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# Pathogenesis of Infective Endocarditis

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## SUMMARY

In vitro studies were conducted to study the early steps of pathogenesis of infective endocarditis caused by *Staphylococcus epidermidis*, which is a significant pathogen in prosthetic valve endocarditis and nosocomial endocarditis.

*Staphylococcus epidermidis* binds to host tissues (pharyngeal epithelial cells, fibrin-platelet clots, cardiac cell lines, surgical sutures and tissue matrix proteins) by lipoteichoic acid present on the bacterial surface. The active component is lipid as deaceylation of LTA removes the adherence properties. The adherence is unrelated to surface charge, hydrophobicity, slime production, encapsulation, biotype and phage type of the strains used. The receptor on the host cells is a glycoprotein.

**Key words:** *S. epidermidis*, pathogenesis, endocarditis, bacterial adherence.

## Introduction

Infective endocarditis (IE) was first described by William Osler in 1885 in his Gustonian Lecture and stated that "The etiological, clinical and anatomical characters of the disease have been fairly well ascertained" (1). However, this statement was an overreach and the disease has been the subject of continuous study since then. There have been advances in the understanding of various aspects of IE. In 1910 Libman and Celler reported in their experience with over 3000 blood cultures, and stated that for IE "The absolute diagnosis must, for the present, rest on the culture of the blood" (2). Nearly a century later blood culture retains a pre-eminent role in the diagnosis of IE. There have been development of new histologic and molecular techniques for detection of intracellular and difficult-to-culture pathogens that cause the disease (3, 4). In 1940s, the basis of successful antibiotic therapy for IE had been established and mortality had decreased from 100% to 30%. The introduction of intracardiac surgery in the 1960s has assumed a central importance in the management of cases where medical therapy fails. Despite rapid medical progress the mortality remains high, approaching 40% at one year.

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The concepts in pathogenesis of IE based upon experimental findings in animal models of IE have been the subject of intense research in 1970s (5, 6). Most patients with IE have pre-existing valve abnormalities. The normal endothelium is resistant to colonization and infection by circulating bacteria. The primary event of IE is bacterial adherence to target tissues and involves both host tissue and bacterial factors. This is followed by establishment, persistence and microbial growth with local tissue damage and extension to adjacent tissues. Any endothelial lesion results in exposure of the underlying extracellular matrix proteins, production of tissue factors, deposition of fibrin and platelets and non-bacterial thrombotic endocarditis (NBTE) which acts as a nidus for bacterial adherence and colonization during transient bacteremia by even less virulent bacteria (e.g. streptococci). This is also possible on physically intact endothelium when virulent, invasive pathogens (e.g. *S. aureus*) can initiate similar process.

The epidemiology of IE is changing due to increase in lifespan, new predisposing factors and increase in number of nosocomial cases. The incidence of community - acquired native valve endocarditis is 1.7 - 6.2 cases per $10^5$ person-years in the developed countries. The risk for prosthetic valve endocarditis is 1 per cent at 12 month and 2-3 per cent at 60 months. Nosocomial endocarditis accounts for 7-29 per cent of all cases of IE seen at tertiary care hospitals (7).

This presentation is a study of various aspects of early events of IE: the physico-chemical basis of adherence of *S. epidermidis*, which has become a significant pathogen in cases of prosthetic valve endocarditis and nosocomial endocarditis (8).

## Results

A prospective study of 60 consecutive cases of IE was carried out during the period of May 1985 to December 1988[9]. There were 40 males and 20 females with a mean age of 28 years. Thirty patients were found to have an evidence of chronic rheumatic heart disease, 8 congenital heart disease, 5 mitral valve pro-lapse and 4 had prosthetic aortic valve. Thirteen patients had IE in a previously normal heart. Positive blood and or tissue cultures were obtained in 44 patients (73%). The commonest infecting organism was *Streptococcus viridans*. Early surgery was performed in 31 patients (21 had severe heart failure, 10 embolisation, 15 failure of medical therapy and 19 had large vegetations (more than 10 mm).

