New Concepts in the Pathophysiology of Infective Endocarditis

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Endocarditis pathogens colonize valves with pre-existing sterile vegetations or valves with minimal endothelial lesions. Inflamed endothelia produce cytokines, integrins, and tissue factor, which in turn attract fibronectin, monocytes, and platelets. Bacteria attaching to such structures further activate the cascade, becoming embedded and protected from host defenses. *Staphylococcus aureus* also actively invade the endothelium, causing apoptosis and endothelial damage. Knowledge of this interplay identifies host factors as potential therapeutic targets. Blocking infection by modulating host factors might be opportune because host factors are conserved. In contrast, interfering with bacterial virulence factors might be more complicated because they vary among different bacteria.

Introduction

Despite improvements in health care, the incidence of infective endocarditis (IE) (2–6 per 100,000 population per year) has not changed over the past two decades [1,2]. This apparent paradox results from progressive modifications in risk factors. Chronic rheumatic heart disease is now rare in industrialized countries. This group of at-risk patients has been replaced with new at-risk patients, including intravenous drug users, elderly people with valve sclerosis, patients with intravascular prostheses, patients exposed to nosocomial bacteremia, and hemodialysis patients [1–3]. As a result, the mean age of patients with IE has increased from 30 years in the 1950s to older than 60 years since the 1990s. IE involves both host and microbial factors. The principal actors of this *liaison dangereuse* are reviewed.

The Critical Role of Host Factors

The valve endothelium

The normal valve endothelium is very resistant to infection. However, mechanical lesions of this endothelium result in exposure of the underlying extracellular matrix proteins, local inflammation, and deposition of fibrin and platelets, which form a nonbacterial thrombotic endocarditis (NBTE) that is prone to bacterial colonization. Congenital cardiac abnormalities cause turbulent blood flow, which may provoke endothelial trauma. Valve scarring and calcification related to rheumatic carditis or valve sclerosis in elderly patients result in endothelial lesions. Degenerative valve lesions are detected in up to 50% of patients older than 60 years of age and may explain the increased risk for IE in the elderly [4]. Extrinsic intervention, such as prosthetic valve replacement or indwelling electrodes or catheters, also promotes endothelial lesions. In addition, *Chlamydia pneumoniae* or cytomegalovirus has been linked to atherosclerosis. Whether these conditions trigger endothelial lesions that promote IE is not known.

IE may also occur without identifiable pre-existing valve lesions. This is particularly true for *Staphylococcus aureus*, which has emerged as the leading cause of IE in recent surveys [1,2]. Local inflammation, which may occur in some circumstances, triggers endothelial cells to express a variety of molecules, including integrins of the β1 family (very late antigen). Integrins are transmembrane proteins that can connect extracellular determinants to the cellular cytoskeleton. Integrins of the β1 family bind circulating fibronectin to the endothelial surface. *S. aureus* and a few other IE pathogens carry fibronectin-binding proteins anchored to their walls. Thus, fibronectin bound by activated endothelia provides an adhesive surface to these circulating bacteria.

Inflammation and coagulation

Inflammation and coagulation are very closely related. Tissue factor (TF), which is a main player in coagulation and vegetation growth, is induced from monocytes and endothelial cells by interleukin (IL)-1 [5], which is itself produced in response to tissue injury. In turn, endothelial cells and monocytes produce proinflammatory cytokines and integrins that favor the construction of the NBTE

and thus the attachment of circulating bacteria. Once attached, microbes further maintain this vicious cycle that promotes growth of the vegetation, in which they are protected from host defenses. A synthetic view of this complex cycle is starting to emerge. However, although microbial characteristics may change according to the infecting species, the host counterparts are conserved. Therefore, critical host components could be convenient targets for adjuvant interventions to antibiotherapy.

