ROLE OF TEICHOIC ACID IN THE BINDING OF *STAPHYLOCOCCUS AUREUS* TO NASAL EPITHELIAL CELLS

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Role of Teichoic Acid in the Binding of *Staphylococcus aureus* to Nasal Epithelial Cells

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The role of teichoic acid in the adherence of *Staphylococcus aureus* to nasal epithelial cells was investigated. Epithelial cells treated with teichoic acid demonstrated decreased binding of *S. aureus*. A 71% reduction in adherence was noted with teichoic acid–treated epithelial cells as compared with controls ($P < 0.001$). Reduction in adherence of *S. aureus* was also noted with lipoteichoic acid (obtained from streptococci)–treated epithelial cells. The data provide evidence that teichoic acid mediates the adherence of *S. aureus* to nasal mucosal cells.

Bacterial adherence to epithelial cells plays an important role in mucous membrane colonization. The mechanisms involved in the binding of bacteria (streptococci and *Escherichia coli*) to epithelial cells have been studied [1–3]. For example, lipoteichoic acid has been found to inhibit the binding of group A *Streptococcus* to oral epithelial cells [1, 2]. However, the mechanism involved in adherence of *Staphylococcus aureus* to nasal mucosal cells has not previously been investigated. Teichoic acids are major cell-wall components of staphylococci and bind spontaneously to mammalian cells [4]. The present study investigates the role of teichoic acid in the adherence of *S. aureus* to human nasal epithelial cells.

Materials and Methods

Preparation of cell wall and teichoic acid. Teichoic acid was extracted and purified according to the method of Baddiley et al. [5]. A broth culture of *S. aureus* 502A was grown for 18 hr in a fermenter, and the cells were centrifuged, washed with 0.9% NaCl, and disrupted in a cold Waring blender (Waring Products, New Hartford, Conn.) containing glass beads (0.11 mm) for 30 sec. The cell walls were separated by ultracentrifugation. Gram-staining was performed on the resulting material to determine cellular degradation.

The degraded material was centrifuged (20,000 *g*) for 30 min to isolate bacterial cells walls, which were washed five times with cold 0.075 M sodium phosphate buffer (pH 7.9) and five times with cold distilled water at 20,000 *g* and lyophilized. The crude cell-wall material was Gram-stained and examined microscopically; it was determined to be free of whole cells. The cell walls were not free of teichoic acid. Teichoic acid was extracted using cold 95% phenol and was recovered in the aqueous phase after centrifugation (2,000 *g*) and purified by methods previously described [5–7]. This material was dialyzed against six changes of distilled water over three days and lyophilized for bacterial adherence studies.

Treatment of nasal epithelial cells with teichoic acid. Nasal epithelial cells washed with phosphate-buffered saline (PBS) were preincubated for 30 min at 37 C with 1.0 mg of a teichoic acid fraction or lipoteichoic acid of group A streptococci or cell-wall material/ml [1, 2]. (The lipoteichoic acid was provided by Dr. I. Ofek, University of Tennessee, Nashville.) The control epithelial cells were treated with PBS only.

The epithelial cells were centrifuged (1,000 *g*) and washed with PBS to remove unattached teichoic or lipoteichoic acids or cell-wall material. These epithelial and control cells were mixed with 10^8 cfu of bacteria to examine their binding capacity [8] to *S. aureus* or group A streptococci. Epithelial cells were stained with crystal violet. The adhering ability of bacteria was determined by counting the bacteria that adhered to epithelial cells.

Results

Bacterial adherence to nasal epithelial cells. Bac-

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Table 1. Bacterial adherence to nasal epithelial cells.

Epithelial cells mixed with	Bacterial counts* Test	Bacterial counts* Control†	Inhibition of adherence (%)
Cell walls from *Staphylococcus aureus* and *S. aureus*‡	33	65	49 ($P < 0.01$)
Teichoic acid and *S. aureus*	13	45	71 ($P < 0.001$)
Lipoteichoic acid (from streptococci) and *S. aureus*	18	45	60 ($P < 0.001$)
Teichoic acid and *Streptococcus pyogenes*	61	74	17 ($P > 0.36$)

* Fifty epithelial cells for each treatment were counted.
† Epithelial cells were mixed with test organisms only.
‡ The epithelial cells were mixed with bacterial cell wall and then incubated with *S. aureus*.

terial attachment to teichoic acid–treated and untreated nasal mucosal cells was compared (table 1). The cells treated with teichoic acid demonstrated a significantly reduced capacity to bind to *S. aureus*—a 71% reduction as compared with untreated control cells. The lipoteichoic acid–treated epithelial cells demonstrated a 60% decreased binding for *S. aureus* as compared with controls. When nasal epithelial cells were treated with teichoic acid and mixed with group A streptococci, the reduction of binding was minimal.