For studies on adherence, 8 clinically significant isolates of *S. epidermidis* obtained from blood cultures of the same number of patients of IE were included in the study. These strains were identified by Staph API System (Table 1). All were non-encapsulated, non-slime producers and did

**Table 1 : Characteristics of 8 strains of *S. epidermidis***

| *S. epidermidis* strain | Biotype | Phage type |
|---|---|---|
| CH1 | 6706113 | Untypable |
| CH2 | 6300113 | 63/138/245/336 |
| CH3 | 6300113 | 63/138/245/336 |
| CH4 | 6306113 | 63/138/245 |
| CH5 | 6706113 | Untypable |
| CH6 | 6300113 | Untypable |
| CH7 | 6300113 | 63/138/245 |
| CH8 | 6700113 | Untypable |

not agglutinate human, guinea pig, rabbit or sheep erythrocytes. These were stored in skim milk at -70°C in aliquots and used fresh for each experiment. Adherence assays were done to pharyngeal epithelial cells (PEC) with and without pre-treatment of bacteria and PEC with various enzymes. The results are shown in Tables 2, 3. Binding was mannose resistant and was not related to surface hydrophobicity and surface charge of bacteria. Adherence to epithelial cells was reduced four to tenfold ($P < 0.01$) on pretreatment of bacteria with lipase while neuraminidase, phospholipase C, trypsin, and sodium periodate did not alter their binding. The surface carbohydrate profile of bacteria was studied by monitoring adherence to Lectin-Sepharoses. The bacteria did not conform to any pattern, and there was no relation to strain variation or adherence property. The pretreatment of PEC with trypsin and sodium metaperiodate produced a marked reduction in bacterial binding, 3 to 25 fold, ($P < 0.01$), but neuraminidase, phospholipase C, and lipase did not have any such

**Table 2 : Effect of lipase pretreatment of *Staphylococcus epidermidis* adherence to pharyngeal epithelial cells**

| *S. epidermidis* strains | No. of bacteria adhering to each PEC | | |
|---|---|---|---|
| | Control bacteria | Lipase-treated bacteria | % Control adherence |
| 1 | 21.8 + 3.6 | 3.0 + 1.1 | 13.8 |
| 2 | 37.9 + 4.3 | 3.9 + 1.3 | 10.3 |
| 3 | 20.9 + 3.0 | 4.1 + 1.4 | 19.6 |
| 4 | 8.0 + 2.6 | 2.1 + 1.3 | 26.2 |
| 5 | 11.1 + 2.0 | 2.6 + 1.0 | 23.4 |
| 6 | 21.7 + 3.0 | 3.7 + 2.0 | 17.0 |
| 7 | 20.8 + 3.1 | 3.9 + 2.1 | 18.7 |
| 8 | 24.0 + 3.1 | 2.7 + 1.2 | 11.2 |

**Table 3 : Effect of periodate and trypsin treatment of human pharyngeal epithelial cells on *Staphylococcus epidermidis* adherence**

| *S. epidermidis* strains | No. of bacteria adhering to | | |
|---|---|---|---|
| | Control cells | Periodate-treated cells | Trypsinized cells |
| 1 | 21.1 ± 3.4 | 2.1 ± 1.7 (9.9) | 2.2 ± 1.4 (11.6) |
| 2 | 38.6 ± 4.7 | 1.5 ± 1.3 (3.9) | 1.7 ± 1.2 (4.5) |
| 3 | 21.6 ± 2.3 | 3.1 ± 1.4 (14.3) | 2.0 ± 1.3 (9.9) |
| 4 | 8.4 ± 2.7 | 2.9 ± 2.0 (34.5) | 2.4 ± 1.8 (30.4) |
| 5 | 11.3 ± 1.8 | 3.4 ± 1.6 (30.1) | 2.6 ± 1.4 (22.4) |
| 6 | 20.4 ± 3.5 | 3.2 ± 1.2 (15.7) | 3.1 ± 1.0 (14.5) |
| 7 | 21.3 ± 4.3 | 4.1 ± 1.4 (19.2) | 3.2 ± 1.6 (15.5) |
| 8 | 24.2 ± 3.9 | 4.0 ± 1.6 (16.5) | 4.2 ± 1.9 (17.9) |

Values represent the average number of adherent bacteria per epithelial cell ± standard error of the mean. Figures in parentheses are percentage of control adherence.