Tissue factor

TF is a procoagulant integral membrane glycoprotein of 47 kD. It is produced on the surface of virtually all cells except for unperturbed endothelia and circulating blood cells [5]. When endothelial damage occurs, TF from underlying tissues reacts with factor VII and factor X, which cleave prothrombin to thrombin, which triggers the polymerization of fibrinogen into fibrin. TF also activates platelets, which are integral components of the vegetation [6]. Although unperturbed endothelia and monocytes do not produce TF, they can be induced to produce it by various agonists, including cytokines (IL-1) and bacterial products [5]. Intravegetation TF activity (TFA) was demonstrated in specimens from experimental IE due to *Streptococcus sanguis*, *S. aureus*, and *Staphylococcus epidermidis*, and the presence of TFA was associated with the presence of the microbes [7,8].

Induction of TFA from the host monocytes

Monocytes are present in the early vegetation, and their binding to fibrin clots elicits TFA in vitro [9]. Moreover, addition of *S. sanguis* to the system increases TFA approximately 10-fold. This occurred in spite of the fact that monocytes could not physically engulf and remove fibrin-adherent streptococci—a phenomenon referred to as "frustrated phagocytosis"—suggesting that some extrinsic bacterial factor was involved. Such microbial factors interacting with innate host defenses have been referred to as modulins. *S. aureus* and *S. epidermidis* can also induce TFA from monocytes [7,8]. Moreover, the contribution of monocytes to intravegetation TFA was confirmed in animals with etoposide-induced monocytopenia [7,8]. Thus, bacteria can encourage vegetation growth by subverting monocytes to produce TFA.

Induction of TFA from the host endothelium

Because *S. aureus* IE might develop on physically intact endothelia, the question arose as to whether these organisms can trigger local vegetation formation by direct induction of endothelial TFA. Veltrop et al. [7] showed that *S. aureus*, but not *S. epidermidis* and *S. sanguis*, could induce TFA expression from cultured endothelial cells. Treating these cells with IL-1 also induced TFA. However, abrogating the IL-1 response with IL-1-receptor antagonist did not abrogate TFA induction by *S. aureus*. Thus, IL-1 and *S. aureus* induce endothelial TFA by distinct mechanisms.

Induction of endothelial TFA with *S. epidermidis* and *S. sanguis* needed the additional presence of monocytes [10]. This required endothelial expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), which depend on pre-existing endothelial activation. Therefore, although *S. aureus* and IL-1 might be enough to initiate endothelial procoagulant activity, *S. epidermidis* and *S. sanguis* depend on a pre-existing endothelial stress (or damage) to trigger the same effect.

Cytokines

Recently, Dankert et al. [11••] highlighted the role of IL-1α in maintaining endothelial inflammation. In experimental endocarditis, the initial valve lesion is produced via a catheter inserted through the valve leaflets and left in place throughout the experiment. Bacteria injected thereafter infect the damaged valves. The authors observed that removal of the catheter 24 hours before bacterial challenge was enough to restore resistance to infection, presumably due to spontaneous endothelial repair. However, animals from which the catheter had been removed could be rendered susceptible to infection again by injection of IL-1α 3 hours prior to bacterial challenge. This 3-hour window corresponded to the time required for IL-1 to induce maximal TFA from endothelial cells in vitro. Thus, IL-1α could resensitize endothelia with minimal lesions to infection susceptibility. Systemic inflammation could be important in moderate valve anomalies as in early degenerative lesions, or following illicit drug injection where impurities are thought to produce minimal lesions to the leaflets' endothelium. Concurrent inflammation due to unrelated diseases could increase susceptibility to IE in these conditions. It would be important to know whether IL-1α has the same effect on nonprimed endothelia, but this is as yet uncertain.

The platelets paradox

Bacterial-induced platelet aggregation is commonly considered to be a pathogenic factor. However, platelets also contribute to anti-infective host defenses by releasing antimicrobial peptides and inflammatory mediators [6]. Platelets harbor three types of granules (α, δ, and λ), which release a variety of mediators involved in adhesion and coagulation (α granules), vascular tone (δ granules), and thrombus dissociation (λ granules) [6]. α Granules also contain an arsenal of antimicrobial peptides, collectively called platelet microbicidal proteins (PMPs), in rabbit platelets and thrombocidins in human platelets. PMPs represent one of the first host-defense barriers in IE, and are released upon platelet stimulation, for instance by thrombin.

