency of C2 were preincubated with each of the three LTA preparations at a concentration of 50 μg/ml and then exposed to increasing concentrations of autologous C2-deficient serum. Erythrocytes sensitized with LTA isolated from *L.*

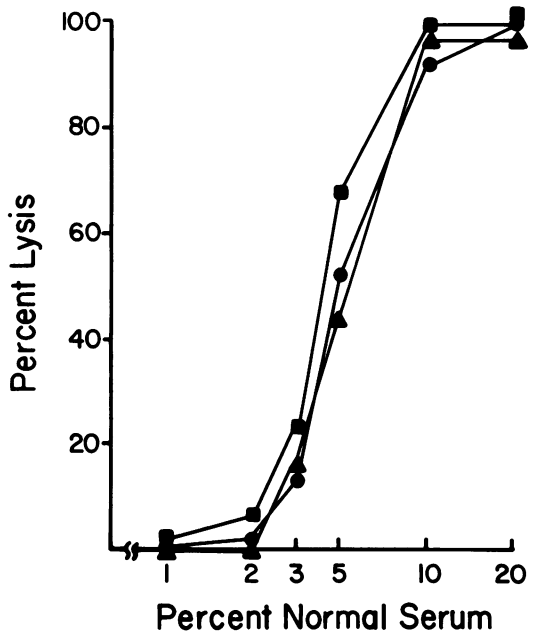

FIG. 1. Lysis of human erythrocytes sensitized with 50 μg of LTA per ml from *S. pneumoniae* (●), *S. pyogenes* (■), or *L. fermentum* (▲). LTA-E were incubated for 30 min at 37°C with increasing concentrations of normal autologous serum. No significant lysis of control erythrocytes was observed.

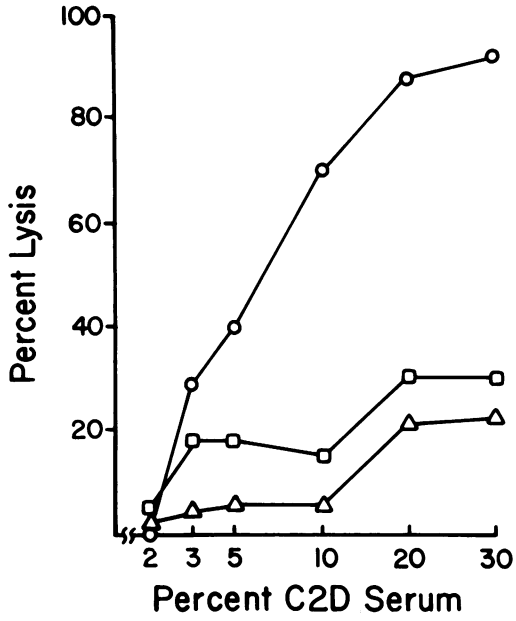

FIG. 3. Lysis of erythrocytes (from a C2-deficient human) sensitized with 50 μg of LTA per ml from *S. pneumoniae* (○), *S. pyogenes* (□), or *L. fermentum* (△). LTA-E were incubated for 30 min at 37°C with increasing concentrations of C2-deficient autologous human serum. No significant lysis of control erythrocytes was observed.

fermentum and *S. pyrogenes* demonstrated significantly less lysis than did erythrocytes sensitized with the *S. pneumoniae* LTA (Fig. 3). Erythrocytes from the C2-deficient patient were also preincubated with varying concentrations of LTA and then incubated with 50% autologous serum. The LTA isolated from *L. fermentum* and *S. pyogenes* produced relatively little lysis, even at doses as high as 100 μg/ml (Fig. 4), whereas a dose-dependent increase in lysis was demonstrated with the pneumococcal LTA, reaching 94% lysis at 100 μg/ml. Erythrocytes sensitized with each of the three

