Detection of Antibody to Staphylococcal Lipoteichoic Acid with a Microenzyme-Linked Immunosorbent Assay

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Sera from individuals with *Staphylococcus aureus* endocarditis and osteomyelitis and from some individuals with other forms of gram-positive endocarditis yielded higher readings in a microenzyme-linked immunosorbent assay against lipoteichoic acid from *S. aureus* than did sera from individuals with other types of serious staphylococcal infection or non-staphylococcal osteomyelitis, or from unselected inpatients.

Detection of antibody to cell components and extracellular products of *Staphylococcus aureus* solely or in combination may be useful in the serological diagnosis of serious *S. aureus* infections. In the United States, only determination of the antibody to the cell wall component, teichoic acid, has been widely adopted by clinical laboratories. It has been generally useful in the serodiagnosis of *S. aureus* endocarditis but not other deep tissue staphylococcal infections (10).

Lipoteichoic acids (LTAs) are membrane components of *S. aureus* (as well as most other gram-positive bacteria). They are glycerolphosphate polymers covalently linked to a lipid moiety. Sugar and alanine substitutions on the glycerolphosphate chains vary from bacterium to bacterium. The antigenicity of LTA probably derives from its transient existence as a surface component during the continuing process of excretion from the intact bacterium (11).

We used purified staphylococcal LTA in a microenzyme-linked immunosorbent assay to examine human sera for antibody to the antigen. Sera from individuals hospitalized with staphylococcal and non-staphylococcal infections, as well as from unselected inpatients, were studied. All sera from infected patients were drawn between 10 and 30 days after positive cultures were obtained. *S. aureus* was identified by the clinical laboratories by the tube catalase and oxidative-fermentation tests, as well as by ability to grow on mannitol-salt agar and in 6% sodium chloride. A novobiocin test to distinguish *Staphylococcus saprophyticus* was performed when indicated. Sera from consecutive but otherwise unselected inpatients who were not admitted with a diagnosis of staphylococcal or other gram-positive infection nor recognized to develop such infections during their hospitalizations were also studied. The diagnoses of the infected individuals studied are listed below.

Eleven hospital inpatients were diagnosed as having acute *S. aureus* osteomyelitis: hematogenous osteomyelitis of the vertebra(e) (four patients), ischium (one patient), and long bone (one patient, acute exacerbation of chronic infection); osteomyelitis following orthopedic surgery (three patients); and osteomyelitis secondary to diabetic vascular insufficiency (two patients). One patient had arthritis of the sternoclavicular joint, secondary to intravenous drug abuse. Six patients had other causes of bacterial osteomyelitis: *Pseudomonas aeruginosa*, secondary to intravenous drug abuse (four patients); *Proteus mirabilis*, posttraumatic (one pa-

tient); and mixed, secondary to diabetic vascular insufficiency (one patient).

Five patients had *S. aureus* prosthetic joint infections. Four had *S. aureus* soft tissue infections: abscesses requiring surgical drainage (three patients) and meningitis secondary to an infected shunt (one patient). Four patients had intravenous catheter-associated bacteremia.

Five cases of other gram-positive endocarditis were diagnosed: *Streptococcus salivarius* (two patients), *Streptococcus faecalis* (two patients), and *Lactobacillus* (one patient).

A strain of *S. aureus*, originally isolated from a patient with bacteremia and stored in portions of rabbit blood at -70°C until required was used for production of LTA. The bacteria were grown in tryptic soy broth for 18 h at 37°C.

LTA was extracted from the pellet by phenol and purified by column chromatography by the method of Fischer et al. (3) with the use of readily dialyzable *n*-octyl- β -D-glucopyranoside at concentrations of 30 mM for initial solubilization and 7.5 mM for elution instead of Triton X-100 in the final chromatographic step.

Microenzyme-linked immunosorbent assay was performed by a microadaptation of the method of Engvall and Perlman (2). Each well of a 96-well polystyrene microtitration plate (Nunc; GIBCO Laboratories, Grand Island, N.Y.) was filled with 200 μ l of sodium carbonate buffer, pH 9.6, containing 5 μ g of LTA per ml and incubated overnight at room temperature in a humid box. Unbound LTA was removed by suction, and the plate was washed three times in phosphate-buffered saline containing 0.05% Tween 20 to reduce nonspecific binding. Each well was drained and filled with 200 μ l of serum diluted 1:500 in phosphate-buffered saline-5% bovine serum albumin-0.02% azide and incubated for 2 h in a humid box at room temperature. An excess of bovine serum albumin was used as diluent for serum and immunoglobulin G to reduce nonspecific binding (8). Negative and positive control sera from a normal individual and an individual with *S. aureus* endocarditis were included on each plate. Results were not evaluated unless these sera gave readings of ≤ 0.04 and between 1.0 and 1.4, respectively. Sera were removed by suction, and the wells were washed as above. Two hundred microliters of goat anti-human immunoglobulin G conjugated to alkaline phosphatase and diluted 1:750 in phosphate-buffered saline-5% bovine serum albumin was then added to each well, and incubation was continued for an additional 2 h. Unbound globulin was removed from wells by suction, and the wells were washed three times as above and then each filled with

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200 μ l of alkaline phosphatase substrate solution, *p*-nitrophenylphosphate (Sigma Chemical Co., St. Louis, Mo.), freshly prepared by solubilizing 40 mg of the substrate in 10 ml of distilled water, then adding 10 ml of glycine buffer (Sigma). Incubation was continued at room temperature for 25 to 30 min (until the positive control appeared by visual inspection to have an optical density at 405 nm of ca. 1.0). The reaction was stopped by the addition of 50 μ l of 2 N NaOH to each well.

