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Wiring the Streptococcal Network for Alternative Lifestyles

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β-hemolytic group A streptococci (GAS) commonly cause infection at the epithelium of the throat or skin of the human host, leading to pharyngitis and non-bullous impetigo, respectively. On rare occasion, the organism gains access to deeper tissue, whereupon it can give rise to a bacteremia without focus, or cause a localized infection (eg., cellulitis) from which it sometimes undergoes systemic spread. The report in this issue by Kansal et al. [1] describes the alternative lifestyles of GAS for invasive infections that are localized versus systemic, and demonstrates the molecular basis for a phenotypic switch that permits the organism to shift from one lifestyle to the other. Characterization of the bacterial factors that are required for different disease states is paramount to understanding invasive disease by GAS, and may also be relevant to streptococcal pathogens of other species.

The resurgence of invasive disease by GAS is partly due to the emergence and global spread of a new strain of M-type 1 (M1) organisms over the past 20 to 30 years [2-4]. During a recent four year period in the USA, M1 isolates accounted for 22% of invasive disease isolates [5], a substantial fraction of the total, considering that > 200 *emm* types have been identified throughout the world [6]. An early clue concerning the molecular basis for recent M1 invasive disease comes from an epidemiological study by Kansal et al. [7] showing an inverse relationship between GAS production of the secreted cysteine protease SpeB, and severe invasive disease. Specifically, M1 organisms recovered from non-severe cases of soft tissue infection and bacteremia are high producers of SpeB, whereas M1 isolates from cases of streptococcal toxic shock syndrome (STSS) display significantly lower SpeB production. Thus, different GAS diseases are associated with distinct and stable phenotypes. SpeB functions by degrading numerous host components and extracellular proteins of GAS, such as M protein, the superantigen SpeA, and the plasminogen activator streptokinase.

The epidemiological findings [7] are supported by experiments demonstrating a phenotypic switch in M1 strains of GAS following passage in mouse-implanted microporous tissue chambers, a model system that aims to mimic a localized infection [8]. The input wild type (WT) strains produce large amounts of SpeB; however, after mouse passage, SpeB production is suppressed among the surviving bacteria whereas SpeA is induced. Subsequent studies show that increased SpeA production is independent of SpeB inactivation [9], pointing to control at the level of transcriptional regulation as opposed to SpeB-mediated degradation of SpeA. Similar findings for a phenotypic switch among M1 strains have been made by several other

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laboratories [10-12]. In each case, GAS delivered subcutaneously (SC) to mice give rise to a localized soft-tissue infection, and organisms recovered from the blood or spleen after undergoing systemic spread have a distinct phenotype.

Mutations in the *covS* gene provide the molecular basis for the phenotypic switch among M1 strains. CovS, the sensor kinase of the CovR/S two component system, modulates the phosphorylation state of CovR via a complex interplay of kinase and phosphatase activity, leading to activation or repression of transcription for ~ 10% of GAS genes [13-16]. Virulence factors whose expression is repressed by CovR/S include the hyaluronic acid capsule and several targets of SpeB degradation, such as SpeA, streptokinase, and the deoxyribonuclease (DNAse) Sda1. The animal passaged variants described by Kansal et al. [1] have frame shift mutations within *covS*. This new report provides evidence for a critical role of CovS by showing that the evolved hypervirulent phenotype can be generated in the WT SpeB-producer strain by targeted deletion of *covS*, and reversed by allelic replacement of mutated *covS* with the WT form.

STSS is a special class of GAS invasive disease, dependent on the superantigens produced by the infecting GAS strain, combined with the genetic susceptibility of the human host. De-repression of *speA* transcription in the *covS* mutant, accompanied by a reduction in *speB* expression and SpeB degrading activity, is consistent with the epidemiological associations between STSS disease and M1 strain phenotypes [7].