**Table 4 : Adherence of homologous and two heterologous strains of *Staphylococcus epidermidis* to PEC pretreated with LTA**

| *Treatment* (μg/ml) | Strains of *S. epidermidis* | | |
|---|---|---|---|
| | CH2 | CH4 | CH8 |
| Hemoglobin 100 | 0.2 ± 0.1[a] | 0.5 ± 0.2 | 0.3 ± 0.1 |
| Dl-α-glycerol-phosphate 100 | 0.5 ± 0.5 | 0.4 ± 0.4 | 0.6 ± 0.3 |
| LTA | | | |
| 3 | 9.2 ± 1.0 | 8.3 ± 0.8 | 3.2 ± 0.9 |
| 6 | 13.8 ± 1.0 | 21.4 ± 1.4 | 11.2 ± 1.3 |
| 12 | 25.4 ± 1.3 | 30.1 ± 1.6 | 20.3 ± 1.2 |
| 25 | 48.2 ± 1.1 | 51.3 ± 1.7 | 46.6 ± 0.9 |
| 50 | 84.4 ± 1.2 | 68.2 ± 0.9 | 73.2 ± 1.7 |
| 100 | 87.9 ± 0.5 | 81.3 ± 1.3 | 84.8 ± 1.4 |
| 200 | 84.7 ± 1.3 | 80.2 ± 1.0 | 78.6 ± 1.9 |

[a] All figures are percentage inhibition of adherence ± SD after treatment of epithelial cells with LTA derived from *S. epidermidis* CH2 strain.

effect. These findings provide evidence that the receptors on the surface of PEC are glycoprotein in nature, while the bacterial adhesin is a lipase-sensitive material (10).

**Table 5 : Inhibition of adherence of *Staphylococcus epidermidis* CH2 to PEC pretreated with LTA of *S. epidermidis, S. aureus* and *S. pyogenes***

| *Treatment* (μg/ml) | Prior treatment of PEC with LTA of | | |
|---|---|---|---|
| | *S. epidermidis* | *S. aureus* | *S. pyogenes* |
| Hemoglobin 100 | 0.2 ± 0.1[a] | 0.8 ± 0.4 | 0.8 ± 0.5 |
| Dl-α-glycerol-phosphate 100 | 0.5 ± 0.5 | 0.2 ± 0.2 | 0.6 ± 0.2 |
| LTA | | | |
| 3 | 9.2 ± 1.0 | 4.8 ± 0.1 | 4.3 ± 0.1 |
| 6 | 13.8 ± 1.0 | 8.8 ± 0.3 | 6.8 ± 0.3 |
| 12 | 25.4 ± 1.3 | 16.1 ± 1.0 | 1.0 ± 1.9 |
| 25 | 48.1 ± 1.1 | 32.1 ± 1.0 | 20.5 ± 1.8 |
| 50 | 84.4 ± 1.2 | 70.0 ± 1.5 | 51.2 ± 1.0 |
| 100 | 87.9 ± 0.5 | 71.7 ± 1.5 | 61.7 ± 1.5 |
| 200 | 84.7 ± 1.3 | 69.9 ± 1.9 | 56.8 ± 1.3 |

[a] All figures are percentage inhibition of adherence ± SD after treatment of epithelial cells with LTA derived from one of the three bacteria.

