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Staphylococcal adherence to chicken cartilage

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Immature chicken cartilage was incubated in a *Staphylococcus aureus* suspension and then washed. Scanning electron microscopy and radiolabel measurements showed increased adherence to cartilage with increasing bacterial concentration. Preheating of the bacteria did not reduce the adherence property, but trypsin treatment did.

Our laboratory has developed chicken models for studies of *Staphylococcus aureus* hematogenous osteomyelitis and septic arthritis closely mimicking the human diseases (Alderson & Nade 1987, Emslie et al. 1983). These studies suggested a specific tropism of the bacteria for the initial infection sites, respectively the growth plate cartilage and the articular cartilage of long bones. Ultrastructural investigations of avian long bones harboring staphylococcal abscesses have shown that bacterial adherence is an important initial step in the pathogenesis of these diseases (Alderson et al. 1986, Speers & Nade 1985). Our in vitro study of staphylococcal adherence to avian cartilage was conducted to verify our previous experimental findings and to gain more insight into the adhesive process.

Materials and methods

One-day-old male broiler chickens (from Diamond Poultry Services) were raised on a diet of "chick starter crumbles" (Milne Feeds) and tap water until 29 days of age. The chickens were then anesthetized with halothane and the proximal tibiae removed and placed into phosphate-buffered saline (PBS). Thin, vertical 2-mm sections were then sliced under PBS and trimmed to 5×10 mm. Slices were used immediately for adherence assays. The strain of *S. aureus* used was isolated from a spontaneous case of chicken tenosynovitis. Primary cultures of the organism from bone swabs were stored at −70° C in brain-heart infusion broth (BHIB) with 10 per cent glycerol. Bacteria for electron microscopy were grown in BHIB with shaking for 6 hours at 37° C, centrifuged (1400×g) for 10 min and suspended in 10 ml of PBS. Quantitative cultures showed 10^9 to 10^{10} colony-forming units (CFU) per ml. To radiolabel bacteria for adherence assays to tissue slices, bacteria were grown in BHIB supplemented with 5μCi/ml of ^{3}H thymidine. Radiolabelled bacteria were then diluted in 10-fold steps in 10 ml volumes. Five tissue slices were used for each concentration of bacteria. For control experiments, bacteria were killed prior to assay by heating at 60° C for 1 h in PBS, while viable bacteria were stored at 4° C in PBS. Viable and dead bacteria were then washed in PBS before assay. Viability checks were performed on the heat-treated bacteria to ensure sterility. Pretreatment of bacteria with 0.25 per cent trypsin was performed in divalent cation-free PBS for 15 min at 37° C with shaking while control bacteria were stored in PBS at 37° C.

All the adherence tests were performed at 37° C with shaking. Tissue slices were submerged in a bacterial suspension for 1 h and individually washed 10 times in PBS. Specimens for scanning electron microscopy were fixed in 0.25 per cent glutaraldehyde for 24 h, dehydrated in a graded acetone series, then critical-point dried. Specimens were mounted on stubs, coated with gold-palladium, and examined on an Edax scanning electron microscope.

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Tissue slices for adherence assays were placed individually into scintillation virals after washing and allowed to dry. Scintillation cocktail (4 ml) was then added and the radioactivity counted over a period of 1 min.

Adherence assays were performed to compare live bacteria with heat-killed bacteria and to consider the effect of trypsin on the adhesive ability of the bacteria. Adherence was expressed as mean counts per minute (CPM) for each concentration of bacteria used.

Results

Scanning electron micrographs of the tissue slices showed that after thorough washing, bacteria still adhered in great numbers to the surfaces of growth plate and articular cartilage (Figure 1).

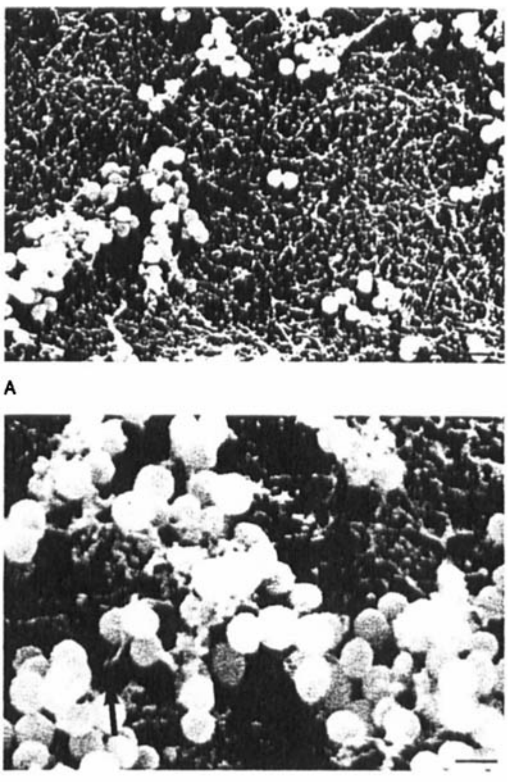

Figure 1. Scanning electron micrographs of cartilage surface of tissue slices after incubation with staphylococci.
A. The bacteria adhered in the form of clumps to all exposed growth-plate and articular-cartilage surfaces. Bar, 5 microns.
B. Inspection of the adherent bacteria showed that the bacteria were closely associated with the collagen fibrils of the cartilage (arrows). Bar, 1 micron.

