Lipoteichoic Acids: A New Class of Bacterial Antigen

Membrane lipoteichoic acids can function as surface antigens of gram-positive bacteria.

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Teichoic acids are a group of phosphate-containing polymers associated with the cell walls and plasma membranes of gram-positive bacteria; they are apparently absent from gram-negative bacteria. Classically, teichoic acids were considered to be polymers of either glycerol phosphate or ribitol phosphate substituted with various glycosyl and D-alanyl ester groups (Fig. 1). This definition has more recently been broadened to include all cell wall, capsular, or membrane polymers containing glycerol phosphate or ribitol phosphate residues (*1*). The extraction, determination of structure, immunological properties, possible functions, and biosynthesis of these polymers have been the subjects of extensive investigations since their discovery and have been equally extensively reviewed (*1–3*).

Several major distinctions can be made between teichoic acids associated with cell walls and teichoic acids associated with the plasma membrane. Cell wall teichoic acids are covalently linked to peptidoglycan and may exhibit the full range of glycerol phosphate–containing and ribitol phosphate–containing structures now included in the definition of the term teichoic acid. They do not occur in all species of gram-positive bacteria and their occurrence may also be dependent upon the growth conditions employed (*4*). Membrane teichoic acids are covalently linked to a glycolipid moiety of the plasma membrane and appear to be always of the classical glycerol phosphate polymer type (*2, 5*). Membrane teichoic acids are more characteristic components of gram-positive bacteria (*6*) than cell wall teichoic acids and their presence is not as dependent upon growth conditions as is the occurrence of wall-associated polymers (*4*). The covalent association of membrane glycerol teichoic acids with glycolipid has led to the use of the term lipoteichoic acid for this group of polymers (*7*).

Little is known of the spatial organization of teichoic acid–peptidoglycan complexes within bacterial cell walls, although studies on a number of bacteria (*8, 9*) have indicated that wall teichoic acid is located at the cell surface, or is located sufficiently close to the surface for reactions to occur between whole organisms and teichoic acid–specific antibodies, plant lectins, or bacteriophages. Reaction of antibodies or other agents with intact cells has usually been taken to imply a surface or somatic location of the antigen or receptor site. This concept of surface location being synonymous with cell wall, however, is difficult to reconcile with the finding that membrane lipoteichoic acids in some organisms may also react serologically at the cell suface. Reference [see (*10*)] is found to "the unexpected absence of the group D antigen from the cell walls of group D streptococci" where the group antigen is a membrane lipoteichoic acid (*11, 12*). *Lactobacillus fermenti* (*13*) and *Streptococcus lactis* (*14*) afford other examples of organisms agglutinable by group antiserums and where the group antigen has been shown to be a membrane lipoteichoic acid.

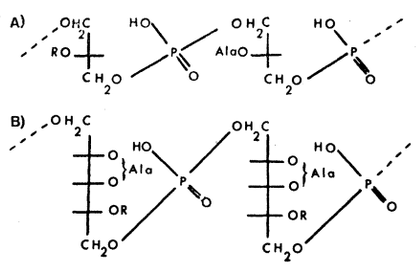

Fig. 1. Generalized structure of (a) glycerol teichoic acids and (b) ribitol teichoic acids (R, H or glycosyl; Ala, D-alanyl). [Courtesy *Bacteriological Reviews*]

The purpose of this article is to consider lipoteichoic acids as surface-reactive antigens as well as membrane components in many gram-positive bacteria, and to discuss some of the biological implications of lipoteichoic acids as a class of potent immunogens.

Lipoteichoic Acids

Lipoteichoic acids have been extracted from a variety of lactobacilli, streptococci, and bacilli, either from whole organisms or membrane fractions obtained from disrupted cells (*2, 5, 15*). A generally applicable procedure is to treat freeze-dried organisms with a mixture of chloroform and methanol to deplete them of lipid and then extract lipoteichoic acid from the dried organisms with hot water; alternatively, the lipoteichoic acid is extracted from whole organisms or membrane fractions by hot 45 percent aqueous phenol (*15*). Gel chromatography of these extracts yields lipoteichoic acid as high molecular weight micellar complexes analogous to the lipopolysaccharide micelles extracted from gram-negative bacteria by similar procedures. Protein, in amounts varying with the organism and extraction procedure, is generally associated with the lipoteichoic acid (*15*), and may represent a fortuitous rather than a specific association of lipoteichoic acid with membrane protein during extraction.

