Virulence factors in streptococcal infective endocarditis

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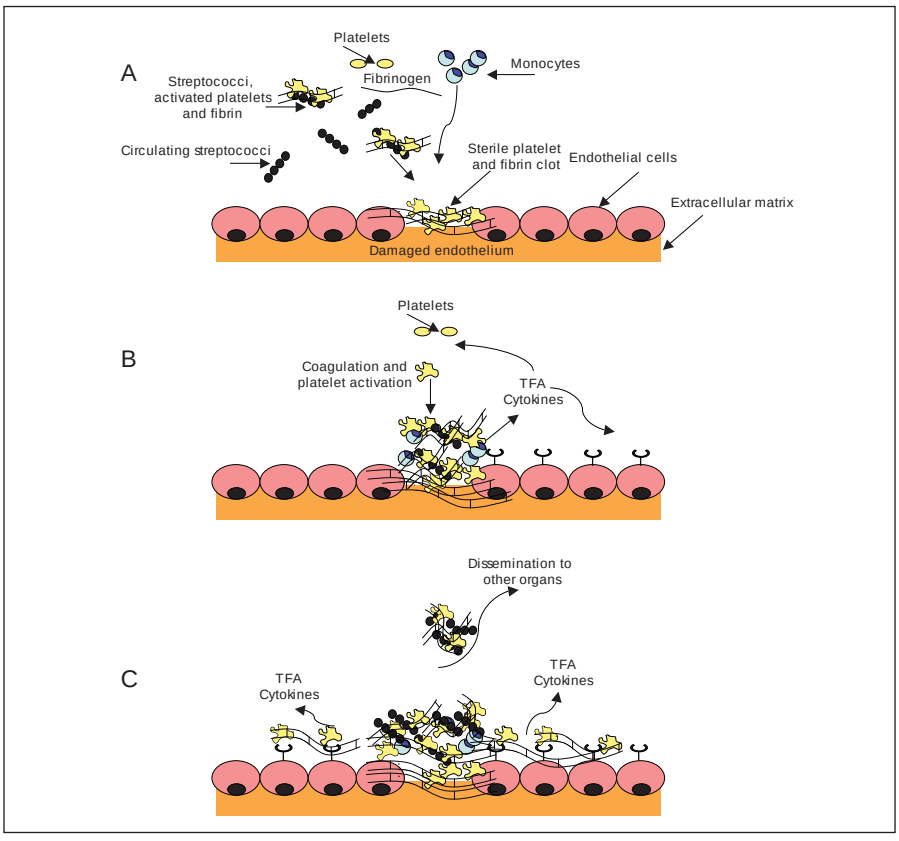
Infective endocarditis (IE) is a life threatening, endovascular infection occurring when bacteria enter the blood stream and adhere to heart valves. Mortality rates remain in the range of 11-27% [1,2]. The most common infecting micro-organisms are now the staphylococci (44%) although streptococci (31%) and particularly the oral streptococci (21%) are still major causative agents. Many different oral streptococci have been isolated from IE cases, the most common being *Streptococcus sanguinis, Streptococcus oralis, Streptococcus gordonii, Streptococcus mitis, Streptococcus anginosus* group and *mutans streptococci* [1,2].

The major predisposing factor in streptococcal IE is pre-existing structural damage to the heart valve exposing the underlying extracellular matrix (ECM) (Figure 1A). The exposed ECM triggers the activation of platelets and the polymerisation of fibrinogen to fibrin. The resultant vegetation contains large quantities of fibrinogen, fibrin, fibronectin, plasma and platelet proteins. Streptococci enter the blood stream through a transient bacteraemia. Circulating streptococci adhere directly to the damaged ECM or the vegetation and subsequent vegetation growth occurs through recruitment of more platelets, fibrin and plasma proteins as well as bacterial growth (Figure 1B). Adherent streptococci attract monocytes and induce them to produce tissue factor (TF) activity and cytokines. These mediators activate the coagulation cascade attracting more platelets and inducing cytokine, integrin and TF production (Figure 1B). Vegetation size is variable and the structures may be small and compact or loose and friable masses. Portions of the vegetation may break off from the main mass and can spread infection to other sites throughout the body (Figure 1C, Figure 2) [3].

