Pathogenesis of Staphylococcal Infection: A Manner of Expression

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(See the article by Loughman et al., on pages 294–301.)

Staphylococcus aureus owes its success as a pathogen in humans to a variety of mechanisms that allow it to thwart, perturb, and evade host defenses [1]. The genes encoding virulence factors, which are not required for structural integrity or for growth or replication, are often located on mobile genetic elements, such as a prophage, pathogenicity island, or plasmid [1, 2]. Some factors (e.g., protein A) are present in virtually all strains, but the presence of others varies considerably from strain to strain. These genes, despite being exogenously acquired, are regulated and highly integrated into the overall cellular program. Knowledge of which genes are turned on and the circumstances under which they are activated is critical for understanding their role in pathogenesis. Much has been learned from in vitro analyses of cells cultured in artificial media. Although such information is extremely useful for characterizing the conditions that affect virulence gene expression, the real clinical question is what is happening at the actual site of infection in humans. Animal models of infection have been used to bridge this gap between the test tube and human infection, but the applicability of these models to human disease is open for discussion because of lack of data from clinical specimens.

In this issue of the Journal, Loughman et al. [3] report their analyses of virulence gene expression in S. aureus cells in pus from cutaneous abscesses (which term “superficial infection”) and from subperiosteal abscesses (i.e., invasive infections) collected from children with community-acquired S. aureus infection. RNA transcript levels were assayed by real-time reverse-transcriptase polymerase chain reaction to determine expression for a panel of virulence genes, some of which (i.e., lukS-PV, lukE, hlgB, hla, spa, and RNAIII) are known to be regulated by agr (accessory gene regulator) and some (i.e., bsaB and arcA) by an unknown regulator [4, 5]. Panton-Valentine leukocidin and arginine catabolic mobile element (encoded by arcA) are considered to be important in human infections caused by the USA300 clone of community-associated methicillin-resistant S. aureus [6, 7]. Two technical points about this approach are worth mentioning. First, transcript levels are average values that obscure the true complexity of responses within a mixed, very heterogeneous population of cells at the site of infection. This may account for the wide scatter of individual data points in some of the figures in their article. Second, the transcript level is not reported; rather, data are the ratio of the transcript level in a population of cells from pus or under specific growth conditions divided by the transcript level in a comparator population of cells. For this study, the comparator population is always one of the following: cells in the stationary-growth phase, grown in tryptic soy broth to an OD of 1.0; cells in the exponential-growth phase, grown in tryptic soy broth to an OD of 0.3; or cells in the stationary-growth phase, washed and transferred to the RPMI cell-culture medium used to conduct polymorphonuclear leukocyte experiments. This must be kept in mind when interpreting the experimental data reported by Loughman and colleagues.

Compared with cells harvested at an OD of 0.3, the expected expression profile of agr autoinduction for cells grown in broth to an OD of 1.0 is a high level of RNAIII; high levels of transcripts for genes encoding exoproteins (e.g., lukS-PV, lukE, hlgB, and hla), which are positively regulated by RNAIII; and low levels of genes encoding surface proteins (e.g., spa), which are negatively regulated by RNAIII. This is essentially what was observed for clinical isolates grown in broth (figures 2A and 3A) and for cells in cutaneous abscesses (figures 1 and 2B), recalling that, in figure 1, RNAIII levels are expressed relative to stationary-phase cells in which RNAIII is up-regulated. Proof that staphylococcal abscess forma-
tion in humans is driven principally by agr is important because it confirms results of animal infection models, which have shown that an intact agr response is required for abscess formation [8], and suggests that a clinical benefit might be gained from blocking this pathway.

Although the study by Loughman et al. [3] may, as they claim, represent the first demonstration of staphylococcal gene expression and regulation directly in human tissue, it is not the first study to have demonstrated staphylococcal gene expression and regulation directly in human tissue, it is not the first study to have looked for it. Goerke et al. [9] assayed sputum specimens from patients with cystic fibrosis who were colonized with S. aureus for RNAIII, hla transcripts (i.e., α-toxin), and spa transcripts (i.e., protein A). They found a low level or sporadic expression of all 3 and concluded that agr was nonessential in cystic fibrosis. S. aureus strains isolated from sputum of patients with cystic fibrosis, however, are defective in agr and virulence gene expression and have attenuated virulence [10]. These differences in agr and virulence gene expression for bacteria during acute infection, such as abscess, and during chronic infection or colonization, in the case of airways in cystic fibrosis, underscores the critical role that host factors and site of infection can have and the flexibility that pathogens have when implementing their virulence programs.

This flexibility is demonstrated by the other major finding of this study that, as in animal models of infection, agr by itself does not completely explain pathogenesis of staphylococcal infection in humans. For example, for cutaneous abscesses, the ratio of exoprotein transcript levels is, on average, 10 times that for stationary cells in culture, even though the average ratio for RNAIII is 1 (i.e., the RNAIII levels are the same in both cell populations) or slightly less. More striking is the difference in protein A gene expression, which is much higher in the population of cells from deep, subperiosteal abscesses (invasive infection) than cells from cutaneous abscesses (superficial infection), even though agr is activated on the basis of RNAIII levels and high levels of exoprotein gene transcripts. The expected effect of agr activation is down-regulation of protein A gene expression. This apparent uncoupling of protein A and agr raises 2 questions: what mechanisms are responsible for over-expression of protein A, and why does this occur in deep but not superficial abscesses?

Although conceptually useful, the notion that a regulatory pathway is controlling expression of virulence genes is a gross oversimplification. A network of numerous global regulatory pathways including agr, sarA, sarS, sarT, sarX, rot, and mgrA and the 2-component systems srrAB, sasRS, and arlRS determines expression of protein A [11–15]. Given this tremendous range of possible responses at the organism’s disposal site, specificity of virulence gene expression depending on location and stage of infection in the host should come as no surprise.

As to the role that protein A is playing, this molecule can interfere with innate host defense in several ways other than its well-known antiopsonic activity due to binding the Fc portion of immunoglobulin. It activates TNFR1, the receptor for TNF-α; it interacts with the epidermal growth factor receptor; it activates platelets by binding von Willebrand factor; and it is a superantigen for B cells [16–20]. It has an important role in other invasive infections, staphylococcal pneumonia, and septic arthritis [21, 22]. Increased expression of protein A in deep but not superficial abscesses suggests that it is important in the pathogenesis of invasive infection in general.

The erosion in the armamentarium of antibiotics has prompted a search for other ways to prevent and treat serious staphylococcal infections. The sheer number of virulence factors, their multiple mechanisms for injuring the host and for avoiding host defenses, and the complexities of the regulatory network that determines which, where, and when these factors are produced make this a daunting challenge. Hopefully, as more is learned about gene regulation and expression in human infections, the list will be pared down to a manageable panel of factors that play a central role in human disease and that can be successfully targeted. It is likely that such information also will confirm much of what is known on the basis of animal infection models. These will be essential tools for developing new strategies and approaches to treat serious staphylococcal infections.

References
11. Oscarsson J, Harlos C, Arvidson S. Regulatory role of proteins binding to the spa (protein A) and sarS (staphylococcal accessory