Structural studies on a number of lipoteichoic acids (*2, 7, 12*) have shown them to consist of $1 \rightarrow 3$ phosphodiester linked chains of 25 to 30 glycerol phosphate residues variously substituted with glycosyl and D-alanyl ester groups. The lipid moiety is a glycolipid, identical to the free glycolipid of the plasma membrane, and is linked through a phosphodiester bond involving a sugar hydroxyl group of the glycolipid and the terminal glycerol phosphate residue of the teichoic acid chain. The partial structures of four lipoteichoic acids are shown schematically in Fig. 2. Some

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uncertainty exists as to the position of one of the fatty acid ester residues. Degradation studies in our laboratories indicate acylation of the terminal glycerol phosphate residue or its substituent glycosyl group (as depicted in Fig. 2), while studies on *Streptococcus faecalis* lipoteichoic acid suggest a phosphatidyl substitution of the glycolipid moiety (*12*). These details of structure notwithstanding, lipoteichoic acids are clearly amphipathic molecules in that each has a long polar glycerol phosphate chain linked to a small hydrophobic lipid portion, and the association of these molecules in solution as micelles can be readily understood. Mild deacylation procedures remove fatty acyl and D-alanyl ester groups, destroying the micellar structure and yielding lower molecular weight glycerol phosphate chains still attached to the glycerol glycoside of the original glycolipid moiety (*7*). The phosphodiester link between the glycerol phosphate chain and the glycolipid appears to be acid-labile in that treatment with trichloroacetic acid (TCA) in the cold, a method commonly employed for the extraction of wall teichoic acids, yields teichoic acid devoid of glycolipid (*2, 7*).

Membrane Association of Lipoteichoic Acids

Historically, membrane-associated teichoic acids were obtained in a lipid-free form by TCA extraction of the intracellular contents of disrupted organisms (*16*) and were, indeed, first referred to as "intracellular teichoic acids." Subsequent studies based on an examination of products resulting from protoplast formation (*10, 17*) led to the introduction of the term "membrane teichoic acid." A detailed investigation on *S. faecalis* (*18*) showed that the teichoic acid could be readily removed from the protoplast membrane by washing with water or salt solutions, suggesting that the teichoic acid was located in or on the external surface of the protoplast membrane. The nature of the attachment remained obscure until the isolation by milder procedures of membrane teichoic acid in the form of lipoteichoic acid (*7*), and it is now presumed that the glycolipid portion of the molecule is in hydrophobic interaction with the lipid bilayer of the plasma membrane (*2, 7*). Studies on isolated protoplasts of *L. fermenti* (*19*) with ferritin-labeled antibody demonstrated the plasma membrane location of lipoteichoic acid. It is evident that magnesium (Mg^{2+}) ions play a role in maintaining this association since protoplasts made in the absence of added Mg^{2+} ions lack detectable lipoteichoic acid (*19*).

It has been shown with *Streptococcus sanguis* that lipoteichoic acid can be extracted from the wall fraction of disrupted organisms (*20*). The assumption that wall preparations are not contaminated with membrane is not always valid, although in this case the component extracted from "wall" with phenol did differ—principally in its lower fatty acid content—from that present in the intracellular fraction.

Immunogenicity of Teichoic Acids

Although the word "antigen" has long been used to indicate a compound inducing antibody formation, this property is now frequently reserved for an immunogen, with an antigen having the restricted definition of reacting with an antibody to give a demonstrable effect such as precipitate. Are teichoic acids immunogenic? To answer this question we need to consider both the method used for detecting immunogenicity and the nature of the preparation injected into the experimental animal. Further, most studies have aimed at obtaining circulating antibodies, and the possibility of a cellular response has received only scant attention (*21*). With respect to a humoral antibody response, immunoglobulins M, G, and A (IgM, IgG, and IgA, respectively) have been detected under different circumstances (*22, 23*). These antibodies differ in their reactivity in different testing procedures, such as the precipitin and hemagglutinin reactions (*24*), and thus conclusions on the relative antibody response to different immunogens can be influenced by the method of detection. It should be noted that lipoteichoic acids will sensitize erythrocytes directly because of their lipid moiety (*2, 25*), whereas acid-extracted teichoic acids, devoid of lipid, will not; thus the very sensitive hemagglutination method can be used for detecting low concentrations of lipoteichoic acid and of antibody.