Pathogenic role in streptococcal IE

S. sanguis has the ability to induce platelet aggregation via two surface-expressed antigens [12]. The class I antigen promotes streptococcal adhesion to platelets (Adh^+

Table 1. Potential *Staphylococcus aureus* MSCRAMMs belonging to the LPXTG-anchoring domain family of wall-associated proteins

Gene	Protein	Function	Potential implication in disease
spa	Protein A	Binds antibody Fc fragment	Experimental sepsis
			Experimental osteoarthritis
clfA	Clumping factor A	Binding to fibrinogen and platelets	Experimental endocarditis
clfB	Clumping factor B	Binding to fibrinogen	Not proven in endocarditis
cna	Collagen-binding protein	Binding to collagen	Experimental arthritis
fnbA	Fibronectin-binding protein A	Binding to fibronectin, fibrinogen, elastin	Experimental endocarditis
		Activates platelets	Cell invasion
fnbB	Fibronectin-binding protein B	Binding to fibronectin and fibrinogen	Undetermined
		Activates platelets	
sdrC	Serine-aspartate repeat protein	Binding to fibrinogen	Undetermined
sdrD	Serine-aspartate repeat protein	Possible binding to fibrinogen	Undetermined
sdrE	Serine-aspartate repeat protein	Possible binding to fibrinogen	Undetermined
pls	Plasmin-sensitive protein	Binding to nasal mucosal cells	Colonization of nasal mucosa
fmtB	Factor affecting methicillin resistance in the presence of Triton X-100	Putative cell-wall building	Affects the expression of methicillin resistance
sasA (sraP)	*S. aureus* surface protein A	Platelet binding	Possible in experimental endocarditis
sasB	*S. aureus* surface protein B	Undetermined	Undetermined
sasC	*S. aureus* surface protein C	Undetermined	Undetermined
sasE	*S. aureus* surface protein E	Implicated in transferring binding	Undetermined
sasF	*S. aureus* surface protein F	Undetermined	Undetermined
sasG	*S. aureus* surface protein G	Binding to nasal mucosal cells	Associated with invasive disease
sasH	*S. aureus* surface protein H	Undetermined	Associated with invasive disease
sasI	*S. aureus* surface protein I	Undetermined	Undetermined
sasJ	*S. aureus* surface protein J	Implicated in transferring binding	Undetermined
sasK	*S. aureus* surface protein K	Undetermined	Undetermined

MSCRAMMs—microbial surface component reacting with adhesive matrix molecules.
(*Adapted from* Siboo et al. [18], Que et al. [40••], and Roche et al. [44].)

phenotype). The class II antigen triggers further platelet aggregation (Agg^+ phenotype). This so-called platelet aggregation-associated protein contains a collagen-like platelet-interacting domain [12]. Studies indicated that an Agg^+ isolate of *S. sanguis* produced more severe infection and larger vegetations than an Agg^- control strain in experimental IE [13]. However, other studies did not find such a correlation. In these studies the Agg^- comparator strains were not characterized for other pro-endocarditis factors, or were less adherent to fibronectin, fibrinogen, and platelet-fibrin clots [13]. Moreover, not all IE streptococcal isolates are Agg^+, indicating that additional factors are involved [14].

Streptococcal-platelet binding was further characterized in *Streptococcus mitis* [15]. Binding was mediated by a complex interaction between at least three loci, *pblA*, *pblB*, and *pblT*. PblA and PblB are surface structures that are likely to act as adhesins. They are encoded by a lysogenic bacteriophage (SM1) of *S. mitis*, suggesting that they might disseminate to other pathogens. PblT is homologous to a membrane transporter of the major facilitator superfamily. Solute-binding proteins of such transport systems have been shown to mediate adherence. However, mutants in these loci have not been tested in experimental IE.