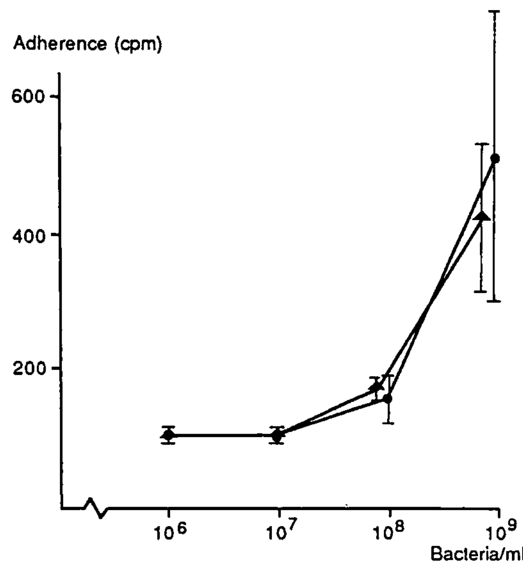

Figure 2. Adherence of the radiolabelled *S. aureus* to tissue slices as determined by CPM. The number of adhered bacteria increased exponentially as the concentration of bacteria was increased in 10-fold amounts. Heat-killed bacteria ● showed no decrease in adherence compared with live bacteria ▲.

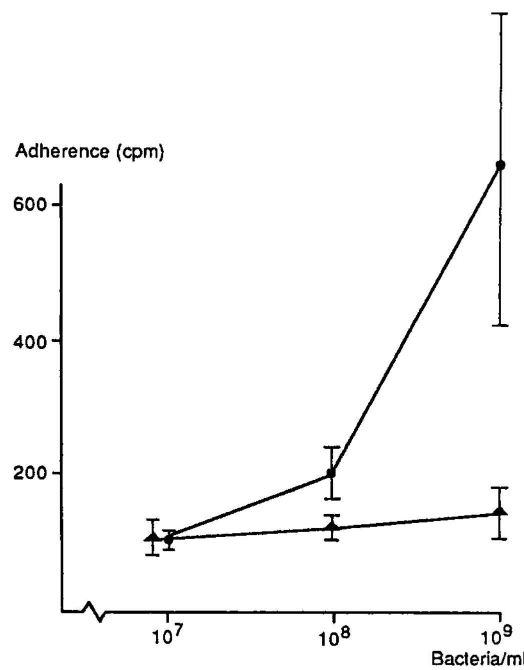

Figure 3. Adherence of radiolabelled bacteria to tissue slices following treatment with trypsin. Trypsin treatment of the bacteria reduced the number of adhered bacteria to background levels ▲ when compared with controls ●.

Upon closer examination the adhered bacterial clumps appeared to be firmly attached to the collagen meshwork of the cartilage.

There was no difference in adhesive ability between viable and heat-killed bacteria; for both populations the number of adhered bacteria rose with the concentration of bacteria (Figure 2). However, when viable bacteria were pretreated with trypsin (Figure 3) their adhesive ability was abolished, providing us with a negative control.

Discussion

The initiation of infection in acute hematogenous osteomyelitis and septic arthritis appears to be the result of specific binding of *S. aureus* to cartilage surfaces.

The loss of the endothelial barrier in metaphyseal tunnels during normal bone growth (Howlett 1980) and the ability of the staphylococci to adhere to the growth-plate cartilage subsequently exposed has already been demonstrated (Speers & Nade 1985). The same strain of *S. aureus* has also been shown to adhere in vivo to the articular cartilage of chickens following intraarticular injection (Alderson et al. 1986). Tissue slices of growth plate and articular cartilage used in our study demonstrate by scanning electron microscopy that this adherence can also be shown with an in vitro model. Vigorous washing techniques can be used with this model to ensure that the nonadherent bacteria are removed. Even after such washing, large numbers of bacteria in the form of clumps remained adhered to the cartilage surface. Attachment appeared to be the collagen meshwork where many bacteria penetrated through the surface. This closely parallels the in vivo morphologic evidence that we previously described (Speers & Nade 1985).

The use of an in vitro radiolabelled assay has advanced our understanding of adherence. We now know that viability of the bacteria is not necessary for adherence. The receptor present on the outer surface of the bacterium is most likely part of the glycocalyx, which completely surrounds the cell (Speers & Nade 1985), and appears to have a proteinaceous element. This receptor, which is sensitive to at least one protease, is also moderately heat resistant, surviving 60° C for 1 hour.

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