Interactions with platelets

Streptococcal strains show three phenotypes in their interaction with platelets. Rapid aggregation with high adhesion (Type I); non-adhesive and longer lag times to aggregation (Type II); and non-aggregating (Type III) [3]. Herzberg [4] has proposed that three components on the surface of *S. sanguinis* strains directly interact with platelets. The platelet aggregation-associated protein (PAAP) is a rhamnose-rich glycoprotein and contains a collagen-like platelet-interactive domain which interacts with a signal-transducing receptor complex on platelets. The adhesin, GspB, binds to sialic acid residues on platelets and an-ecto-ATPase which converts ATP to ADP, a natural agonist of platelets [4]. In addition, a cell surface protein SrpA, a serine-rich glycoprotein, has been identified which mediates adhesion to platelets via platelet glycoprotein GPIb [5] (Table 1.). Platelets without GPIb (Bernard-Soulier disease) fail to aggregate with *S. sanguinis.* Platelet aggregation types I and II require fibrinogen binding to its platelet receptor GPIIb/IIIa. In this case the bacteria do not bind directly to GPIIb/IIIa as aggregation is not inhibited by GPIIb/IIIa antagonists.

Figure 1. Steps in the colonisation of the heart valve.



Also type II aggregation is dependent on IgG binding to the platelet low-affinity IgG receptor, FcγRIIA and complement assembly on the bacterial surface [3, 6]. The activation and aggregation of platelets in the presence of streptococci is part of the host's defence against infection resulting in the release of platelet microbicidal proteins (PMP). Streptococci isolated from the blood of IE cases have been found to be more resistant to killing by PMP than those isolated from non-IE bacteraemia indicating an important role for PMP in IE (Table 1.) [7].

Adhesins

The role of fibronectin binding in IE has been demonstrated using insertional mutagenesis to generate mutants with diminished fibronectin binding ability. In the animal model the infection rate for mutant strains, the vegetation weight and the number of organisms per vegetation were significantly reduced [8,9]. The FimA protein of *Streptococcus parasanguinis* has homologues widely spread among the oral streptococci and is able to bind to fibronectin. Vaccination with FimA produced antibodies in rats that protected against challenge by *S. mitis, S. mutans* and *Streptococcus salivarius,* although the ability of antibody to penetrate the endocarditis vegetation to reach embedded bacteria is limited. Coupled with the findings from a study with *Staphylococcus aureus* IE mutant strains that also differed in their ability to bind fibronectin, the data suggests that the adhesion of bacteria to fibronectin is important in the colonisation of the IE vegetation.

LraI family proteins and the *sloABCR* genes are constituents of operons encoding ATP-binding cassette transport systems. LraI has been identified as having adhesive functions, similar to FimA. The *sloABCR* genes are high affinity Mn and lower affinity Fe transporters, the loss of which causes a reduction in the rate of IE. Many streptococci have no requirement for either Mn or Fe for growth. However, under aerobic conditions Fe can generate toxic hydroxyl radicals by a Fenton-type reaction. Mn does not generate hydroxyl radicals and has been demonstrated to act as an antioxidant. Transport of Mn in IE may therefore protect against oxidative damage.

Sialic acid residues are found on many cellular surfaces. *S. gordonii* produces a high molecular weight protein, Hsa, containing a long serine-rich region. Adhesion of deletion mutants to platelets was significantly reduced, as was adhesion to neuraminidase treated platelets by the parent strain. Additionally, Hsa has also been implicated along with glucosyltransferases and the hydrophobicity/coaggregation proteins CshA and CshB in the invasion of endothelial cells by several species of streptococci (Table 1) [10]. Killing of endothelial cells during cell culture experiments was a function of peroxidogenesis and acidogenesis, but endothelial cells could be protected and invasion by streptococci promoted by increased buffering capacity and the addition of catalase [10].

S. sanguinis also produces heat and trypsin sensitive secreted components that induce human peripheral blood monocytes to synthesise pro-inflammatory cytokines. Two proteins have been isolated and one identified as a manganese dependent superoxide dismutase (SOD); the other protein could not be sequenced and is unknown. The activity of these proteins suggests that the protein or proteins were binding to the CD14/Toll-like receptor complex (Table 1.) [11].