Pathogenic role in staphylococcal IE

S. aureus organisms bind platelets directly or indirectly via several surface proteins, including fibrinogen-binding and fibronectin-binding proteins, as well as a newly described serine-rich protein called SraP (Table 1) [16–19]. Adhesion of *S. aureus* to activated platelets may also occur via thrombospondin, von Willebrand factor, protein A, and/or C1q bridging.

Sullam et al. [20] compared an Agg$^+$ parent *S. aureus* to a Tn*551*-insertional mutant with decreased platelet-binding and aggregation in experimental endocarditis. The parent strain was up to 100-fold more infective than the mutant, as determined 48 hours after challenge. The organisms were comparable for other relevant virulence properties, including their susceptibility to platelet peptide-induced killing, and their ability to bind to fibrinogen and fibronectin. This provides strong evidence for the implication of *S. aureus*–induced platelet aggregation in IE pathogenesis. The same laboratory showed that fibrinogen-binding protein A (ClfA) was involved in this interaction.

The antimicrobial effects of platelets

Conversely, experimental IE due to an *S. sanguis* strain was more severe in thrombocytopenic rabbits than in control animals with normal platelet counts [21]. The infecting organism extensively aggregated platelets, but was rapidly killed by PMPs in vitro. Dankert et al. [22] confirmed the role of PMPs in experimental IE by using a series of PMP-susceptible and PMP-resistant streptococcal isolates. PMP-susceptible streptococci colonized more effectively damaged valves than PMP-resistant strains 5 minutes after bacterial challenge. However, they were rapidly eradicated from the vegetations during the following 24 to 48 hours, whereas PMP-resistant strains persisted. Therefore, although bacteria may subvert platelets to develop the vegetation, they need to resist PMP-induced killing to take advantage of this procoagulant effect.

The mechanism of action of PMP was clarified in *S. aureus*. Thrombin-induced PMP-1 (tPMP-1), the principal PMP released from rabbit platelets, is a cationic molecule of relatively small size (8.036 Da). It permeabilizes the cytoplasmic membrane in a voltage-dependent manner without classic pore formation [23], which is different from other types of bactericidal peptides. Recently, resistance to PMP-induced killing was associated with the *dltABC* and *mrpF* loci, the products that are responsible for alanylation of surface teichoic and lipoteichoic acids, and lysinylation of membrane phosphatidyl glycerol [24]. Both of these surface structures are positively charged. They are likely to repel the cationic PMPs and, thus, protect the bacterium.

Clinical implication of PMPs

Experimental studies support the protective role of PMPs in intravascular infections [21,22]. In humans, *S. aureus* isolates from intravascular infections were more often resistant to PMPs than those isolated from other types of infections [25]. PMPs may also play a role in therapy. tPMP-1 acted synergistically with several antibiotics—in particular, cell-wall active drugs—both in vitro and in vivo [14], and the effect was observed against both PMP-susceptible and PMP-resistant isolates. Thus, development of PMP analogues for therapy may be envisioned.

Because platelets are involved in IE, investigators explored the effect of adjuvant antiplatelet therapy in the disease. Limited clinical observations suggest that aspirin might prevent vegetation growth and decrease cerebral septic emboli in human IE [26]. Nicolau et al. [27] studied the effect of aspirin and ticlopidine, two antiplatelet drugs with different modes of action, in rabbits with *S. aureus* experimental IE. Both drugs synergistically decreased the vegetation size, and also moderately decreased vegetation bacterial titers. However, the PMP-resistance phenotype of the infecting organism was not reported.