Further experiments were done to characterize the adhesin on the surface of bacteria. Lipoteichoic acid (LTA) was extracted, purified, characterized and then used for various blocking experiments. The results are shown in Tables, 4, 5, 6. These experiments show that prior treatment of pharyngeal epithelial cells with lipoteichoic acid derived from *Staphylococcus epidermidis* produced a marked inhibition of adherence of the homologous strain and two heterologous strains. The inhibition was dose-dependent and saturable with 100 μg/ml of LTA. However, pretreatment of PEC with deacylated LTA did not block the adherence of the three strains tested. A similar but less marked blocking effect on the adherence of *S. epidermidis* to PEC was also observed with LTAs derived from *S. aureus* and *Streptococcus pyogenes*. On treatment of bacteria with substances capable of binding to LTA, such as polyclonal mouse anti-LTA antibodies or with human albumin, a marked inhibition of bacterial adherence was observed. Immunofluorescence studies showed that anti-LTA antiserum bound readily to the surface of bacterial cells. These findings provide clear evidence that the lipid component of LTA located on the bacterial surface is centrally involved in the adherence of *S. epidermidis* to human mucosal cells (11).

**Table 6 : Adherence of pretreated *Staphylococcus epidermidis* to PEC**

| *Treatment* | % Inhibition of adherence of *S. epidermidis* | | |
|---|---|---|---|
| | CH2 | CH4 | CH8 |
| Human albumin (μg/ml) | | | |
| 1 | 15.0 ± 1.0 | 18.3 ± 0.9 | 12.4 ± 0.7 |
| 10 | 32.1 ± 1.2 | 28.4 ± 1.3 | 36.4 ± 1.6 |
| 100 | 81.2 ± 0.8 | 76.3 ± 1.4 | 68.4 ± 0.9 |
| 1000 | 87.5 ± 0.5 | 83.4 ± 1.1 | 84.3 ± 1.3 |
| Anti-LTA serum (Titer 1/12,800) | | | |
| 1:5 | 68.9 ± 1.2 | 58.1 ± 2.0 | 56.3 ± 1.7 |
| 1:10 | 56.6 ± 1.1 | 43.4 ± 1.8 | 40.6 ± 1.2 |
| 1:40 | 40.3 ± 1.5 | 30.3 ± 1.1 | 31.3 ± 1.4 |
| Anti-heat-killed serum (Titer 1/3200) | | | |
| 1:5 | 38.8 ± 1.5 | 30.3 ± 1.3 | 31.4 ± 0.9 |
| 1:10 | 28.9 ± 1.2 | 20.4 ± 1.0 | 20.3 ± 0.9 |
| 1:40 | 3.0 ± 1.0 | 5.3 ± 0.8 | 6.4 ± 0.9 |

Haemoglobin, dla-glycerophosphate and normal mouse serum were negative controls

The next set of similar experiments were done using fibrin-platelet clot as the

substrate. The results are shown in Tables 7, 8. The conclusions from these experiments were that adherence was

**Table 7 : Effect of pretreatment of *S. epidermidis* on adherence to platelet-rich clots**

| Pretreatment with | % Inhibition of adherence of *S. epidermidis* | |
|---|---|---|
| | CH2 | CH5 |
| Lipase (10 mg/ml) | 85.7 ± 0.9 | 78.5 ± 1.2 |
| Human albumin (µg/ml) | | |
| 0.1 | 6.9 ± 0.3 | 9.1 ± 0.7 |
| 1 | 10.7 ± 0.7 | 15.9 ± 1.0 |
| 10 | 28.6 ± 1.0 | 20.6 ± 1.2 |
| 100 | 68.7 ± 1.2 | 59.7 ± 1.3 |
| 1000 | 78.8 ± 1.0 | 75.5 ± 1.0 |
| Mouse anti-LTA serum | | |
| 1.5 | 73.6 ± 1.1 | 80.6 ± 1.3 |
| 1.10 | 50.1 ± 0.8 | 42.6 ± 0.9 |
| 1.40 | 30.6 ± 1.0 | 31.3 ± 1.4 |
| Normal mouse serum 1:5 | 7.6 ± 0.4 | 10.9 ± 0.7 |

reduced four-to six fold ($P < 0.001$) on pretreatment of bacteria with lipase, while neuraminidase, trypsin, phospholipase C, and sodium periodate did not alter their