Absorbance at 405 nm was read on a Titertek Multiskan (Flow Laboratories, McLean, Va.). Data were evaluated by a one-way analysis of variance with subsequent pairwise comparison of the group means by the Tukey method for group comparisons.

Individuals with *S. aureus* osteomyelitis had more immunoglobulin G antibody activity to staphylococcal LTA than did three other groups: unselected inpatients ($P < 0.001$), individuals with osteomyelitis due to other bacteria, and individuals with either abscesses requiring surgical drainage or intravenous catheter-associated bacteremia due to *S. aureus* (Fig. 1). Antibody activity in the staphylococcal osteomyelitis patients was similar to that in individuals with *S. aureus* endocarditis or other gram-positive endocarditis.

Like teichoic acid antibody, LTA antibody is not specific to staphylococcal infection. Its presence, however, does suggest the presence of either endocarditis or osteomyelitis, which usually have distinguishing clinical characteristics.

S. aureus osteomyelitis may provide difficulty in diagnosis for other reasons. First, the patient may have osteomyelitis, but the precise bacterial etiology might be unclear, or

second, the patient may have staphylococcal soft tissue infection, but whether adjacent bone is also infected might be unknown. In either of these circumstances, being able to observe the presence of elevated antibody activity to staphylococcal LTA might be useful. In our study, individuals with non-staphylococcal osteomyelitis did not have unusually high antibody activity to LTA, despite the fact that a number of our non-staphylococcal osteomyelitis patients were drug abusers, a group generally recognized to have frequent staphylococcal infections. In adults, osteomyelitis due to facultative streptococci is rare, and elevated LTA antibody in an individual with osteomyelitis would suggest staphylococcal etiology. Patients in our study who had soft tissue abscess but not bone infection due to *S. aureus* lacked elevated levels of antibody activity to LTA.

Other investigators have not found teichoic acid antibody or other serological tests to be helpful in the diagnosis of staphylococcal osteomyelitis. The teichoic acid antigen used by most laboratories has been a crude sonicate of whole bacteria, thus containing a mixture of cell wall and membrane teichoic acids. The presence of titratable antibody by double diffusion in agar correlates well with the diagnosis of endocarditis but not of osteomyelitis. The counterimmunoelectrophoresis technique with undiluted serum yields more positive results but often also yields an unacceptably high percentage of positive results in presumably uninfected individuals. Wheat and White (10), reviewing seven studies reported before 1978, found 38% of individuals with bone or joint infections to have teichoic acid antibody. Jackson et al. (5) found that of seven individuals with osteomyelitis, all had

FIG. 1. Antibody to staphylococcal LTA detected by microenzyme-linked immunosorbent assay in unselected inpatients and in patients with infections. (A) *S. aureus* osteomyelitis (11 patients) and arthritis (1 patient; asterisk); (B) unselected inpatients (30 patients); (C) other osteomyelitis (6 patients); (D) *S. aureus* prosthetic joint infections; (E) *S. aureus* endocarditis (6 patients); (F) *S. aureus* soft tissue infection (5 patients) and intravenous-catheter-associated bacteremia (4 patients; asterisks); (G) other gram-positive endocarditis (5 patients). Vertical bars indicate means and 95% confidence intervals for the means.

titratable teichoic acid antibody by counterimmunoelectrophoresis. However, all of 15 individuals with soft tissue staphylococcal infection did also. Larinkari (7) found teichoic acid antibody in 4 of 16 patients with acute *S. aureus* osteomyelitis and in 7 of 22 patients with chronic *S. aureus* osteomyelitis. Positivity was defined as a titer by gel diffusion that was $\geq 1:4$. Such a titer was identified in 6 of 11 patients with acute osteomyelitis and in none of seven with chronic osteomyelitis in the study of Tuazon (9). Nine of the patients with acute osteomyelitis and three of seven with chronic osteomyelitis had detectable antibody by counterimmunoelectrophoresis.

Doyle et al. (1) reported the presence of passive hemagglutinating antibody to erythrocytes sensitized by a *Bacillus subtilis* cell wall antigen, poly(glycerolphosphate), in sera of normal individuals, as well as those with staphylococcal infections. Hemagglutination was inhibited by that antigen but not by *S. aureus* poly(ribitolphosphate). Recently Granström et al. (4) reported a microenzyme-linked immunosorbent assay response to a purified trichloroacetic acid extract of disrupted *S. aureus*. Glycolipid moieties are generally removed from membrane LTAs by acidic conditions used for cell wall extraction (6), and Granström et al. indicated that their antigen was a ribitol teichoic acid from the cell wall. Few of the individuals studied with chronic *S. aureus* osteomyelitis had elevated levels of antibody. Individuals with acute osteomyelitis were not evaluated.

In patients with *S. aureus* bacteremia, determination of teichoic acid antibody has been helpful in identifying those patients with *S. aureus* endocarditis. LTA antibody determination would appear to add little information in that situation. Determination of LTA antibody appears to be helpful, however, in the diagnosis of staphylococcal osteomyelitis.

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