Aspirin also significantly decreased vegetation weight, vegetation bacterial titers, and renal septic emboli in *S. aureus* experimental IE [28]. In these experiments, preexposure of platelets to aspirin and preexposure of *S. aureus* to salicylic acid (the principal metabolite of aspirin in vivo) decreased platelet adherence and aggregation as well as bacterial adherence to fibrin and fibrin-platelet matrices in vitro. One reason is that salicylic acid can block the expression of global regulator *agr*, thus altering the expression of *S. aureus* pathogenic factors [28]. Thus, aspirin acts both on the platelets and on the microbe. Experiments also indicated that aspirin, but not ticlopidine, was synergistic with vancomycin in increasing valve sterilization during experimental therapy. Whether this is also true for streptococci is as yet not determined. However, although platelet antiaggregants have a potential efficacy, extrapolating these experimental results to the human situation must be tempered by the risk of hemorrhage related to anticoagulation during acute IE [29].

The role of transient bacteremia

Medicosurgical procedures in nonsterile anatomic sites may provoke transient bacteremia. However, mastication and tooth brushing do so as well [30,31]. The duration of bacteremia after dental manipulation is short (≤ 15 minutes), and its magnitude is not very different during various dental procedures compared to normal activity [31]. It was reported to be 1 to 10 colony-forming units (CFU)/mL versus 10 CFU/mL during mastication and dental extraction, respectively [30]. It was estimated that the cumulative numbers of circulating bacteria (in CFU/mL/y) resulting from 1) mastication, 2) tooth brushing two times per day, and 3) dental examination were 5.6 million times, 154,000 times, and 48 times greater, respectively, than that provoked by a single tooth extraction [32]. This probably explains why most cases of IE are not preceded by medicosurgical procedures. Moreover, if the cumulative exposure to circulating bacteria is a greater risk factor for IE than the magnitude of bacteremia during dental procedures, then the recommendation for antibiotic prophylaxis should be revisited. This issue remains to be solved.

Besides bacteremia of dental origin, two increasingly frequent "health care–associated" bacteremia are of concern: bacteremia in hemodialysis patients and nosocomial

bacteremia. IE is up to three times more frequent in hemodialysis patients than in the general population [3] and more than 50% of the cases are due to *S. aureus*. Nosocomial IE accounted for 22% of 109 cases in one study [2]. Many patients had debilitating conditions, but less than 50% had known cardiac predisposing factors. The predominant pathogens were staphylococci and enterococci, which were frequently associated with catheters and/or medicosurgical procedures, and mortality was more than 50% [2]. It was estimated that more than 10% of nosocomial *S. aureus* bacteremia was responsible for subsequent IE. Therefore, these new conditions are important.

Microbial Factors

S. aureus, *Streptococcus* spp., and enterococci are responsible for more than 80% of all IE cases [1,2]. These organisms possess surface adhesins that mediate attachment to the vegetation and may modulate inflammation. These adhesins are collectively referred to as MSCRAMMs (microbial surface component reacting with adhesive matrix molecules) [33].

MSCRAMMs of viridans streptococci

Some streptococcal MSCRAMMs that were studied in experimental IE were reviewed [14]. Glucans are surface polysaccharides produced from dietary sucrose by bacterial glucosyltransferase (Gtf) and fructosyltransferase (Ftf) enzymes. Glucan production increases streptococcal adherence to damaged valves and fibrin-platelet clots, and triggers the production of IL-6 in vitro [34]. MSCRAMMs' role in experimental IE was demonstrated by using conditions favoring or not favoring the production of these surface polysaccharides, as well as in *gtf* and *ftf* inactivated mutants of *Streptococcus mutans*. However, *gtf* inactivation did not decrease experimental infectivity in all streptococci, indicating that glucans are not uniformly required.

FimA is a pathogenic factor in *Streptococcus parasanguis*. Its inactivation greatly decreased the ability of *S. parasanguis* to adhere to immobilized fibrin, and to cause IE in rats. Moreover, immunization of animals with FimA conferred protection against subsequent experimental IE with the original strain [35]. FimA belongs to a family of oral mucosal adhesins, including SsaB of *S. sanguis*, ScaA of *Streptococcus gordonii*, PsaA of *Streptococcus pneumoniae*, and EfaA of *Enterococcus faecalis*. FimA-like proteins were found by cross-hybridization in a number of streptococci, suggesting that immunization could confer cross-protection against several organisms. Further experiments are required to confirm this hypothesis.