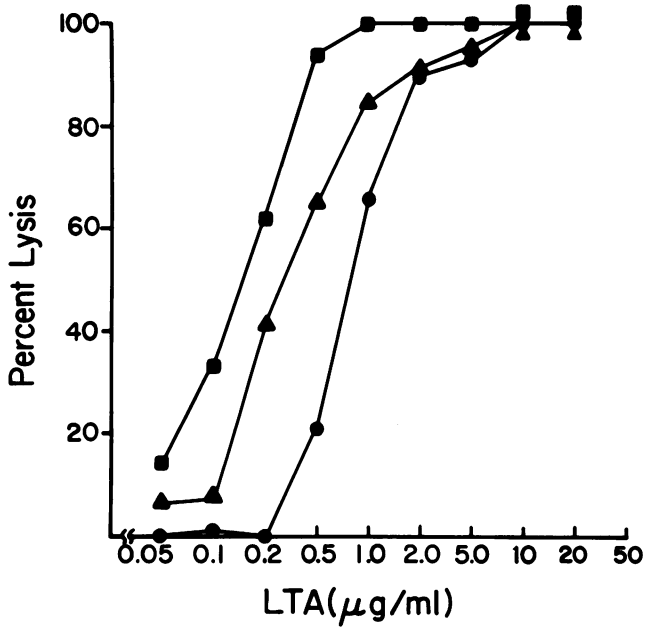

FIG. 2. Lysis of human erythrocytes sensitized with increasing doses of LTA from *S. pneumoniae* (●), *S. pyogenes* (■), or *L. fermentum* (▲). LTA-E were incubated for 30 min at 37°C with 50% autologous normal human serum. No significant lysis of control erythrocytes was observed.

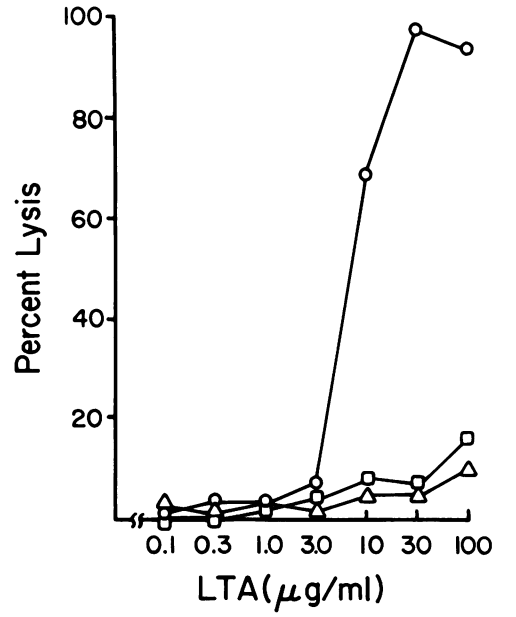

FIG. 4. Lysis of erythrocytes (from a C2-deficient human) sensitized with increasing doses of LTA from *S. pneumoniae* (○), *S. pyogenes* (□), or *L. fermentum* (△). LTA-E were incubated for 30 min at 37°C with 50% C2-deficient autologous human serum. No significant lysis of control erythrocytes was observed.

LTA preparations (100 μg/ml) and then incubated in 50% C2-deficient serum which had been heated at 56°C for 1 h or treated with 0.01 M EDTA demonstrated no significant lysis (<4%).

The C2-deficient serum was then reconstituted with various concentrations of purified human C2 and tested to determine whether it was able to support the lysis of LTA-sensitized erythrocytes. Erythrocytes were preincubated with each of the three LTA preparations at 3 or 30 μg/ml, washed, and then incubated with 50% autologous serum to which various concentrations of C2 had been added. Lysis of sensitized erythrocytes was enhanced for all three species of LTA by the addition of human C2 (Fig. 5). Not shown in Fig. 5 are the results for pneumococcal LTA at 30 μg/ml, which yielded 100% lysis without C2 reconstitution, as expected.

Role of immunoglobulin in lysis of LTA-sensitized erythrocytes by autologous complement. Erythrocytes from a newly diagnosed, untreated patient with agammaglobulinemia were preincubated with each of the three different LTA preparations (50 μg/ml). The sensitized cells were then incubated with increasing concentrations of autologous serum, both pretreatment serum (IgG, 22 mg/dl) and serum obtained after intravenous infusion of gamma globulin (IgG, 951 mg/dl). The erythrocytes sensitized with either the *L. fermentum* LTA or the *S. pyogenes* LTA demonstrated little if any lysis in the immunoglobulin-deficient (pretreatment) serum, even at concentrations as high as 50%. In contrast, the erythrocytes sensitized with pneumococcal LTA showed increasing amounts of lysis with increasing concentrations of immunoglobulin-deficient serum. In autologous serum reconstituted with IgG, significant augmentation of lysis was observed for all three species of LTA, with each of the LTA preparations reaching complete lysis in 10% autologous serum (Fig. 6).

Agglutination studies with LTA-sensitized erythrocytes were performed to determine the LTA antibody titers in the agammaglobulinemic and IgG-reconstituted sera. No detectable titers (<1:2) to any of the three LTA preparations were found in the pretreatment (IgG, 22 mg/dl) serum. After reconstitution with pooled immunoglobulin (IgG, 951 mg/dl), titers of 1:8 were obtained for each of the LTA preparations.

