Mediation of Staphylococcal Adherence to Mucosal Cells by Lipoteichoic Acid

MARY M. CARRUTHERS* AND WILLIAM J. KABAT

Department of Medicine, Northwestern University Medical School and Veterans Administration Lakeside Medical Center, Chicago, Illinois 60611

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Staphylococcal lipoteichoic acid markedly reduced adherence by Staphylococcus aureus to buccal cells in vitro, suggesting that lipoteichoic acid mediates adherence by that bacterium. Adherence inhibition by lipoteichoic acid was lost after deacylation of the preparation, suggesting that fatty acids on the molecule are essential to binding.

Binding of the membrane teichoic acids or lipoteichoic acids (LTA) of gram-positive bacteria to mammalian cell surfaces in the form of hemagglutination reactions with erythrocytes has long been recognized. More recently, surface interaction of LTA with other mammalian cells has been studied. Beache and Ofek have established a direct role for LTA from Streptococcus pyogenes in binding of that bacterium to epithelial cells (4). It has been suggested that a similar mechanism is responsible for adherence of another major mucosal pathogen, Staphylococcus aureus, to mucosal surfaces. Evidence presented to support this suggestion, however, is less clear. Aly and co-workers reported that teichoic acid from S. aureus inhibited adherence of that bacterium, but not that of S. pyogenes, to nasal mucosal cells (1). Because Aly's preparation was called teichoic rather than lipoteichoic acid and was isolated from bacterial cell walls, it would appear to lack the glycolipid which has been considered to be essential for membrane binding by LTA (12). The lack of effect of Aly's teichoic acid preparation on S. pyogenes adherence is consistent with absence of the functional glycolipid structure. Thus, it seems possible that a component other than LTA in Aly's cell wall preparation was responsible for the adherence inhibition he observed.

The S. aureus strain used in this study was isolated from a patient with bacteremia, and S. pyogenes IRP 41, used for some of the experiments, was kindly donated by M. McCarty of Rockefeller University, New York, N. Y.

Bacterial LTA was isolated by the method of Fischer et al. (7), with the substitution of 30 mM n-octyl-β-D-glucopyranoside (Sigma Chemical Co., St. Louis, Mo.) for Triton X-100 in the reconstitution of the preparation for final chromatography and 7.5 mM of the same detergent in the eluting buffer. For some experiments, LTA was deacylated by incubation with methanolic KOH. Erythrocyte binding activity was measured by an adaptation of the method of Hewett et al. (8), and antiserum to LTA was produced in rabbits by a modification of the method of Fiedel and Jackson (6).

S. aureus and S. pyogenes were grown in tryptic soy broth and Todd-Hewitt broth, respectively, in stationary culture for 18 h at 37°C; S. pyogenes was incubated in 5% CO2. Bacteria were washed once in saline and resuspended to their original volume in saline. Buccal cells from the same two normal donors were obtained, pooled, washed four times in saline, harvested by centrifugation, and counted in a hemacytometer. In some experiments, S. aureus was preincubated with 100 µg of trypsin per ml in Earle's basic salt solution for 15 min at 37°C; 1 mg of soybean trypsin inhibitor was added, and the preparation was held for 5 min, washed twice, and used in the buccal cell procedure. About 105 epithelial cells were resuspended in Earle's basic salt solution or LTA-Earle's basic salt solution, and bacteria in an approximate CFU to cell ratio of 500:1 were added (final volume, 1 ml). Tubes were incubated with shaking for 1 h at 37°C. Cells were applied to microscope slides, air-dried, stained with Giemsa stain, and read.

The number of bacteria adherent to 25 buccal cells under test conditions was divided by the mean number of untreated bacteria adherent to 25 cells, and the result was expressed as a percentage. The number of bacteria adherent to washed buccal cells which were not incubated with bacteria (always less than 10 per buccal cell) was subtracted from each determination. Adherence by untreated S. aureus was usually 110 to 120 bacteria per cell. Each experiment was performed a minimum of three times. Staphylococcal LTA inhibited adherence by that bacterium to buccal mucosal cells (Table 1). Inhibi-

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* Present address: Department of Pathology, University of Colorado, Denver, Colo. 80235.
tion was similar with concentrations of LTA from 25 to 500 μg/ml. Inhibition of adherence by *S. pyogenes* to mucosal cells by the staphylococcal LTA preparation was similar, although a higher concentration of LTA was required. LTA from *S. pyogenes* also inhibited adherence by either bacterium (Table 2). Again, adherence by *S. aureus* was affected at lower concentrations than by *S. pyogenes*. Buccal mucosal cells were not uniform in their ability to bind *S. aureus*, and the number of bacteria adherent to cells on control slides varied from experiment to experiment. Similar variability has been observed and analyzed by Andersson et al. in studies of pneumococcal adherence to pharyngeal cells (2).

Adherence inhibition as well as hemagglutination inhibition activity by staphylococcal LTA was destroyed by alkaline hydrolysis of the preparation, compatible with a dependence of both activities on the presence of fatty acids in the molecule.

*S. aureus* adherence to buccal mucosal cells in the presence of 25 or 250 μg of decacylated LTA was 82 or 108% of adherence in the absence of LTA. The erythrocyte-binding activity of KOH-treated LTA was <1:2. Treatment of the bacteria with 100 mg of trypsin before incubation with cells decreased bacterial adherence by 51%.

The staphylococcal LTA molecule consists of 28 to 30 units of substituted glycerolphosphate linked to a glycerol which contains two fatty acid substituents (5). Fatty acids, which constitute less than 5% of LTA by weight, appear to be essential to the epithelial cell-binding properties of the molecule. Removal of the fatty acids by alkaline hydrolysis abolishes adherence inhibition by LTA. Similar activity of staphylococcal and streptococcal LTA in inhibiting mucosal cell adherence by both bacteria would be expected, given the similar structure of their LTA (5, 10), and parallels the competitive binding for human erythrocyte membranes by LTA from several bacteria, as reported by Beachey et al. (3). Because fatty acid substituents on the LTA of the same or different bacteria are known to vary (12), the binding which occurs seems likely to be due to physicochemical forces, i.e., hydrophobic interaction, rather than a specific structural configuration. Hydrophobic interaction has been implicated in the adsorption of K88 antigen-bearing enteropathogenic *Escherichia coli* (11).

The negligible amount of protein in our LTA (less than 200 ng in inhibitory concentrations) suggests that a bacterial protein is not directly involved in binding. However, the reduction in adherence by pre-trypsinization of the bacteria is consistent with an effect by trypsin-sensitive bacterial components on access to LTA. The tendency of amphiphilic molecules such as LTA toward complex formation is well known (12), and Ofek et al. have presented evidence that, on the surface of *S. pyogenes*, LTA forms complexes with M protein (9). The protein-LTA arrangement on *S. aureus* is not known.

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**LITERATURE CITED**


