Unique Teichoic Acid Isolated from the Cell Walls of a Strain of Staphylococcus aureus

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Teichoic acid antigen extracted from cell walls of a strain of Staphylococcus aureus has been shown to have the unique structure of glycero phosphate, with N-acetyl-D-galactosamine as the immunodominant substituent.

The teichoic acids isolated from the cell walls of Staphylococcus aureus have been shown to be polymers of ribitol 1,5-phosphate with N-acetyl-D-glucosamine a common substituent at C4, and alanine found on either C2 or C3 (6). The amino sugar moiety can be linked in either the alpha or beta configuration, and has been shown by Sanderson et al. (10) and Morse (8) to be the immunodominant group which determines serological specificity. The teichoic acid antigens of S. aureus cell walls do not cross-react with the teichoic acids from S. epidermidis (8). The latter have been shown to be polymers of glycerol linked 1,3 by phospho-diester linkages. The C2 is available for substitution, and, not infrequently, glucose is found linked in either the alpha or beta configuration, and is also the important determinant of antigenic specificity (6).

This current concept of the structure of teichoic acid antigens isolated from the cell walls of staphylococci is based in reality upon careful studies of relatively few strains. It has been suggested in the past (2) that these antigens may not be as homogeneous in structure as originally thought. We report here the isolation and characterization of a teichoic acid antigen from clean cell walls of a strain of S. aureus which has the unique structure of glycero phosphate with N-acetyl-D-galactosamine as the immunodominant substituent.

MATERIALS AND METHODS

The strain of S. aureus used in these studies was designated in our laboratory as strain 406 and was obtained as a clinical isolate from M. Silverman, Veterans Administration Research Hospital, Chicago, Ill. It was gram positive, showed the typical morphology of staphylococcus, grew well on Staphylococcus no. 110 medium (Difco, Detroit, Mich.), and produced a golden-yellow pigment. It was shown to be coagulase positive, clotting 1:5 diluted normal rabbit plasma in less than 1 h. It was also positive for clumping factor, fermented glucose, and mannitol, and produced alpha, beta, and delta toxins as determined by its hemolytic activity on appropriately prepared blood agar (4). It was susceptible to all the antibiotics tested, which included: neomycin, 5 µg; streptomycin, 2 µg; chloramphenicol, 5 µg; novobiocin, 5 µg; erythromycin, 2 µg; penicillin, 2 µg; nafcillin, 1 µg; ampicillin, 2 µg; kanamycin, 5 µg; lincomycin, 2 µg; cloxacillin, 1 µg; nortrin, 1 µg; aureomycin, 5 µg; and furandantin, 2 µg.

Other organisms mentioned in relation to antiserum or from which teichoic acids were prepared for comparative purposes were described (9). The organisms were grown in Brain Heart Infusion broth (Difco, Detroit, Mich.) supplemented with 5 g of glucose per liter, at 37°C for 18 to 20 h with gentle agitation. The teichoic acid was extracted and partially purified from enzymatically digested cell walls by the method of Sanderson et al. (11). The final product was dissolved in water at a concentration of 100 µg per ml and passed through a G-50 Sephadex column equilibrated with water, to remove some nucleic acid fragments which contaminated the preparations. The fractions from the column were analyzed for phosphorus by the method of Matsuno and Slade (7), and thefractions containing the purified teichoic acid were pooled, lyophilized, and stored at -20°C.

Samples of the purified 406 teichoic acid were hydrolyzed for 3 h at 100°C in 2 N HCl. The HCl was removed by evaporation to dryness, and the hydrolysate was redissolved in water and spotted on a precoated thin-layer chromatographic plate (Merck Silica Gel) which had been activated for 20 min at 110°C. The chromatograms were developed with chloroform-methanol-ammonia (17%) in a ratio of 2:2:1, and the spots were visualized with an alkaline silver nitrate reagent (5) for the sugars and a 0.5% ninhydrin in butanol aerosol spray (Ninhydrin; Nutritional Biochemicals Corp., Cleveland, Ohio) for amino sugars and amino acids. Amino acids and amino sugars were also determined by analysis on a Beckman model 116 amino acid analyzer by the method of Matsuno and Slade (7). Hydrolysates were also silylated by the method of Matsuno and Slade (7), and 2 µliters
of the silylated material, representing 11 µg of the hydrolysate, was injected into a model 1400 Varian Aerograph gas chromatographic apparatus with a stainless-steel column (121.92 by 0.3175 cm) packed with 3% S.E. 52 coated on chromosorb W. The initial temperature of the column was 100°C, and the instrument was operated on a linear program which resulted in a temperature increase of 4°C per minute. The method of Belcher et al. (1) was used to quantitate galactosamine, and glycerol was determined by gas chromatography by the method of Matsuno and Slade (7).