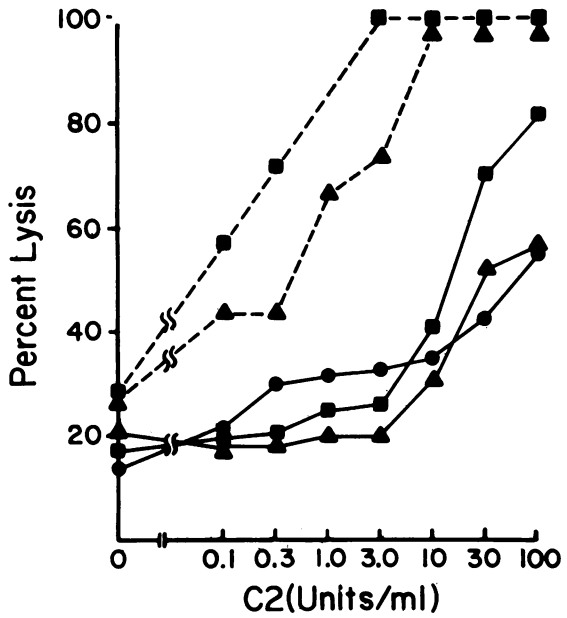

FIG. 5. Lysis of erythrocytes (from a C2-deficient human) sensitized with LTA from *S. pneumoniae* (●), *S. pyogenes* (■), or *L. fermentum* (▲), at 3 (——) and 30 μg/ml (– – –) after reconstitution of C2-deficient autologous human serum with increasing doses of C2. No significant lysis of control erythrocytes was observed.

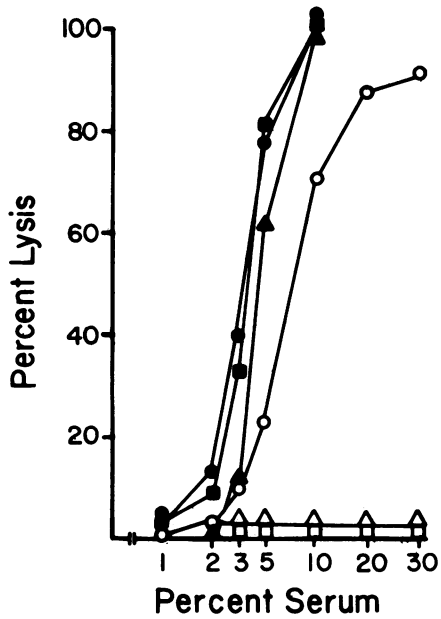

FIG. 6. Lysis of erythrocytes (from an immunoglobulin-deficient human) sensitized with LTA from *S. pneumoniae* (○, ●), *S. pyogenes* (□, ■), or *L. fermentum* (△, ▲). LTA-E were incubated for 30 min at 37°C with increasing concentrations of immunoglobulin-deficient autologous human serum (open symbols) or with immunoglobulin reconstituted autologous human serum (filled symbols). No significant lysis of control erythrocytes was observed.

DISCUSSION

LTA from gram-positive bacteria are shed from the cell membrane during culture (15, 17, 22) and presumably also during the course of infections. Once shed by the bacteria, the LTA can bind to erythrocytes (3) and other mammalian cells (2, 19, 24). This binding can lead to the activation of the classical or alternative pathways of complement in heterologous sera (7, 11, 12). Recently, it has been shown that pneumococcal cell membrane LTA can render sensitized host erythrocytes susceptible to lysis by autologous complement via activation of either the alternative or classical pathway (12). The present study examined whether this ability of *S. pneumoniae* LTA to sensitize host cells for lysis by autologous complement was a general property of LTA. We also studied whether the activation of complement occurred via the classical or the alternative pathway and what role natural antibody might play.

The LTA preparations from each of the three gram-positive bacterial species were able to sensitize human erythrocytes for lysis in autologous serum. Optimal concentrations of autologous sera and bacterial LTA preparations were determined and used to provide maximally favorable conditions for erythrocyte lysis. That complement activation was required for autologous lysis was demonstrated by the inhibition of lysis by pretreating the serum with EDTA or heat. Two previous studies have examined the ability of homologous, but not autologous, complement to lyse host cells (10, 20). In one study, desialidated erythrocytes and

nucleated tumor cells (10) were used as targets, whereas in the other study, erythrocytes coated with lipopolysaccharide and sensitized with antibody were used (20). In each case, lysis by homologous complement was markedly reduced, compared with lysis by heterologous complement. Presumably, intrinsic control mechanisms exist in the host cell to prevent or retard lysis by autologous complement. The binding of a critical number of LTA molecules to the cell surface seems to overcome this inhibition and promote complement activation and lysis. The results of the present study indicate that LTA is able to sensitize host cells for lysis by autologous complement and that this is a general property of LTA from gram-positive bacteria.