The intravenous injection into rabbits of whole bacterial cells that contain

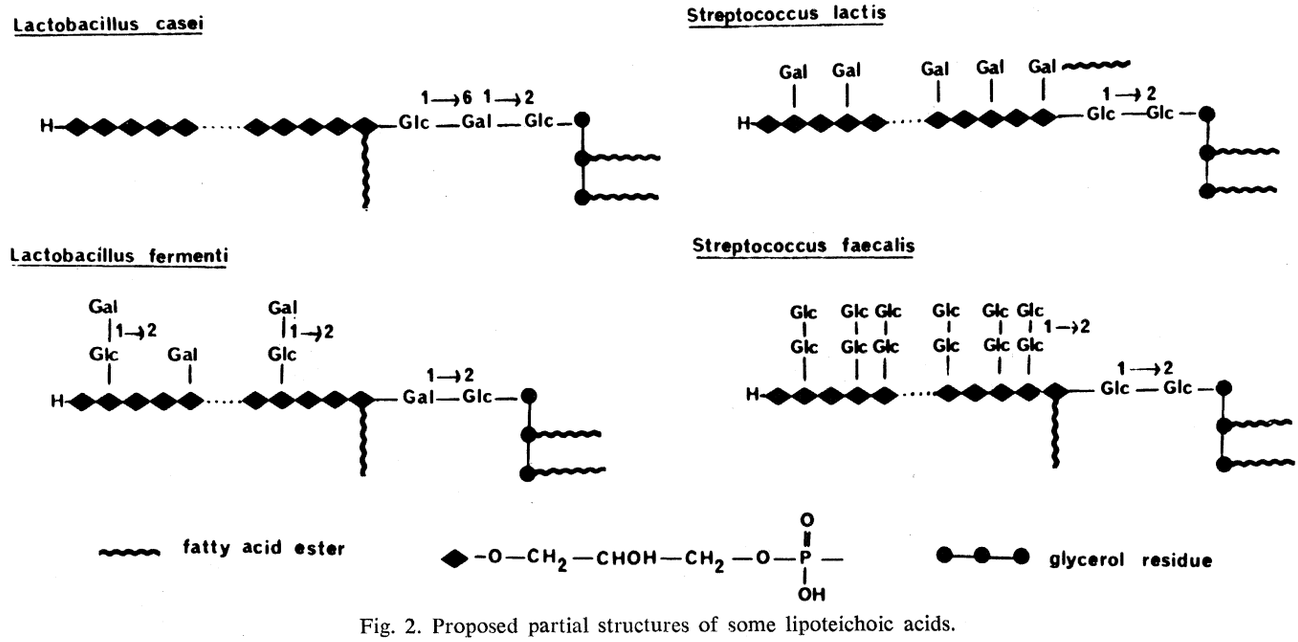

Fig. 2. Proposed partial structures of some lipoteichoic acids.

a wall teichoic acid will generally lead to a humoral response specific for the teichoic acid component (*22, 26, 27*). Whether the similar injection of whole cells will result in the formation of antibodies to membrane lipoteichoic acid seems to depend both on the particular organism and on the individual rabbit. Early studies on the streptococcal group D antigen, which is now known to be the membrane lipoteichoic acid (*11, 12*), showed the difficulty of obtaining suitable antiserums when whole organisms were injected (*28*); injection of disintegrated organisms, however, yielded potent antiserums against lipoteichoic acids that could be used for grouping the organisms (*28*). With *Lactobacillus plantarum*, where the wall ribitol teichoic acid is the group antigen, injection of whole organisms always gave antibodies to the ribitol teichoic acid while a few rabbits also produced antibodies to the membrane lipoteichoic acid (*22*); injection of cell wall preparations containing associated membrane lipoteichoic acid invariably led to the formation of antibodies specific for the membrane component (*22*) as well as antibodies specific for the wall ribitol teichoic acid.

These observations indicate that the membrane lipoteichoic acid is a potential immunogen. It is generally assumed that antibodies are more likely to be formed against a component on the bacterial surface and thus, where the membrane lipoteichoic acid is the group antigen, we should expect it to be prominent on the surface. As will be described later, evidence has been obtained of a surface location for the group antigen of *L. fermenti*; in contrast, there is only a scant amount of membrane lipoteichoic acid on the surface of *L. casei*, and injection of whole cells does not lead to the production of antibodies to lipoteichoic acid (*2, 13*).