Hyperimmune rabbit antisera were prepared by repeated intravenous injection of washed, autoclaved organisms in saline suspension, as previously described (3). The method used in the hapten inhibition studies has also been described (9).

RESULTS

It was observed that teichoic acid from the 406 strain of *S. aureus* did not react in gel diffusion or in fluid systems with many human sera or with antisera prepared against any of several strains of *S. aureus* or *S. epidermidis*, and reacted only with its homologous antiserum. Conversely, it was shown that hyperimmune rabbit antiserum prepared against the 406 strain of *S. aureus* reacted only with the homologous 406 purified teichoic acid, and not with teichoic acid prepared from several other strains of *S. aureus*. Typical results of the latter reaction are shown in Fig. 1. It is known from previous work reported from this laboratory (9) that teichoic acid from the Copenhagen strain of *S. aureus* has both the alpha- and beta-linked N-acetylglucosamine specificity, whereas the Foggie and Smith diffuse strains of *S. aureus* were of the beta-specific type. These results suggested that the 406 strain teichoic acid had a unique antigenic structure.

Chemical analyses of the 406 strain purified teichoic acid showed that it contained 9.9% phosphorus, 30.2% glycerol, and 22.8% galactosamine. On a molar basis the glycerol to phosphorus ratio was about 1:1, and the glycerol to galactosamine ratio was 1.0:0.37, suggesting that the galactosamine was not attached to every glycerol unit. Thin-layer, gas chromatographic, and amino acid analyzer analysis confirmed that it contained glycerol, galactosamine, and alanine. No ribitol or anhydroribitol, the main products of acid hydrolysis of other *S. aureus* teichoic acids, could be detected by thin-layer or gas chromatography, even after treatment of the hydrolysate with alkaline phosphatase. The alkaline phosphatase treatment of the acid hydrolysate produced a great increase in the glycerol detected by gas chromatography over that detected in nonalkaline phosphatase-treated hydrolysates, indicating the attachment of phosphate to glycerol.

FIG. 1. Gel diffusion pattern between 406 hyperimmune rabbit antiserum and purified teichoic acids from several strains of Staphylococcus aureus. Center well contains antiserum. Peripheral wells contain: 1, Copenhagen teichoic acid (100 µg); 2, Smith diffuse teichoic acid (100 µg); 3, Foggie teichoic acid (100 µg); 4, 406 teichoic acid (100 µg).

To determine the specificity of the 406 teichoic acid-antiserum system, hapten inhibition studies were done. The results of these experiments are shown in Fig. 2, where it is clear that the only effective inhibitor of the reaction between 406 teichoic acid and its homologous antiserum was N-acetyl-D-galactosamine.

DISCUSSION

From the results of these and other studies carried out in the past 10 years in this laboratory (personal observations), it would appear that the majority of strains of *S. aureus* do show the typical ribitol phosphate-N-acetylglucosamine-ala

tine teichoic acid present in their cell walls. The occurrence of atypical strains of *S. aureus* with regard to their teichoic acid structure cannot be discounted, however. The 406 strain represents one of the series of 14 strains of *S. aureus* isolated from clinical specimens and was the only one to lack ribitol and to contain galactosamine. It did not react with heterologous antisera against various *S. aureus* teichoic acids known to contain ribitol and have N-acetyl-D-galactosamine as the immunodominant group. Other evidence has also
suggested that the teichoic acid extracted and purified from the Foggie strain of *S. aureus* is not typical in that it contains a relatively high content of glucose, a constituent not found as commonly in *S. aureus* teichoic acid as in the teichoic acid from *S. epidermidis* strains. Further studies to determine the distribution of strains of *S. aureus* having unique teichoic acid antigens would seem warranted, since there is some evidence (3) that these antigens may play a role in immunity to staphylococcal infection.

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**LITERATURE CITED**