Experiments were also performed to determine the pathway(s) by which complement activation occurred. A previous study found that pneumococcal LTA is able to sensitize host cells for lysis by autologous complement via the alternative pathway, although lysis is facilitated in the presence of the classical pathway (12). In the present study, erythrocytes obtained from a C2-deficient patient were sensitized with each of the LTA preparations and incubated with autologous sera. The results of the present study indicate that LTA isolated from *L. fermentum* and *S. pyogenes* were relatively poor activators of the alternative pathway compared with pneumococcal LTA.

The present study also evaluated the role of immunoglobulin in the ability of autologous complement to lyse erythrocytes sensitized with LTA isolated from *S. pyogenes, L. fermentum*, and *S. pneumoniae* by using serum deficient in immunoglobulin. Erythrocytes sensitized with pneumococcal LTA were lysed in the agammaglobulinemic autologous serum, whereas erythrocytes sensitized with the other two LTA preparations were not lysed in the agammaglobulinemic serum. On reconstitution of the autologous agammaglobulinemic serum with IgG, lysis was achieved for all three species of LTA. Although erythrocytes sensitized with pneumococcal LTA could be lysed by autologous complement in the absence of immunoglobulin, lysis was still enhanced by immunoglobulin reconstitution. The other LTA preparations were dependent on physiologic concentrations of antibody in the serum for complement-mediated lysis to proceed.

The results of the present study indicate that the ability to render host cells susceptible to damage or lysis by autologous complement is a general property of LTAs of diverse chemical structure from a number of different gram-positive bacteria. The present study also indicates that the primary mechanism or pathway by which the LTA activates the autologous complement may differ, depending on the bacterial species from which the LTA is obtained. Whereas pneumococcal LTA was able to activate the alternative pathway and did not depend on significant levels of immunoglobulin, the LTAs from *S. pyogenes* and *L. fermentum* depended on antibody and an intact classical pathway. The reason for the difference in the ability to produce complement-mediated lysis via the alternative pathway is unknown but may reside in the unique chemical nature of pneumococcal LTA.

In a recent study, pneumococcal LTA was shown to sensitize erythrocytes for destruction by autologous complement in vivo (12). LTA-sensitized rat erythrocytes, when reinfused into normocomplementemic autologous donors, undergo rapid intravascular hemolysis. Complement-depleted rats sequester LTA-E in the reticuloendothelial system, but do not exhibit detectable cell lysis. It is therefore possible that gram-positive bacteria release LTA into the local environment, where it binds to host cells and interacts with autologous complement to result in host cell destruction.

The capacity of gram-positive bacterial LTA to promote autologous complement activation and lysis or damage to host cells could have important implications in the interaction of microorganism and host during the course of natural bacterial infections. The potential for complement-mediated damage to host cells sensitized with LTA might explain some of the tissue damage seen during and after bacterial infections.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-07007, AI-11637, AI-13550, AI-10085, DE-03487, and DE-06039 from the National Institutes of Health.

We thank Stephanie Hilberg for her assistance in the preparation of the manuscript.