In the foregoing studies on the production of antibodies to both wall and membrane teichoic acids, either whole organisms or crude cell fractions were used. Classically, teichoic acids have been extracted with dilute acid, generally cold TCA, with the aim of obtaining pure polymers (*3, 16*). Such preparations are not immunogenic, even when injected subcutaneously with Freund's complete adjuvant, probably because of their low molecular weight; immunogenicity can be restored by forming complexes with methylated bovine serum albumin, or cetylpyridinium chloride (*8*). As described earlier, the membrane lipoteichoic acid can be extracted as a high molecular weight micellar lipoteichoic acid–protein complex. This preparation is immunogenic when injected subcutaneously with Freund's complete adjuvant (*2, 13, 29*); the protein content appears to be important because when the protein content of the complex is lowered its immunogenicity is decreased.

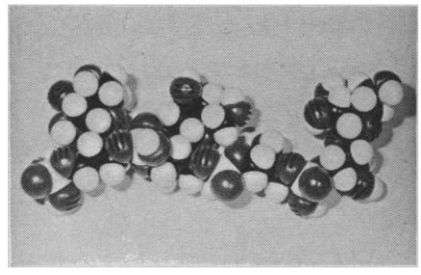

Fig. 3. A space-filling model of a sequence of four glycerol phosphate residues joined by $1 \rightarrow 3$ phosphodiester bonds; three of the glycerol moieties are substituted with α-D-glucopyranosyl residues on carbon 2. The model shows that such a sequence can have a conformation in which all the glucosyl residues are on one side of the molecule.

The detection of antibodies to teichoic acids in human serums has found only a limited application to epidemiological studies. Because of its importance as a pathogen there have been several studies on the occurrence of antibodies to *Staphylococcus aureus* wall teichoic acids (*27, 30*). With membrane lipoteichoic acids, however, there do not appear to be any unequivocal reports showing the detection of antibodies specific for the lipoteichoic acid from a particular organism; where antibodies have been detected their specificity is generally directed against the common glycerol phosphate backbone component (*31*).

Specificity of Antibodies

Lipoteichoic acids contain a number of potential immunological determinants, namely, the glycerol phosphate backbone sequence, the carbohydrate and D-alanyl substituents, and the glycolipid (*2, 32*). Antibodies specific for the glycolipid component have not been detected and only rarely have antibodies specific for D-alanine been obtained (*33, 34*). Whether the antibodies are specific for the glycerol phosphate sequence or the carbohydrate substituents is influenced by the degree of substitution (*2*), and may also relate to whether they are G or M immunoglobulins (*35*).

Antibodies to the group-specific lipoteichoic acids from groups D and N streptococci and group F lactobacilli (Fig. 2) are generally specific for the carbohydrate substituents. Antibodies to *L. casei* lipoteichoic acid (Fig. 2) are specific for the glycerol phosphate sequence and will cross recat with a variety of lipoteichoic acids (*2, 29*). However, the picture is not quite as simple as it might first appear since lipoteichoic acids containing carbohydrate substituents may also elicit an antibody response specific for the glycerol phosphate backbone. This can be explained in terms of antibodies being formed against one "face" of the immunogen—a concept applied to both teichoic acids (*2*) and lipopolysaccharides (*36*)—and a molecular model of a glucosyl-substituted glycerol phosphate sequence (Fig. 3) certainly shows that the molecule can assume a configuration in which all the glucose residues are on the same side.

Lipoteichoic Acids as Surface Antigens

A comparison of the reaction of whole organisms of *L. fermenti* and *L. casei* with antibodies specific for lipoteichoic acid provided evidence for lipoteichoic acid being exposed at the cell surface (*19*). By conventional procedures, cells of *L. fermenti*, but not *L. casei*, adsorbed and were agglutinated by antiserums to lipoteichoic acid. A more sensitive method of detecting adsorbed antibody, involving electron microscopy of organisms that had been treated successively with antiserum and ferritin conjugated to goat antibodies to rabbit IgG, showed the apparent difference between the two organisms to be quantitative rather than absolute: *L. casei* showed some surface adsorption of teichoic acid antibody but it was irregular in distribution and significantly less than the confluent labeling shown by *L. fermenti* (*19*). In order to explain these differences in surface activity of the lipoteichoic acids from these and other organisms we proposed a wall-membrane model (*2, 19*) in which lipoteichoic acid molecules were depicted as embedded in the plasma membrane at their hydrophobic glycolipid ends while the long polar glycerol phosphate chains extended by intercalation into the polysaccharide-peptidoglycan network of the cell wall. In some cases, it was suggested, these chains came near enough to the surface of the cell wall for their distal ends to act as surface antigens. Factors on which this latter

