Salicylic acid: an old dog, new tricks, and staphylococcal disease

Mathias Herrmann

Department of Bacteriology and Hygiene, Institute of Medical Microbiology and Hygiene, University of Saarland, Homburg/Saar, Germany

Aspirin has been shown to cause a reduction in the virulence of *Staphylococcus aureus*–associated endocarditis. A new study (see the related article beginning on page 222) reveals that salicylic acid, the major metabolite of aspirin, acts at the level of transcription to downregulate the production of fibrinogen, fibronectin, and α-hemolysin — virulence factors necessary for bacterial replication in host tissues and, now, potential therapeutic targets.

One hundred and twenty years after its initial description as the pathogen that causes sepsis and abscesses (1), *Staphylococcus aureus* remains a dangerous organism. Staphylococcal endocarditis is on the rise (2) and still causes significant mortality (3). The methicillin-resistant *S. aureus* (MRSA) epidemic has entered a new era due to the spread of MRSA into the community (4) and acquisition of new resistance cassettes with the potential for genetic transfer (5). The advent of fully vancomycin-resistant, methicillin-resistant clinical isolates (6) has further weakened the available armamentarium against this pathogen.

Importance of staphylococcal attachment and invasion in endovascular disease

*Staphylococcus aureus* is a nonmotile microorganism with a particular propensity to colonize biologic or artificial substrates using a battery of pathogenicity factors (7), allowing for specific bacterial attachment. This can be followed by cellular invasion and subsequent tissue degradation. Several lines of evidence clearly indicate that the interaction with host proteins and platelets is instrumental in the development of disease. A plethora of bacterial factors — either wall bound (8) or secreted (9, 10) — mediate binding of and attachment to ECM molecules such as fibronectin, fibrinogen, collagen, and vWF. Work with deletion mutants and complemented heterologous hosts has demonstrated the particular role of adhesins that recognize fibronectin (such as fibronectin-binding protein A) and fibrinogen (such as clumping factor A, ClfA), allowing for cellular invasion and production of experimental endocarditis (11, 12), and *gfp* reporter assays from endocarditis models clearly indicate that activation of global regulators that coordinate adhesin and toxin expression, such as *agy* and *sar*, occurs in vivo (13, 14).

Distinctive effects of acetylsalicylic acid and salicylic acid on platelets and bacteria

A particular role of platelets in the pathogenesis of staphylococcal endocarditis has been suggested since the early observation by Durack of bacterial interaction with fibrin-platelet matrices at sites of nonbacterial thrombotic endocarditis (15) and the series of reports by Clawson et al. on the interaction of *S. aureus* with purified platelets (16). In the early 1990s, experiments with surface-activated platelets suggested to our group the importance of fibronogen and *S. aureus* clumping factor in the bacteria–platelet interaction (17). These observations were subsequently confirmed and extended by use of a low-platelet-binding mutant expressing a mutated ClfA protein (18) that displays diminished virulence in an endocarditis model (19), and by identification of the secreted fibrinogen-binding proteins Coa and Efb in phage-display panning assays (20) (Figure 1).

While these observations pointed toward complex but, according to their adhesive function, rather propathogenic events at the bacteria–endocardium interface, the role of platelets had to be reevaluated after the discovery that they function as specialized inflammatory cells (21) in response to secretion of antimicrobial peptides. In fact, paradoxically, hyperexpression of α-toxin by *S. aureus* results in diminished virulence in experimental endocarditis, possibly because of the release of platelet microbicidal proteins (22).

The attributed role of platelets in the disease process that results in endovascular infection has prompted a number of researchers to interfere with platelet function for prevention or treatment of endocarditis. Acetylsalicylic acid (ASA, aspirin) has been used in vitro and in a number of experimental models to reduce vegetation sizes and to mitigate the course of disease (23–25). Similar effects have also been observed by Kupferwasser et al. (26). However, when they studied its metabolite, salicylic acid (SAL), in parallel to ASA, they made the interesting observation that pretreatment of bacteria with SAL reduced attachment to the valvular epithelium to an even greater extent than administration of ASA. This observation was accompanied by the in vivo finding that SAL-pretreated *S. aureus* cells bound to...
a lesser degree to platelets and to fibrin-platelet and fibrin matrices than did untreated bacteria and elicited platelet aggregation in a prolonged reaction time. Since SAL lacks the key effect of ASA on platelet function consisting of acetylation of the platelet COX-1, the observed in vivo attenuation and in vitro adherence studies delineated distinct effects of ASA and SAL on platelets and microorganisms, respectively.