MSCRAMMs of *S. aureus*

S. aureus is extremely well equipped with both MSCRAMMs and secreted factors that mediate tissue colonization and invasion. Most described MSCRAMMs (Table 1) are covalently attached to the peptidoglycan via sortase processing of LPXTG proteins [36]. Other protein MSCRAMMs are loosely attached to the wall, including coagulase, extracellular adherence protein [37], and the recently described autolysin Aaa [38]. Nonprotein adhesins that promote experimental IE include teichoic acids, which mediate binding to platelets and endothelial cells [24,39].

This intricate context makes it difficult to analyze the pathogenic role of individual gene products by classic gene-inactivation and gene-complementation experiments. To circumvent this problem, systems were developed to express individual staphylococcal determinants in surrogate bacteria missing the rest of the redundant staphylococcal environment. Using a system developed in *Lactococcus lactis*, Que et al. [40••] demonstrated that *S. aureus* ClfA was necessary and sufficient for early valve colonization and infection in rats with experimental IE, but not sufficient for invasive and persistent disease. Lactococci equipped with ClfA had a more than 100-fold increased ability to colonize damaged valve over the control parent. However, they were progressively eradicated from the vegetations within 72 hours [40••]. This spontaneous healing is not characteristic of IE and was most likely due to the susceptibility of *L. lactis* to PMP-induced killing.

In the same study, fibronectin-binding protein A (FnBPA) promoted both early valve colonization and persistent infection. FnBPA is a peculiar MSCRAMM carrying at least three binding specificities—to fibronectin, to fibrinogen, and to elastin [41–43]. Constructing truncated proteins expressing only specific FnBPA-binding regions confirmed that fibrinogen binding was necessary and sufficient for early valve colonization, as observed with ClfA. In addition, fibronectin binding was necessary for further invasion and persistence. This invasive phenotype was associated with the capacity of fibronectin binding to trigger active internalization of staphylococci into eukaryotic cells, both in vitro and in vivo [40••]. However, truncated FnBPA expressing only fibronectin binding was unable to colonize damaged valves at first, and required the simultaneous expression of fibrinogen binding to provoke infection [40••]. Moreover, cooperation between fibrinogen-binding and fibronectin binding could occur both in *cis*, when the two domains were present on the same protein as in FnBPA, and in *trans*, when both fibrinogen-binding and fibronectin-binding domains were present on two separate surface molecules simultaneously expressed by separate plasmids in lactococci [40••]. Thus, interadhesin cooperation might also occur between other MSCRAMMs, adding even more flexibility to the already wealthy set of *S. aureus* surface determinants.