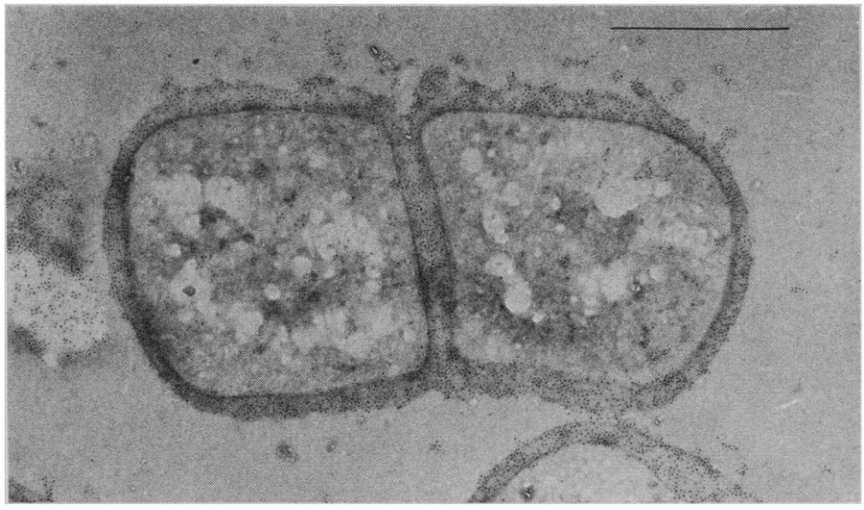

Fig. 4. Electron micrograph of a thin section of *Lactobacillus plantarum*. The section was treated with rabbit IgG specific for the glycerol phosphate sequence of lipoteichoic acid and then labeled with ferritin conjugated to goat antiserum to rabbit IgG. Bar equals 0.5 micrometer.

condition might depend were suggested to include (i) the thickness of the cell wall and degree of peptidoglycan cross-linking, (ii) the length of the glycerol phosphate chain of the lipoteichoic acid, and (iii) the conformation of the chain within the ionic environment of the cell wall.

Recently, we have been able to prepare thin sections of bacteria while preserving the antigenic reactivity of their lipoteichoic acid in situ (*37*). By labeling these sections with rabbit IgG specific for the glycerol phosphate backbone and then treating them with ferritin-labeled goat antibodies to rabbit IgG, we have shown the label extending from the outer surface of the membrane through the cell wall and in some cases beyond the outer boundary of the cell into the external environment (Fig. 4).

Intercalation of a membrane component, lipoteichoic acid, into the cell wall matrix also has implications in the interpretation of the serological properties of isolated cell walls or their extracts. The observation that injection of cell wall preparations from *L. plantarum* and *L. casei* (*22*) can lead to the production of antibodies specific for a membrane component described above raises the question of how we define the cell wall: Do we regard it as only an operational term for a cellular fraction or do we give the restricted definition of peptidoglycan and covalently linked polymers? Although proteolytic enzymes were employed in early studies on "purified" cell wall, the tendency now is to depend on washing disrupted cells with salts and then subjecting them to differential centrifugation (*38*). As was shown with *L. plantarum*, the latter procedure will almost certainly leave membrane lipoteichoic acid in the wall fraction and even treatment with trypsin may leave residual lipoteichoic acid sufficient to induce antibody formation (*22*). The production of antibodies reacting with glycerol teichoic acid by injecting animals with cell wall preparations does not necessarily mean, therefore, that the teichoic acid is present as a wall polymer in the sense of being covalently linked to peptidoglycan.