Carbohydrate metabolism

To survive, streptococci must be able to acquire a source of carbohydrate for growth. Streptococci have a variety of glycosidases available to degrade the complex glycoproteins found enmeshed in vegetations. Their glycosidases have been shown to be up-regulated when grown in more neutral pH conditions found on a heart valve as opposed to the acid conditions found in dental plaque [12]. In vivo expression technology (IVET) has been employed in *S. gordonii* and identified four loci involved in the metabolism of β-glucosides which are expressed *in vivo* on heart valves (Table 1.) [13]. Three of the identified loci are phosphoenolpyruvate-dependent phosphotransferase systems (PTS) and the fourth, the *gom* locus, is involved in multiple sugar metabolism. The mammalian ECM is rich in glycosaminoglycans which contain beta-linked repeating sugar units. The *gom* locus contains 15 genes, including five sugar degrading enzymes; a fucosidase, two mannosidases, an *N*-acetyl-β-hexosaminidase and a β-glucosidase. The *N*-acetyl-β-hexosaminidase, *(bhsA* or

Figure 2. Cardiac vegetation from a case of Infective endocarditis. (Photograph courtesy of David Newell, Anatomical Pathology, Royal North Shore Hospital).

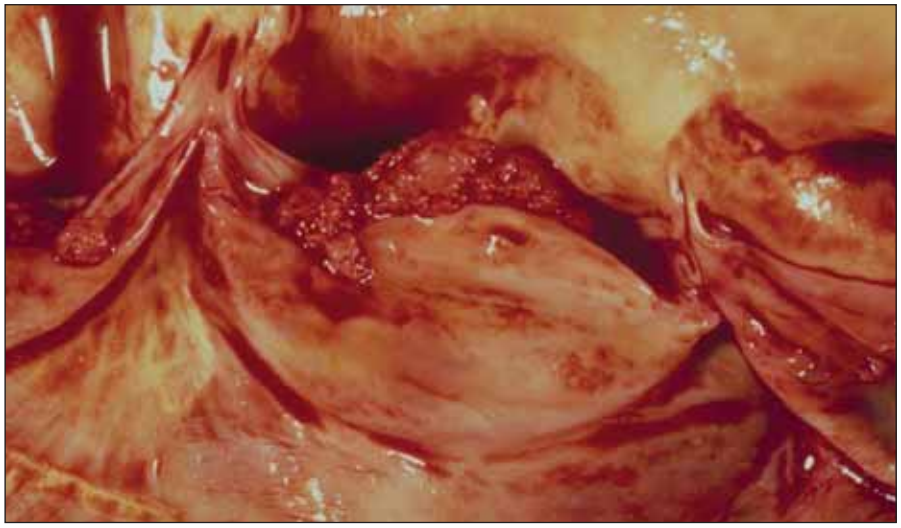


Table 1. Streptococcal Virulence factors.

Virulence Factor	Function
Platelet Microbicidal Protein Resistance (PMPr)	Resistance factor to killing by PMP.
GspB (and homologues)	Platelet adhesion, binds to α2-3-linked sialic acid residues.
PAAP	Platelet aggregation-associated protein.
Ecto-ATPase	Production of ADP a natural agonist for platelets
SrpA	GPIb binding protein, induces platelet aggregation in presence of fibrinogen.
IgG	Binds to platelets, induces platelet activation in the presence of streptococci.
Fibronectin binding protein	Adhesion to cellular fibronectin.
Exo-polysaccharide production – glucosyltransferase, fructosyltransferase.	Glycan synthesis. Invasion of endothelial cells.
FimA (and homologues)	Fimbrial adhesin. Adhesion to fibronectin and fibrin.
*slo*ABCR, LraI family	High affinity Mn and Fe transport system, adhesin. Either Mn or Fe required for growth in presence of oxygen. LraI (Lipoprotein receptor antigen) adhesin similar to FimA.
Hsa	Sialic acid binding protein. Invasion of endothelial cells, adhesion to platelets and polymorphonuclear leukocytes (PMM).
CshA and CshB	Hydrophobicity, coaggregation, invasion of endothelial cells.
Mn dependent SOD and unknown 190kDa protein.	Binding to CD14 /Toll-like receptor on monocytes, induction of pro-inflammatory cytokines.
Bgl, esc, bfb, gom, loci	β-glucoside metabolism. *In vitro* adhesion, biofilm formation, growth and *in vivo* colonization and bacterial growth.

gcnA) has been cloned, sequenced, inactivated and crystallised[14]. Inactivation of the gene had no significant effect on *in vitro* growth with a variety of carbohydrate substrates. The gom locus was only induced in vivo in the rabbit model of IE and not under *in vitro* conditions. This may mean that the correct substrate required for induction of this system has not yet been applied to *in vitro* growth conditions[13].

In addition to the above mechanisms, IE also leads to the destruction of the tissue underlying the heart valve and, as yet, these processes are not well understood. Infective endocarditis is therefore a multifactorial disease with many different virulence factors contributing to the progression of the disease.

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