Role of other protein-MSCRAMMs in *S. aureus* IE

S. aureus staphylococci carry up to 21 LPXTG-attached surface proteins, of which 10 have a known adhesin function

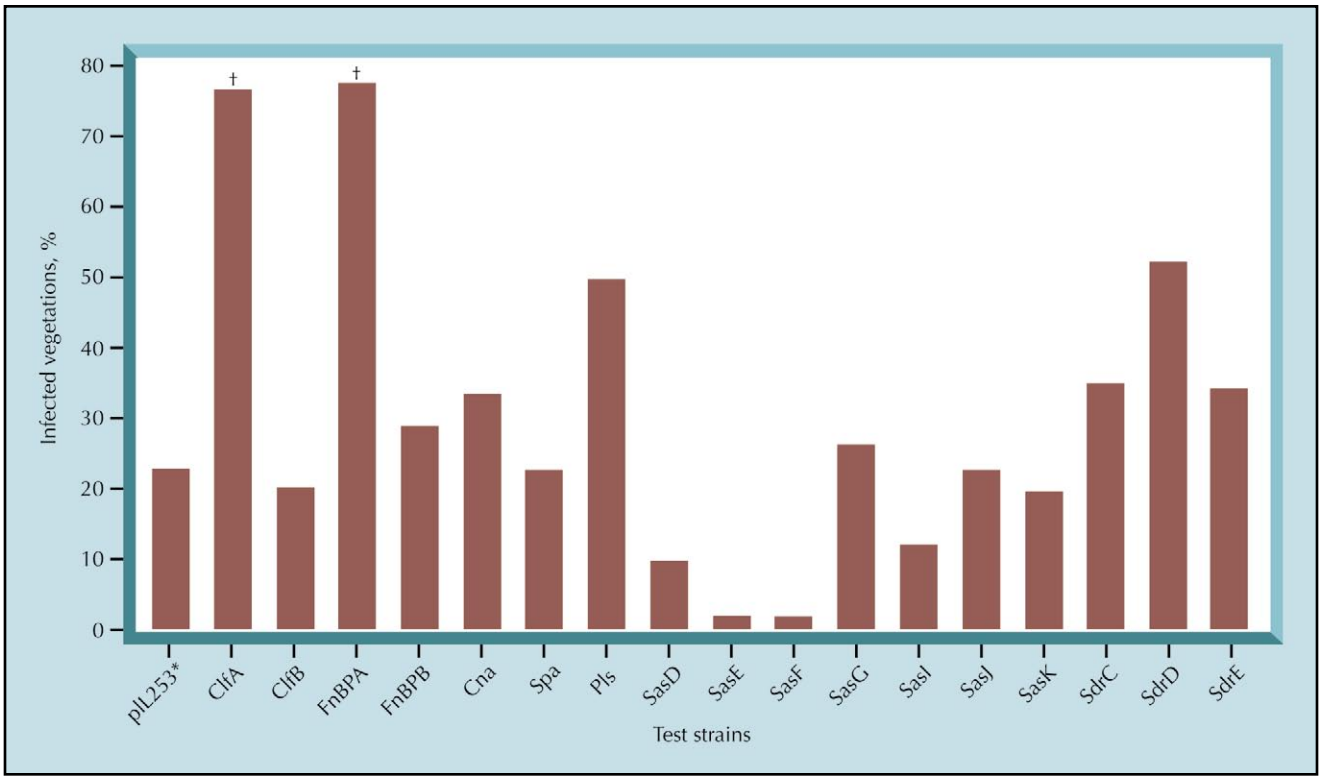

Figure 1. Induction of experimental endocarditis with lactococcal recombinants expressing 17 of the 21 LPXTG wall-attached proteins of *Staphylococcus aureus* (Table 1). Groups of 10 rats with catheter-induced aortic vegetations were challenged with 10^6 colony-forming units of each of the recombinant organisms, and the proportion of valve infection was assessed 16 hours later. The test strains are indicated at the bottom of each column. pIL253 (*asterisk*) stands for the control *Lactococcus lactis* carrying an empty expression vector. The other 17 abbreviations represent the name of the staphylococcal protein expressed by each of the tested recombinants. *Daggers* indicate that the percentage of infected vegetations was statistically different from that of the pIL253 control strain ($P < 0.05$) [47].

(Table 1) [44]. In ongoing work (Widmer et al., Unpublished data), we succeeded in cloning and expressing 17 of them in the lactococcal system (Fig. 1). Protein expression was assessed by mass spectrometry and the recombinant lactococci were tested for in vitro adherence and in vivo infection in rats with experimental IE (Fig. 1). Adherence tests confirmed known properties of ClfA, ClfB, FnBPA, FnBPB, and collagen-binding protein (Cna). In addition, new properties were detected, such as adherence to fibrinogen by plasmin-sensitive protein (Pls), and adherence to keratin by ClfA, FnBPA, and Sdr. However, in spite of these functional redundancies, only ClfA and FnBPA significantly ($P < 0.05$) promoted infection in experimental IE (Fig. 1).

It was surprising that ClfB and FnBPB, which both bind fibrinogen, did not promote valve infection. One hypothesis is that their fibrinogen-binding sites became saturated by soluble fibrinogen in the plasma before binding to the vegetation [45]. It is also relevant that ClfB did not affect experimental IE in *S. aureus*–deleted mutants. Nevertheless, although the contribution of other adhesins is not entirely excluded, these results underline the critical role of ClfA and FnBPA in endovascular infection.