Detection of Extracellular Lipoteichoic Acid

The results with ferritin-labeled antibodies to lipoteichoic acid show that the lipoteichoic acid can function as a surface component. Partial release of lipoteichoic acid from whole organisms can be obtained by washing the cells with saline or by subjecting them to the conditions of "cold shock" (*15*). It is therefore possible that part of the lipoteichoic acid detectable at the cell wall surface is no longer in association with cell membrane. There is also a long history (*2, 39, 40*) of reports describing erythrocyte-sensitizing antigens that were obtained from saline washings or the culture fluid of gram-positive bacteria; these antigens, frequently referred to as "Rantz antigens" (*41*), have properties that are now recognized to be those of lipoteichoic acid. The detection of such antigens in saline extracts or culture fluid has depended on their ability to sensitize erythrocytes which are then agglutinated by specific antiserums; the extent to which the culture fluid can be diluted while still retaining activity gives a measure of the antigen concentration. Using this method with antiserums specific for lipoteichoic acid and knowing that the minimum concentration of lipoteichoic acid for cell sensitization is 2.5 micrograms per milliliter (*25*), one can obtain evidence that culture fluid from oral streptococci and lactobacilli may contain up to 50 μg/ml of lipoteichoic acid—an amount comparable to the amount of lipoteichoic acid in the cell mass (*42*); lipoteichoic acid has also been isolated from culture fluid (*42*). It would therefore seem likely that part at least of the lipoteichoic acid detectable as a surface component is a transient component—released from the membrane but not yet released from the cell.

Whether or not the existence of extracellular lipoteichoic acid indicates that the acid is actually excreted, in the absence of cell lysis, or is the result of turnover occurring in the wall, or membrane, or both, as in many gram-positive bacteria under conditions of rapid growth in rich media, remains to be determined. It is interesting to note that, apart from the erythrocyte-sensitizing extracellular lipoteichoic acid found in cultures of *L. fermenti* and *L. casei*, nonmicellar and nonerythrocyte-sensitizing glycerol teichoic acid, in varying amounts, has also been isolated (*42*). These polymers have a much lower fatty acid ester content than lipoteichoic acid and may represent partial degradation products. The "wall" lipoteichoic acid reported from *S. sanguis* (*20*) may have a similar origin.

Common Antigens

The relatively simple hemagglutinin method has lent itself to the survey of a large number of bacterial strains by examination of culture fluids or saline extracts of the organisms; antiserums that were frequently of unknown specificity were used in several studies that showed that a wide variety of gram-positive bacteria contain cross-reacting antigens (*39, 40*). The results were often interpreted, however, as indicating that all strains contained the same antigen—a common antigen. Lipoteichoic acids carrying different carbohydrate substituents can be detected by antibodies specific for the backbone (*2, 29*), and by the earlier definition these different polymers would be regarded as common

antigens. However, all that is being detected is a common glycerol phosphate sequence. This conclusion has also been supported by studies that used the precipitin reaction to test acid extracts of a wide variety of gram-positive bacteria (*43*), though in this reaction wall glycerol teichoic acids could also be contributing to the reaction. The hemagglutinin method can detect concentrations of antigen considerably lower than those detectable by the precipitin method, a difference that has not always been appreciated in the interpretation of results (*44*).

Frequently, human serums contain antibodies to glycerol teichoic acid that are detectable by the hemagglutinin reaction. Generally, these serums will react with lipoteichoic acids carrying different glycosyl substituents, and once again are specific for the glycerol phosphate sequence (*31*). It is also quite likely that whereas the primary stimulus for the formation of glycerol phosphate "backbone" specific antibodies may come from one particular organism, subsequent stimuli could come from a variety of unrelated organisms. Such hyperimmune serums may account for some of the biological false-positive reactions for syphilis (*45*), and also for the death of the recipient of blood contaminated with a *Bacillus* species (*40*), a case in which the recipient's red blood cells were rendered "polyagglutinable" by the absorption of extracellular lipoteichoic acid derived from the bacillus. A further point is that where such cross-reacting antibodies could be present and be detectable by the procedures employed, it is not necessarily justified to conclude (*31, 46*) that "antibodies reacting with" is synonymous with "antibodies produced against" a bacterial strain.

Group Antigens

The term "group antigen" has generally been taken to denote a specific component that is common only to a group of bacteria possessing other similar characteristics and is used as a method for differentiating such a group; inherent in the definition is (i) the requirement that there be a demonstrable reaction with antibodies, and (ii) the assumption that the antibodies were produced against the component. However, evidence on the latter point is often lacking, and in specific situations we may need to draw a distinction between a component that fulfills both criteria and one that only reacts with antiserums. The complication can arise that one carbohydrate polymer is able to react with antibodies produced against a second polymer carrying the same serological determinant or determinants.

Table 1. Specificity of lipoteichoic acids reacting with grouping serums; glc, glucopyranosyl residue; gal, galactopyranosyl residue.