**SAL modulates key regulatory steps in pathogenesis**

In this issue of the JCI, Kupferwasser and colleagues (27) extend these exciting findings. In a careful approach using a number of strains with functional gene regulator deletions and complementations in various genetic backgrounds, they demonstrated an acid stress–independent, SAL-mediated activation of the alternative staphylococcal stress response gene 

Figure 2

Potential roles of salicylic acid (SAL) in the pathogenesis of *S. aureus* endovascular infection. (I) SAL pretreatment of *S. aureus* results in overexpression of SigB-dependent genes in both an rsbU-positive and an rsbL-negative background, suggesting SigB activation independent of the anti-α-factor RsbU. (II) Activation of the sarAP3 promotor appears to contribute to decreased expression of active SarA protein, putatively via inhibitory activity of the sarAP3 gene product. Again, SAL appears to contribute to SarA reduction in a sarAP3-additive fashion. (III) As SarA controls expression of RNAII and RNAIII, the net effect of SAL is a mitigation of the *agr* response. Since α-toxin (*hla*) expression depends on both *sar* and *agr*, and expression of wall-bound adhesins is also in part controlled by *sar*, the mitigation of both adhesin and toxin expression in SAL-treated microorganisms appears to depend on a combined *sar-agr* effect; yet other regulators may also be involved. (IV) In addition to *hla*, the expression of other secreted molecules such as the extracellular adhesive protein Eap depends on *agr* and *sar* and may be downregulated under the influence of SAL. Expression of the polysaccharide intracellular adhesin (PIA) is suggested to depend on SigB, which may positively control expression of icaADBC, with consequences not yet fully understood in *S. aureus* endovascular infection. The *S. aureus* adhesins (FnBPA, FnBPB, ClfA, and ClfB) recognize fibronectin and fibrinogen, which are presented on ECs and platelets.

Potential directions of future research

The study by Kupferwasser et al. (27) leaves a number of open questions.
Firstly, is there any role of SAL in established endocarditis? As downregulation of attachment factors may be of prime importance to prevent initial steps of pathogenesis, SAL may come too late for a salutary effect in treatment. In fact, the above-mentioned “paradoxical” effect due to hyperexpression of α-hemolysin may be abrogated, resulting in diminished release of platelet microbicidal proteins. Secondly, how do the findings regarding agr suppression relate to the findings of others, that in a serum milieu (32) or in established infection (33), agr expression is already largely diminished, and other regulators such as sae may play a more prominent role in the in vivo infection? Lastly, what is the mechanism of the effect of SAL on S. aureus regulation? SAL is known to exert a multitude of effects on various eukaryotic and prokaryotic cells. More specifically, SAL treatment enhances resistance of S. aureus to fluoroquinolones and fusidic acid. Even more interesting, SAL inhibits biofilm production in Staphylococcus epidermidis (34), apparently because of multiple effects on proteaceous and non-proteaceous cell wall and cell surface components (35). Biofilm production in S. aureus (36) and S. epidermidis (37) has been demonstrated to depend on the icaADBC gene cluster that confers production of the polysaccharide intercellular adhesin (PIA, also known as PS/A). Expression of the icaADBC gene cluster is environmentally controlled and, at least in part, regulated by sigB. sigB expression, on the other hand, is controlled by a cascade of sgg activators and inhibitors (38).

The observations by Kupferwasser et al. (27) shed substantially more light on the patchwork of information concerning the effect of SAL on staphylococci, and they relate it to its potential as a therapeutic compound. Given this exciting new prospect for a widely used and established drug, additional research into the molecular events that result from staphylococcal exposure to SAL is now warranted.

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