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Streptococcus pyogenes under pressure

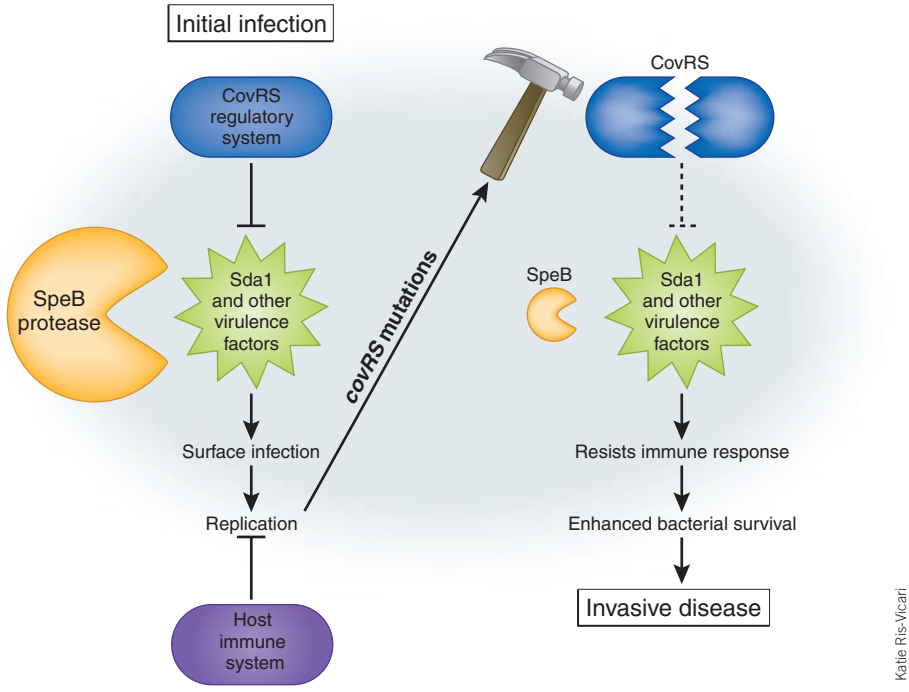
Claire Turner & Shiranee Sriskandan

Contemporary M1 strains of *Streptococcus pyogenes* have acquired a DNase gene that improves the virulence of the bacterium, but its expression is repressed by the CovRS regulatory system. Walker *et al.* report that the bacteria are under selective pressure to mutate the *covRS* locus to maintain DNase expression for invasive infection (pages 982–986).

It may seem surprising that the acquisition of a single gene might exert selective pressure sufficient to force global phenotypic change in a bacterium. In this issue, Walker *et al.* report that contemporary *Streptococcus pyogenes* isolates with a recently acquired DNase gene adopt a ruthless strategy to protect expression of this new recruit, by sacrificing an important regulatory gene[1].

S. pyogenes causes disease ranging from surface infections to deep tissue invasive infections, such as necrotizing fasciitis ('flesh-eating bacteria') and septic shock. During invasive disease, *S. pyogenes* upregulates expression of a number of genes in order to survive phagocytic killing by human neutrophils[2]. The control of virulence regulatory sensor (CovRS) system has a pivotal role during invasive infection[2], by regulating the critical virulence factors that combat the immune response[3].

In recent decades, M1 serotype *S. pyogenes* strains have emerged as the most common cause of invasive disease in many developed countries. During invasive infections, contemporary M1 isolates can develop mutations within the *covRS* regulatory locus. These mutations release bacterial virulence factors from repression, allowing the bacteria to overcome the neutrophil immune response and aid their dissemination within the host[2]. Following the mutation of CovRS, the transition from non-invasive to invasive disease isolate is marked by upregulation of an array of virulence genes but also simultaneous downregulation of a streptococcal protease, SpeB[2]. Walker and colleagues show that *covRS* mutations are selectively enriched during infection, but only in *S. pyogenes* strains that express the Sda1 DNase[1]. The authors suggest that the Sda1 DNase provides the sole driving force for this important mutation.



Katie Ris-Vicari

Figure 1 Model explaining mutation of *Streptococcus pyogenes* regulatory locus during invasive infection. Bacterial virulence factors are needed to resist killing by the host and allow the bacteria to replicate and survive *in vivo*. The CovRS regulatory system, however, represses the expression of these virulence factors. The SpeB protease can also degrade virulence factors, further diminishing invasiveness. During replication, mutations arise in bacteria: those that favor survival in a particular niche will be selected. During invasive infection, mutations that increase resistance to killing by the host will be favored. Mutations in the *covRS* locus allow enhanced production of virulence factors needed for resistance to phagocytic killing and invasive infection.