LITERATURE CITED

1. **Aikan, M. L., and E. H. Beachey.** 1978. Excretion of lipoteichoic acid by group A streptococci: influence of penicillin on excretion and loss of ability to adhere to human oral mucosa cells. J. Clin. Invest. **61**:671–677.
2. **Beachey, E. H.** 1981. Bacterial adherence: adhesion receptor interaction mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
3. **Beachey, E. H., J. B. Dale, W. A. Simpson, J. D. Evans, K. W. Knox, I. Ofek, and A. J. Wicken.** 1979. Erythrocyte binding properties of streptococcal lipoteichoic acids. Infect. Immun. **23**:618–625.
4. **Briles, E. B., and A. Tomasz.** 1973. Pneumococcal Forssman antigen, a choline-containing lipoteichoic acid. J. Biol. Chem. **248**:6394–6397.
5. **Brissette, J. L., G. D. Shockman, and R. A. Pieringer.** 1982. Effects of penicillin on synthesis and excretion of lipid and lipoteichoic acid from *Streptococcus mutans* BHT. J. Bacteriol. **151**:838–844.
6. **Brundish, D. E., and J. Badilley.** 1968. Pneumococcal C substance, a ribitol teichoic acid containing choline phosphate. Biochem. J. **110**:573–582.
7. **Fiedel, B. A., and R. W. Jackson.** 1978. Activation of the alternative complement pathway by a streptococcal lipoteichoic acid. Infect. Immun. **22**:286–287.
8. **Goebel, W. F., T. Shedlovsky, G. I. Lavin, and M. H. Adams.** 1943. The heterophile antigen of pneumococcus. J. Biol. Chem. **148**:1–15.
9. **Harris, T. N., and S. Harris.** 1953. Agglutination by human sera of erythrocytes incubated with streptococcal culture concentrates. J. Bacteriol. **66**:159–165.
10. **Houle, J. J., and E. M. Hoffman.** 1984. Evidence for the restriction of the ability of complement to lyse homologous erythrocytes. J. Immunol. **133**:1444–1452.
11. **Hummell, D. S., A. J. Swift, A. Tomasz, and J. A. Winkelstein.** 1985. Activation of the alternative complement pathway by pneumococcal lipoteichoic acid. Infect. Immun. **47**:384–387.
12. **Hummell, D. S., and J. A. Winkelstein.** 1986. Bacterial lipoteichoic acid sensitizes host cells for destruction by autologous complement. J. Clin. Invest. **77**:1533–1538.
13. **Hyatt, A. C., K. M. Altenburger, R. B. Johnston, Jr., and J. A. Winkelstein.** 1981. Increased susceptibility to severe pyogenic infections in patients with an inherited deficiency of the second component of complement. J. Pediatr. **98**:417–419.
14. **Jackson, D. E., W. Wong, M. T. Largen, and G. D. Shockman.** 1984. Monoclonal antibodies to immunodeterminants of lipoteichoic acids. Infect. Immun. **43**:800–803.
15. **Joseph, R., and G. D. Shockman.** 1975. Synthesis and excretion of glycerol teichoic acids during growth of two streptococcal species. Infect. Immun. **12**:333–338.
16. **Knox, K. W., and A. J. Wicken.** 1973. Immunological proper-

ties of teichoic acids. Bacteriol. Rev. **37**:215–257.
17. **Markham, J. L., K. W. Knox, A. J. Wicken, and M. J. Hewett.** 1975. Formation of extracellular lipoteichoic acid by oral streptococci and lactobacilli. Infect. Immun. **12**:378–386.
18. **Mayer, M. M.** 1961. Complement and complement fixation, p. 133–240. *In* E. A. Kabat and M. M. Mayer (ed.), Experimental immunochemistry, 2nd ed. Charles C Thomas, Publisher, Springfield, Ill.
19. **Ofek, I., E. H. Beachey, W. Jefferson, and G. L. Campbell.** 1975. Cell membrane-binding properties of group A streptococcal lipoteichoic acid. J. Exp. Med. **141**:990–1003.
20. **Okada, H., H. Tanka, and N. Okada.** 1983. Prevention of complement activation on the homologous cell membranes of nucleated cells as well as erythrocytes. Eur. J. Immunol. **13**:340–344.
21. **Rantz, L. A., E. Randall, and A. Zuckerman.** 1956. Hemolysis and hemagglutination by normal and immune sera of erythrocytes treated with a nonspecies-specific bacterial substance. J. Infect. Dis. **98**:211–222.
22. **Shockman, G. D.** 1981. Cellular localization, excretion, and physiological roles of lipoteichoic acid in gram-positive bacteria, p. 21–40. *In* G. D. Shockman and A. J. Wicker (ed.), Chemistry and biological activities of bacterial surface amphiphiles. Academic Press, Inc., New York.
23. **Simpson, W. A., I. Ofek, and E. H. Beachey.** 1980. Binding of streptococcal lipoteichoic acid to the fatty acid binding sites on serum albumin. J. Biol. Chem. **255**:6092–6097.
24. **Simpson, W. A., I. Ofek, C. Sarasohn, J. C. Morrison, and E. H. Beachey.** 1980. Characteristics of the binding of streptococcal lipoteichoic acid to human oral epithelial cells. J. Infect. Dis. **141**:457–462.
25. **Wicken, A. J., J. W. Gibbens, and K. W. Knox.** 1973. Comparative studies on the isolation of membrane lipoteichoic acid from *Lactobacillus fermenti*. J. Bacteriol. **113**:365–372.
26. **Wicken, A. J., and K. W. Knox.** 1970. Studies on the group F antigen of lactobacilli: isolation of a teichoic acid-lipid complex from *Lactobacillus fermenti* NCTC 6991. J. Gen. Microbiol. **60**:293–301.
27. **Wicken, A. J., and K. W. Knox.** 1975. Lipoteichoic acids: a new class of bacterial antigen. Science **187**:1161–1167.
28. **Wicken, A. J., and K. W. Knox.** 1980. Bacterial cell surface amphiphiles. Biochim. Biophys. Acta **604**:1–26.