Genus	Group	Strain	Determinant	References
Lactobacillus	A	*helveticus*, NCIB 8025	α-D-glc	(*49*)
Lactobacillus	F	*fermenti*, NCTC 6991	α-D-gal	(*13*)
Streptococcus	D	*faecalis*, 39	glc-α-1 → 2-glc	(*11, 12*)
Streptococcus	N	*lactis*, ATCC 9936	α-D-gal	(*47*)
Streptococcus	Serotype *a*	*mutans*, AHT	β-D-gal	(*52*)

In only a few instances do we find that lipoteichoic acids fulfill one or both of the requirements for a group antigen, and that they have also been fully characterized serologically and chemically (Table 1). Their apparently limited ability to induce the formation of group-specific antibodies could depend on a number of factors: (i) the extent to which they function as surface components; (ii) their relative concentration as compared to other potential immunogens which may be present in greater amounts (such as cell wall polysaccharides); and (iii) whether or not the antibodies produced are specific for glycosyl substituents, a factor that would, in turn, depend on both the degree of substitution of the polymer and the individual animal response. Membrane lipoteichoic acids are also at a disadvantage in procedures for detecting antibodies, because the frequently used acid-extraction procedures yield a product that reacts only weakly in the precipitin reaction compared with the intact lipoteichoic acid (*13*), and the relative concentration of lipoteichoic acid in such extracts is less than that of wall polysaccharides or teichoic acids.

The membrane lipoteichoic acids from group D streptococci (*2*) and group F lactobacilli (*2, 13*) have received the most attention. In group F lactobacilli, however, the cell wall polysaccharide is only weakly immunogenic (*13*), while the polysaccharides of some group D streptococci enable a number of serotypes to be distinguished (*2*).

Of the antigens listed in Table 1, the streptococcal group N antigen is the most recent to have had its specificity defined. Studies in which the isolated lipoteichoic acid and the quantitiative precipitin method were used showed that the determinant is an α-D-galactopyranoside (*47*), not galactose phosphate as originally suggested (*14*); the results with galactose phosphate can be accounted for on the basis of the nonspecific inhibition of the precipitin reaction by ionized compounds (*2, 48*).

Examples of antigens reacting with grouping serums but not necessarily inducing antibody formation are provided by the teichoic acids of group A lactobacilli and of *Streptococcus mutans* of serotype *a*. With the group A lactobacilli both the wall and membrane components are α-D-glucosyl–substituted glycerol teichoic acids and, until this was realized (*49*), there were differing views on whether the wall (*50*) or the membrane component (*51*) was the grouping antigen. The amount of the wall teichoic acid is approximately ten times that of the membrane component (*49*), so that one might expect the wall component to be the major contributor to serological reactivity; however, whether antibody formation is induced by only one or both of the components is not known. With *S. mutans* serotype *a*, studies on different strains have yielded different products reacting with grouping antiserums: the lipoteichoic acid described in Table 1 (*52*) and a polysaccharide obtained by aqueous extraction of whole cells (*53*). The relation between these products and the unequivocal identification of the component inducing antibody formation remains unresolved.

Teichoic Acid Antibodies and Human Cell Constituents

The frequent existence of humoral antibodies to teichoic acids or lipoteichoic acids in humans raises the question of whether these antibodies can cross react with human cell membrane constituents and subsequently participate in complement mediated lysis. Cross-reactions of this kind could be of importance in such tissue-damaging sequelae to streptococcal infections as rheumatic fever or glomerulonephritis,

both of which are believed to have an immunological etiology (*54*). Cross-reaction and complement fixation between cardiolipin and rabbit antibodies specific to the glycerophosphate sequence of lipoteichoic acids have been clearly demonstrated and could explain some of the biological false-positive reactions obtained in cardiolipin–complement fixation tests for syphilis (*45*). The possibility also exists of cross-reactions occurring between teichoic acid antibodies specific for glycosyl groups and similar sugar moieties of eukaryotic membrane ceramides or glycoproteins. It is interesting to note in this connection that antiserum to type XIV pneumococcal polysaccharide cross reacts with a galactose-containing ceramide from human erythrocyte membrane (*55*).

Properties of the Lipid Component of Lipoteichoic Acids

Factors associated with the ability of lipoteichoic acids to sensitize erythrocytes were recently reviewed (*2*), and continue to receive attention (*56*). In several studies, where the presence of lipid as an integral part of the sensitizing antigen was not realized, the results were interpreted as indicating that the D-alanine component was required for erythrocyte sensitization (*56, 57*). However, because alanine-containing glycerol teichoic acid will not sensitize erythrocytes unless esterified with fatty acids (*34*), and alanine-free lipoteichoic acid will sensitize erythrocytes (*25*), the lipid component is concluded to play the essential role (*2*). Presumably it forms hydrophobic bonds with erythrocyte membrane lipids, a mechanism also proposed for the adsorption of lipopolysaccharides to erythrocytes (*58*).

