Anatomical Evidence of Microbial Biofilms in Tonsillar Tissues

A Possible Mechanism to Explain Chronicity

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Context: Bacteria within biofilms are resistant to host defenses and antibiotics. The presence of bacterial biofilms within the tissue and crypts of inflamed tonsils may explain the chronicity and recurrent characteristics of some forms of tonsillitis.

Objective: To determine if microbial biofilms occur within clinically abnormal tonsils.

Design: In this study, we evaluated the histomorphological appearance of 19 human tonsils for evidence of biofilm formation using light and transmission electron microscopy.

Subjects: Human tissues were collected during surgical tonsillectomy. Fifteen specimens were removed because of a history of repeated infections, and 4 were removed because of hypertrophy and obstruction.

Interventions: No interventions were used in this study.

Results: Gram-positive and gram-negative bacteria were seen within otherwise acellular deposits among crypts of 11 of 15 infected tonsils. Regions of accumulated bacteria possessed the ultrastructural appearance of typical amorphous polysaccharide biofilm matrix. Small clusters of bacterial colonies were seen in 3 of 4 tonsils removed because of hypertrophy.

Conclusions: There is strong anatomical evidence for the presence of bacterial biofilms in chronically diseased tonsils. Because sessile bacteria within biofilms are resistant to host defenses and antibiotics, bacterial biofilms within tonsils may explain the chronicity and recurrent nature of some forms of tonsillitis.


Tonsillitis is one of the most common infectious diseases of childhood. In spite of the widespread use of antibiotics for this disease, it is often recalcitrant and recurrent. Tonsillectomy is often performed when antibiotic therapy fails to ameliorate this disease. More than 400,000 tonsillectomies were performed in the United States in 1996, making it one of the most common surgical procedures performed on children.

The failure of antibiotic therapy to eradicate susceptible organisms causing tonsillitis has been an enigma. For example, in spite of adequate treatment of tonsillitis due to β-hemolytic streptococci with penicillin, 20% of tonsillitis cases are bacteriologically positive after treatment. Although the increasing incidence of β-lactamase–producing organisms recovered from tonsils may allow better bacterial survival, tonsillar infections may recur even when they are caused by organisms shown to be susceptible in vitro. These observations have led us to hypothesize that bacteria from biofilms within the crypts of chronically infected tonsils can resist eradication by antibiotics and host defenses. In this study, we evaluated tonsils removed during routine tonsillectomy for anatomical evidence of biofilm formation.

METHODS

TONSIL SPECIMENS

Tonsils were obtained during routine tonsillectomy and placed in 10% neutral buffered formalin. Specimens were obtained from individuals with recurrent tonsillitis (n=15) as well as from individuals undergoing tonsillectomy for snoring or sleep apnea (n=4). The Human Studies Review Committee of Washington University in St Louis, St Louis, Mo, approved the study, and subjects provided written consent to donate tissue for the study.
LIGHT MICROSCOPY

Tonsils were initially placed in 10% neutral buffered formalin and then were cut into 2- to 4-mm-thick slices, which were transferred to a fixative consisting of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1M phosphate buffer for 24 hours at 4°C. Alternatively, samples of tonsil were placed directly into a non-aqueous solvent (Fluorinert FC-72; 3M, St Paul, Minn) with 1% osmium to preserve the polysaccharide matrix of biofilms, which readily dissolves in aqueous solutions. All specimens were then dehydrated in graded solutions of acetone and embedded in epoxy resin (Epon-Araldite; Electron Microscopy Sciences, Fort Washington, Pa). Several semithin sections (0.5-1.0 µm) were collected at a variety of depths of the sample and counterstained with toluidine blue and basic fucshin. An alternate group of sections was gram stained using a commercially available staining kit (Protocol Gram Stain Set; Biochemical Sciences Inc, Swedesboro, NJ). The sections were examined with an upright light microscope (Olympus BH-2; Olympus America Inc, Melville, NY), and images were captured with a digital photo system (Sony DKC-5000; Sony Corporation of America, New York, NY).

TRANSMISSION ELECTRON MICROSCOPY

Tissue samples were fixed and dehydrated as described above and then embedded in epoxy resin. Blocks were thin sectioned for transmission electron microscopy on an ultramicrotome (Reichert Ultracut; Leica Inc, Deerfield, Ill). Thin sections for transmission electron microscopy were then taken from regions containing suspected biofilms and counterstained with uranyl acetate–lead citrate. They were examined and photographed using a transmission electron microscope (Hitachi H-7500; Hitachi High Technologies America, Pleasanton, Calif) with digital imaging capabilities. The contrast and brightness of the images were optimized for publication with image-editing software (Adobe Photoshop 7.0; Adobe Systems Inc, San Jose, Calif).