Role of gene regulation

The expression of surface and secreted factors is orchestrated by the global regulators *agr* (accessory gene regulator), *sar* (staphylococcal accessory regulator), and other determinants, including *sigB* [46]. Together, *sar* and *agr* coordinately regulate the expression of adhesins during exponential growth and the secretion of soluble factors in the postexponential growth phase in vitro. Moreover, they were shown to be critical for infectivity in experimental IE and other models in vivo [47,48].

Regulation of adhesions

Inactivating *agr* alone decreased pathogenicity in experimental-model subcutaneous abscesses, where exoprotein production is likely to be important [48]. Nevertheless, *agr* inactivation did not have much influence on the course of experimental endocarditis, where bacterial surface adhesins are critical for valve colonization [47]. The logic to this result is that *agr* inhibits adhesin production in the late exponential phase, whereas the negative mutant does not. Therefore, although the negative mutant is hampered in exoprotein production, it is still fully equipped with surface-bound colonizing determinants. The effect of *agr* deletion could be partially restored by concomitant activation of the reciprocal regulator *rot*, thus highlighting the complexity of the network.

However, inactivating *sar* decreased infectivity in experimental IE because it also decreased the expression of surface adhesins [47], whereas overexpression of

sigmaB increased early infectivity because it increased the expression of fibrinogen binding [49]. This further underlines the role of fibrinogen binding in IE induction.

Regulation of exoproteins

Regulation of adhesin expression is important because it affects colonization. However, regulation of exoproteins and toxin expression is critical as well. As mentioned, FnBPA-positive lactococci or *S. aureus* can invade perivegetation endothelial cells after colonization. After endothelium invasion, the recombinant lactococci can divide, but mostly remained trapped inside the cells. In contrast, after internalization, *S. aureus* can lyse the cells and spread further [40••].

Cell lysis is mediated by hemolysins, which are positively regulated by *agr* [46]. Yet, although hemolysins are useful for escaping the host cell, they are dangerous when the bacteria become exposed to blood cells. Staphylococcal mutants overexpressing α-hemolysin produced less severe experimental IE than did parent strains, presumably because they lysed and activated platelets, which consequently released PMPs [50]—thus, the importance of hemolysin regulation, as hemolysins are only expressed at high cell density and are turned off in scattered growing cells [46].

Conclusions

IE is an exquisite model of bacteria–host interactions. At first glance, it is seems simple. Microbes equipped with specific adhesins can stick to pre-existing endovascular thrombi, in which they get embedded and are protected from host defenses. However, digging into these mechanisms indicates that the microbes are equipped with an array of determinants that do not only attach to extracellular matrix molecules but also directly to endothelia and platelets, which become activated and promote vegetation formation and growth. These interactions involve serial reactions with host components, including fibrinogen, fibronectin, von Willebrand factor, complement, and antibodies in the case of platelet activation. Bacteria can also invade endothelial cells, where they can either reside, thus evading host defenses and antibiotics, or escape to spread further after having lysed the host cell. Surviving the intracellular and extracellular milieus requires tight regulation of an array of pathogenic genes that are aimed at specific environmental-dependent tasks.

On the side of the host, monocytes, endothelial cells, and platelets coordinately reunify both healing and antimicrobial processes. It is likely that these reactions successfully abort numerous episodes of bacteremia and early valve colonization. This could explain the inoculum dependency of IE, where a threshold number of bacteria is required for successful infection. However, these healing and defense systems can also become subverted by the bacteria.

Current interventions against IE primarily target the microbes. Although this is sound, knowing the host players that are subverted might help identify parallel targets of the host components. These have the advantage of being conserved, as opposed to microbial factors that vary depending on the infecting organisms. They will include modulating inflammation, expression of intercellular adhesion molecules, and platelet functions.

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