From the above we make a formal analogy between lipoteichoic acids and lipopolysaccharides. Both are amphipathic molecules capable of attachment to cell membranes. The lipid moiety of lipoteichoic acid may be regarded as a glycolipid or as a phosphatidyl glycolipid, but it is certainly simpler in structure than lipid A of lipopolysaccharides. Much of the well-known endotoxic activity of lipopolysaccharides appears to be due to the presence, in lipid A, of hydroxyacyl esters (*59*). Such substituents are absent from lipoteichoic acids and it is therefore not surprising that lipoteichoic acids do not share all of the properties of lipopolysaccharides. Lipoteichoic acids are not pyrogenic for rabbits, nor are they lethal for mice (*2*); and they do not stimulate B cell transformations (*60*). On the other hand, they do give a positive localized as well as a generalized Schwartzman reaction in rabbits, the latter being accompanied by the bilateral necrosis of the kidneys characteristic of a lipopolysaccharide-induced reaction; lipid-free teichoic acid is without effect (*2*). Lipoteichoic acid, but not lipid-free teichoic acid, will also stimulate bone resorption in tissue cultures, although the amounts required are approximately tenfold greater than for lipopolysaccharide (*61*). Lipopolysaccharide has been implicated in the resorption of bone in human periodontal disease (*62*), and it has been suggested (*2*) that lipoteichoic acid from gram-positive organisms in plaque and gingival pockets could play a similar role in alveolar bone loss. *Streptococcus pyogenes* lipoteichoic acid has been reported to suppress the immune response to sheep erythrocytes in mice but enhance antibody production to lipopolysaccharide. This property was first ascribed to the glycerol phosphate backbone of lipoteichoic acid (*63*) but, more recently, a preliminary report indicates that the active moiety is the lipid component (*64*).

Concluding Remarks

It is now being realized that membrane lipoteichoic acids are important and presumably essential components of most gram-positive bacteria. Much of the impetus for studying them, however, has come from their immunological properties. Historically it was the group D streptococcus that provided the first example of such an antigen, though the group F lactobacillus antigen was the first to be shown to contain the glycolipid component, and to be located in or on the plasma membrane. The presence of this glycolipid component also accounts for observations, extending over 20 years, of erythrocyte-sensitizing antigens present in, on the surface of, or in the culture fluid from a variety of gram-positive bacteria, because it is the glycolipid component which accounts for the ability of the lipoteichoic acid to sensitize erythrocytes.

The recognition of the membrane association of these lipoteichoic acid antigens has been hampered by a mechanistic view of the bacterial cell surface, which precluded the presence of membrane components as surface components. This view probably arose from a too strict interpretation of the word "wall" in "bacterial cell wall." Basic to this problem is the question of how to define the cell wall. As has been shown with *L. plantarum*, "cell wall" preparations obtained by various procedures can still contain membrane lipoteichoic acid that is detectable immunologically (*22*). Rogers and Perkins (*65*) have concluded that "There is no ideal answer to this dilemma, since there is no absolute definition of a cell wall." As the definition is therefore dependent on individual interpretation, we may need to distinguish between "cell wall" and "cell surface," using the latter term to ensure that membrane components are not precluded from consideration.

The indication that a wide variety of gram-positive bacteria contain a cross-reacting surface component might be regarded as conflicting with the practice of serological classification. However, a serological classification is based on a subjective assessment of observations, with the aim being to use specific serums obtained against specific bacterial strains as a means for dividing organisms into meaningful groups. Initially, a serological classification is empirical and only later does a rational basis emerge—in the case of the group D streptococci there was a gap of 20 years before the antigen was identified, and only then was it possible to explain a number of puzzling features about this group.

It will be noted that the examples of membrane lipoteichoic acids functioning as specific antigens are confined to two genera, *Streptococcus* and *Lactobacillus*, and that the number of genera examined by chemical or serological procedures is rather limited. With the help of the methods new defined for obtaining antibodies to lipoteichoic acid and for the subsequent detection of the antigen-antibody reaction, investigators will probably be able to add more examples to the present list of group-specific lipoteichoic acids.

References and Notes

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