The adherence of *S. aureus* to nasal epithelial cells pretreated with whole cell walls of *S. aureus* was also investigated. Whole cell-wall–treated epithelial cells demonstrated a 49% reduction of *S. aureus* binding as compared with the controls.

Optimal concentration of teichoic acid to inhibit binding of *S. aureus* to nasal mucosal cells was determined. Maximal inhibition of *S. aureus* occurred between 1 and 1.5 mg/ml of teichoic acid (figure 1).

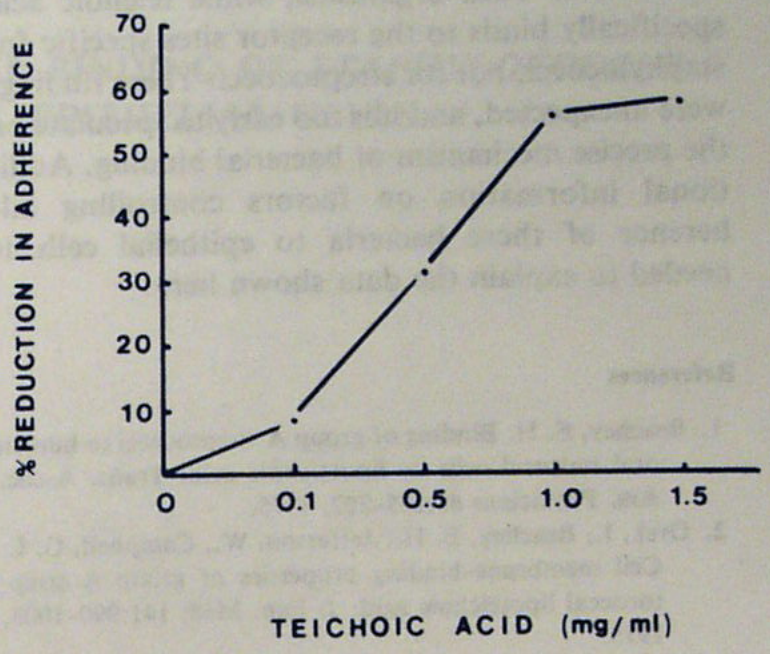

Figure 1. Determination of optimal concentration of teichoic acid to inhibit adherence of *Staphylococcus aureus* to nasal epithelial cells.

Discussion

This study provides evidence that either lipoteichoic acid or teichoic acid binds to nasal epithelial cells and inhibits the adherence of *S. aureus* to these cells. A 71% reduction in adherence was noted with teichoic acid–treated epithelial cells ($P < 0.001$) and a 60% reduction with lipoteichoic acid–treated cells ($P < 0.001$), as compared with control cells.

Lipoteichoic acid is located on the surface of group A streptococci and mediates in their binding to oral mucosal cells. Pretreatment of the oral epithelial cells with lipoteichoic acid inhibits the binding of streptococci to these cells [1, 2].

The cell walls of *S. aureus* strains thus far studied have been shown to contain polymers of ribitol connected by a phosphate diester linkage with side chains of D-alanine and various proportions of N-acetylglucosamine [5, 9]. The phosphate groups and the amino groups in the alanine ester residue of teichoic acid have a profound effect on cation binding [10], providing a suitable ionic environment. It has been suggested that the polysaccharide fibers of the bacteria, for the most part negatively charged, can form a polar bond with host-cell polysaccharides, also negatively charged, by way of divalent positive ions in the medium [11]. Lectin, a protein that carries receptor sites for the bacteria and the host cells, can also form a bridge between them.

Pretreatment of epithelial cells with teichoic acid had little effect on the binding of streptococci. On the other hand, when epithelial cells were treated with lipoteichoic acid, a decrease in adherence to *S. aureus* and streptococci was noted. This result suggests that lipoteichoic acid of streptococci combines with epithelial receptor sites

common to both organisms, while teichoic acid specifically binds to the receptor sites specific for staphylococci, not for streptococci. These findings were unexpected, and it is too early to speculate on the precise mechanism of bacterial binding. Additional information on factors controlling adherence of these bacteria to epithelial cells is needed to explain the data shown here.

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