**Table 8 : Adherence of *S. epidermidis* to platelet-rich clots pretreated with LTA and deacylated LTD**

| Pretreatment with (µg/ml) | | % Inhibition of adherence of *S. epidermidis* | |
|---|---|---|---|
| | | CH2 | CH5 |
| LTA | 3 | 6.4 ± 1.3 | 4.0 ± 0.3 |
| | 6 | 11.1 ± 1.6 | 7.1 ± 1.2 |
| | 12 | 20.3 ± 1.9 | 11.3 ± 1.2 |
| | 25 | 34.2 ± 3.7 | 29.2 ± 1.9 |
| | 50 | 60.4± 3.2 | 51.2± 2.1 |
| | 100 | 73.4 ± 2.9 | 66.7± 3.2 |
| | 200 | 76.3± 3.9 | 70.2± 3.6 |
| DLTA | 100 | 10.8± 1.0 | 6.6 ± 1.1 |

binding. Pretreatment of bacteria with substances known to bind lipoteichoic acid (LTA), such as human albumin and anti-LTA antibodies, also resulted in a four-fold ($P < 0.001$) reduction in adherence. Prior incubation of clots with free LTA, but not with deacylated LTA, produced a fourfold ($P< 0.001$) decrease in the adherence of homologous and heterologous strains of *S.*

**Table 9 : Adhesion of two strains of *S. epidermidis* to three cardiac cell lines**

| Cell Line | Number of bacteria adherent per well | |
|---|---|---|
| | CH2 | CH5 |
| Girardi | 3,712.8 ± 40.4 | 2,310.4 ± 47.9 |
| HR 9 | 192.7 ± 18.3 | 182.6 ± 11.7 |
| CPAE | 154.3 ± 10.7 | 110.1 ± 14.4 |

*epidermidis.* A similar reduction was also observed when LTAs derived from *Staphylococcus aureus* and *Streptococcus pyogenes* were used. These data provide evidence that the lipid moiety of LTA has a central role in the adherence of *S. epidermidis* to fibrin-platelet clots in vitro (12).

**Table 10. Adherence of bacteria to surgical sutures**

| Bacterial Strains | Adherent bacteria $X10^8$ per $cm^2$ (mean ±SD) | | | |
|---|---|---|---|---|
| | Poly-propylene | Polyester | Polyester coated with polybutylate | |
| | | | White | Green |
| *S. epidermidis* | | | | |
| 1 | 4.6 ± 0.7 | 11.6 ± 1.3 | 46.3 ± 4.4 | 45.1 ± 4.2 |
| 2 | 4.1 ± 0.5 | 12.5 ± 1.5 | 40.3 ± 3.4 | 41.2 ± 3.5 |
| 3 | 4.4 ± 0.5 | 14.3 ± 0.9 | 40.6 ± 3.1 | 41.4 ± 3.3 |
| 4 | 5.0 ± 0.4 | 10.4 ± 1.0 | 41.5 ± 3.6 | 40.7 ± 3.5 |
| *S. aureus* | | | | |
| 1 | 3.8 ± 0.4 | 11.3 ± 0.9 | 39.8 ± 3.3 | 40.4 ± 3.3 |
| 2 | 4.3 ± 0.4 | 12.0 ± 0.9 | 37.9 ± 3.5 | 37.4 ± 3.3 |
| *Strep. sanguis* | | | | |
| 1 | 3.9 ± 0.3 | 4.2 ± 0.6 | 5.1 ± 0.6 | 5.0 ± 3.3 |
| 2 | 3.6 ± 0.3 | 4.6 ± 0.5 | 5.3 ± 0.5 | 5.4 ± 0.6 |

The studies were extended to 3 cardiac cell lines (Tables 9, 10, 11). These clearly showed that LTA is an important though not the sole adhesin for bacterium-host cell receptor interaction in this experimental model (13).