RESULTS

We considered dense accumulations of bacteria embedded in an amorphous matrix to be biofilms. Of the tonsils with a history of infections, 11 of 15 showed evidence of biofilms. Of enlarged tonsils that were removed for obstruction and did not have active current infection, 3 of 4 had evidence of biofilms.

Biofilms most commonly occurred within cysts of tonsils and appeared as large, dense accumulations of mixed bacteria. Inflammatory cells were commonly seen at the periphery of these bacterial colonies but not within the mass (Figure 1). Gram stains revealed gram-positive and gram-negative organisms, although gram-positive cocci were predominant (Figure 1, inset). Some biofilms were several millimeters in diameter and could be seen with the naked eye. Some of the colonies appeared to be more complex, with infoldings. With electron microscopy, bacterial colonies were seen as densely packed bacteria with a varying morphological appearance, including rod-shaped and spherical profiles, and a variety of capsular staining patterns. On close inspection, the bacteria were embedded in a homogeneous, amorphous background substance that was well preserved in solvent-processed tissues (Figure 2).

COMMENT

Bacteria that cause human disease are generally thought of as existing as freely motile organisms. However, most bacteria, typically Escherichia coli, Haemophilus influenzae, and Pseudomonas, Staphylococcus, and Klebsiella species, have the capacity to adhere to surfaces at a solid/liquid interface. Once adherent, the bacteria produce a complex polysaccharide matrix in which they become embedded. Colonies of bacteria then enlarge and, through a process called quorum sensing, form large colonies of sessile bacteria. Sessile bacteria within biofilms assume an altered phenotype in which they do not divide. These biofilms are ubiquitous in nature and are found in virtually every natural body of water in the world. Over the last 10 years, biofilms have been recognized as a signifi-
cant cause of persistent infections, including dental plaque, pneumonitis due to cystic fibrosis, chronic cystitis, osteomyelitis, otitis media, and cholesteatoma.6,11 The most important characteristic of bacterial biofilms is that bacteria within these colonies become highly resistant to antibiotics and host defenses.12 For example, a strain of *Klebsiella pneumoniae* had a minimum inhibitory concentration of 2-ug/mL ampicillin in aqueous suspension, but the same strain grown as a biofilm withstood ampicillin concentrations of 5000 ug/mL.4

It is likely that attachment of bacteria to tonsillar epithelium is the first step in biofilm formation.13 Adherent bacteria, especially within tonsillar crypts, can form large mixed colonies of bacteria. The bacteria in these biofilms, while protected from host defenses and antibiotics, continue to metabolize and form endotoxin. The elaboration of local endotoxin within tonsillar crypts may lead to chronic inflammation. Furthermore, when local environmental conditions are favorable, the bacteria within the biofilm may become motile, causing an acute infection to recur.14

In this study, we examined tonsils that had been removed because of recurrent infection as well as enlargement and obstruction. It is thought that hypertrophied tonsils have increased pathogenic bacteria compared with nondiseased tonsils.19 Indeed, when aspirates of normal tonsils20 are compared with those of hypertrophied tonsils, the hypertrophied tonsils have more bacterial isolates. A study that compared tonsillar microbial flora in hypertrophied tonsils and tonsils with recurrent infection found that the 2 groups did not differ significantly in the number of isolates per patient, of either aerobic (3.8 vs 4.3) or anaerobic (5.2 vs 4.7) bacteria.17 Therefore, the enlarged tonsils in the present study may not represent normal controls.

The finding of biofilms in tonsils without a clinical history of infection does raise the possibility that biofilm formation within the crypts of tonsils is part of a normal process of immune surveillance and that mixed bacterial biofilms are part of the normal flora of human tonsils. The most appropriate control tissues would be tonsillar tissue from age-matched control subjects who never had upper respiratory infection, but such tissues were not available for this study.

The anatomical evidence of mixed bacterial biofilms within the crypts of tonsils that have been removed because of recurrent infection or hypertrophy raises the possibility that bacteria sequestered from host defenses and antibiotics may explain the recalcitrant nature of some cases of recurrent tonsillitis. Accepted for publication October 16, 2002.

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REFERENCES