Phagocytic killing of Gram-positive bacterial pathogens such as *S. pyogenes* requires deposition of complement on the bacterial surface, specific antibodies directed against bacterial surface proteins and effective recruitment of neutrophils to the site of infection. *S. pyogenes*, however, has an array of mechanisms to evade host neutrophil defenses, including M protein to counter phagocytosis, a capsule of hyaluronic acid to evade immune cells, and cell envelope proteinases to cleave neutrophil chemoattractants. Recently, streptococcal DNases were reported to also play a

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role in combating neutrophil-mediated bacterial killing by degrading neutrophil extracellular traps (NETS), which are strands of extracellular DNA with antimicrobial activity that project from the neutrophil surface[4,5]. The CovRS system is able to repress all of these genes, although the sensor triggers for Cov activation and de-activation remain poorly characterized[2].

Walker *et al.* showed that an *S. pyogenes* strain that had a functional *sda1* DNase gene underwent mutation of *covRS* during an *in vivo* infection[1]. They found that an isogenic strain without a functional *sda1* gene, however, did not undergo the same mutation. When the *sda1* gene was replaced, the *in vivo* susceptibility to *covRS* mutation returned. Put simply, during *in vivo* infection, selection for the *covRS* mutation was completely dependent on the presence of a functional *sda1* DNase gene.

Why might the *sda1* gene be so influential? In contemporary M1 isolates, Sda1 is the principal DNase and, in addition to interfering with antimicrobial NETs, can increase overall virulence in invasive disease[4,5]. Mutation of *covRS* increases activity of Sda1 in two ways: by de-repression of *sda1* transcription and by switching off transcription of the gene encoding the SpeB protease that would degrade Sda1 protein[2,6]. Thus, mutations in *covRS* may be selected in order to preserve critical Sda1 activity during host invasion by *S. pyogenes* (**Fig. 1**).

In contrast to the fivefold increase in Sda1 expression, SpeB protease expression was downregulated by a factor of 10,000 in the strain that had undergone *covRS* mutation[1]. Downregulation of the SpeB protease may further benefit the bacterium in invasive disease, as SpeB is known to degrade a number of streptococcal factors that are critical to virulence[6,7]. Whether the *covRS* mutations are beneficial because they increase the transcription of anti-phagocytic factors, or because they switch off SpeB protease expression, remains unclear.

Adaptation of *S. pyogenes* to escape the immune response and promote invasive infection is not a new concept. Decades ago, medical microbiologists noted increased encapsulation of *S. pyogenes* isolates obtained from patient blood cultures. More recently, blood culture isolates were reported to have increased SpyCEP activity[8] to battle neutrophil recruitment. A genetic basis for the phenotypic change observed in capsule expression was first reported by Engleberg *et al.*, who described mutations in *covRS* arising *in vivo*[9]. More recently, Sumby *et al.* demonstrated that mutations in *covRS* arose in contemporary M1 isolates passaged through mice. These mutations affected an array of virulence factors, including, increasing strain invasiveness[2]. Interestingly, Walker *et al.*[1] and Sumby *et al.*[2] report various mutations in *covS*, suggesting that there is no single 'hot spot' for mutation in this regulatory locus. It is not known, however, whether mutations resulting in truncation of the sensor kinase CovS have greater impact than single amino acid changes. Mutations in the response regulator CovR may have an even greater effect[10]. What's more, the basis for the dramatic and seemingly complete shut down of SpeB protease resulting from *covRS* mutations remains unclear.

The findings of Walker *et al.* raise several more questions. *S. pyogenes* classically expresses up to four serologically distinct DNases A–D. Sda1 is serologically similar to DNase D (ref. 4), and may represent the return of an evolutionarily older DNase. If Sda1 is a recently acquired phage-derived DNase and exerts the selective force required for the *covRS* mutation, is the pressure on *covRS* a purely modern phenomenon restricted to M1 strains with Sda1? Observations from other streptococcal serotypes would suggest not[10]. *covRS* mutations are beneficial even to strains without Sda1 DNase. In those strains, other virulence factors that are repressed by CovRS and are known to influence bacterial clearance could act as the driving force for *covRS* mutation.

Finally, Walker *et al.* showed that *covRS* mutations can arise *in vivo*, enhancing virulence in wild type strains that have *sda1*, but do not arise when the *sda1* gene is mutated[1]. This raises the rather worrying possibility that specific invasive properties attributed to a virulence gene may, in fact, result not solely from that gene, but from the pleiotropic effects of an associated regulatory gene mutation. The potential impact of the *covRS* mutation in *S. pyogenes* virulence should not be underestimated, given that the mutation affects over 10% of the transcriptome and potentially all isolates may acquire mutations in the locus during *in vivo* passage[1,2]. The difference in pathology observed between infections caused by wild-type strains and isogenic mutants thus not only may stem from loss of one gene product, but may be compounded by a failure to undergo mutation of *covRS*. Therefore, the decrease in virulence may be exaggerated. And, as shown by Walker *et al.*, the process of genetic complementation will not help identify which possibility is true[1]. Only careful comparison of strains going into and coming out of a host will identify the extent to which this is—and has been—a problem.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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