**Table 11 : Adherence of 2 antibiotic-treated strains of *S. epidermidis* to pharyngeal pathelial cells**

| | Strain 2 | Strain 5 |
|---|---|---|
| *Cephalothin* | | |
| ¼ MIC | 83.3 (< 0.05) | 98.7 (NS) |
| ½ MIC | 54.7 (< 0.001) | 56.8 (0.001) |
| *Cloxacillin* | | |
| ¼ MIC | 141.7 (<0.01) | 64.3 (<0.001) |
| ½ MIC | 97.6 (NS) | 77.2 (<0.001) |
| *Vancomycin* | | |
| ¼ MIC | 81.0 (<0.05) | 55.1 (< 0.001) |
| ½ MIC | 21.8 (<0.001) | 15.1 (<0.001) |
| *Rifampicin* | | |
| ¼ MIC | 57.7 (<0.001) | 82.9 (< 0.05) |
| ½ MIC | 48.5 (<0.001) | 49.9 (<0.001) |

All values are expressed as the adhesion index with their p. values in brackets
NS= Difference not significant.

In-vitro bacterial binding to extracellular matrix proteins was studied and the results are shown in Table 12. *S. epidermidis* strains bind well to immobilized fibronectin, laminin, and vitronectin but not to fibrinogen and collagen IV (Author's unpublished data). The adherence of bacteria to sutures used in cardiac surgery was studied by in vitro quantitative determination with [$^3$H]-leucine-labeled *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus sanguis* (14). The adherence per unit area for staphylococci was least for monofilament polypropylene (prolene), 3 times higher ($p< 0.05$) for braided polyester (Mersiline) and greatest (10 times, $p< 0.005$) for braided polyester sutures coated with polybutylate (ethibond). Mean values for the adherence of streptococci were low for all the sutures. Sutures pretreated with human plasma showed a 12-37% increase in bacterial adherence. In view of these observations, it is suggested that: (a) the preferential adherence of staphylococci to intra-cardiac sutures may be one of the explanations for its being the commonest cause of early prosthetic valve endocarditis, (b) there is a need for a careful selection of sutures used in cardiac surgery and (c) the described *in vitro* assay for bacterial adherence may be used for monitoring the development of better designed sutures and the effect of incorporation of antibiotics in the sutures (14, 15).

**Table 12 : In vitro adhesion of 2 strains of *S. epidermidis* to extracellular matrix proteins**

| Immobilised | No. of adherent bacteria | |
|---|---|---|
| | $CH_2$ | $CH_5$ |
| Fibronectin | 28.6 ± 3.2 | 6.3 ± 1.2 |
| Laminin | 21.4 ± 3.1 | 9.6 ± 1.7 |
| Vitronectin | 16.2 ± 4.1 | 10.2 ± 1.1 |
| Fibrinogen | 3.0 ± 2.1 | 2.1 ± 0.3 |
| Collagen IV | 5.1 ± 1.3 | 4.0 ± 1.0 |

## Conclusions

The primary event in the pathogenesis of IE is bacterial adherence to host tissues. The present studies clearly show that *Staphylococcus epidermidis* is capable of adhering to pharyngeal epithelial cells, fibrin-platelet clots, tissue proteins, surgical sutures used in cardiac surgery and human cardiac cell line. The slime production, bacterial capsule, hydrophobicity, surface charge, mannose resistance and adherence

to lectin-sepharoses has no effect on the adherence property. The adhesin on the surface of staphylococci was identified to be lipoteichoic acid, the lipid being an essential component as deacylation caused loss of adherence. The receptor on the surface of host cells was found to be glycoprotein.

Lipoteichoic acid has been reported to be the principle adhesin of group A & B streptococci & *S. aureus* as well. LTA is complexed to proteins on bacterial surface in such a way that fatty acid ends of LTA molecules are exposed at the outer ends to interact with specific receptors on host tissues. LTA appears to be the principal adhesin of various pathogens that frequently cause IE.

Immunization against IE is impractical because of a variety of causative pathogens. However, common adherence factors seem to exist between the pathogens. LTA is a common adhesin, shared by streptococci and staphylococci, the most common cause of IE. Impregnation of LTA analogues in the prosthetic valves may be able to prevent prosthetic valve endocarditis. Anti-LTA vaccine is another alternative.

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