Distinct pharyngeal and invasive transcriptome profiles have been defined for recent M1 clinical isolates recovered from cases of pharyngitis and invasive disease [11]. M1 strains having a pharyngeal transcriptome profile are more virulent in a mouse model of soft-tissue infection following SC injection, whereas organisms having the invasive transcriptome profile are more virulent in a mouse model of bacteremia following intraperitoneal injection. Following SC injection of mice with a pharyngeal transcriptome profile strain, bacteria recovered from the spleen have mutations in *covS* and exhibit the invasive transcriptome profile [11].

Several virulence factors under CovR/S control act in concert to facilitate the transition between localized soft tissue infection and systemic spread. Transgenic mice expressing human-derived plasminogen are highly sensitive to the effects of streptokinase produced by GAS [17]. Localized M1 GAS soft tissue infection in humanized plasminogen transgenic mice, as achieved by SC injection, leads to systemic spread of the organism only if the *speB* gene has been inactivated [18]. Otherwise, SpeB degrades the “spreading factor” streptokinase, which is necessary for plasmin formation and degradation of host tissue.

During localized soft tissue infection, GAS become caught in neutrophil extracellular traps (NETs) composed of DNA and granule components that directly damage the bacteria [19]. Importantly, the newly emerged M1 clone differs from earlier M1 strains in its content of bacteriophage genes that encode SpeA and the DNase Sda1 [4,20,21]. The phage-encoded Sda1 degrades NETs and is responsible for larger skin lesions following SC injection of an M1 strain [19]. In fact, Sda1-mediated degradation of NETs, which is allowed to proceed only in the absence of SpeB, provides the selective force for systemic spread of *covS* mutants [22].

SpeB production normally peaks during stationary phase, characteristic of a high bacterial density. Even if a *covS* mutation arises by a chance genetic event, the SpeB produced by the WT parental organisms is likely to diffuse and degrade virulence factors secreted by neighboring GAS cells, such as Sda1. It stands to reason that in order for a *covS* mutant to successfully escape from a NET, SpeB production by WT organisms needs to be inhibited or

down-regulated via a different regulatory pathway, and/or the new *covS* mutant also acquires a growth advantage so that the WT is out-competed.

An important issue revolves around whether the newly emerged M1 clone has a higher intrinsic virulence due to a superior ability to break through host tissue barriers or alternatively, the association of M1 GAS with invasive disease is largely a function of its high prevalence at the oropharynx. In large population-based surveys in the USA, there is a near equal proportion of invasive disease and pharyngitis cases (18 to 19%) due to M1 organisms [23,24]; this trend is supported by other studies [25]. Thus, when considered as an undifferentiated group, M1 organisms are highly successful in transmission via a respiratory route, yet may gain access to deep tissue at a frequency no higher than that for strains of many other M types. To help to firmly establish the general importance of *covS* mutations in GAS invasive disease, more extensive surveys that use precise definitions for GAS disease, and incorporate more detailed molecular analysis of the organisms, are needed.

The *covS* mutations that aid in GAS escape into the bloodstream provide a selective advantage that may be limited to the short term, because the hypervirulent mutant bacterium dies along with the human host. Data appear to indicate that *covS* mutants are rarely recovered from the oropharynx [11]. The phenotypic switch observed for *covS* mutants might be mimicked, at least in part, through signal transduction during superficial infection by WT strains, leading to de-repression of CovR effects. Environmental signals received by CovS include sub-inhibitory concentrations of the human-specific antimicrobial peptide (AMP) LL-37 [26], produced by epithelial and inflammatory response cells [27]. CovS signaling is apparent, but at a decreased level with the rhesus monkey-derived counterpart of LL-37, and is completely absent with mouse-derived cathelicidins. Thus, AMP stimulation of CovS is probably lacking in mouse models of GAS disease, although low Mg++ concentrations may provide a substitute signal [26]. LL-37 stimulation leads to up-regulation of transcription for hyaluronic acid capsule biosynthesis genes in GAS strains of several M-types. Likewise, the M1-derived *covS* mutants display increased capsule gene transcript levels relative to WT. A large mucoid capsule not only promotes GAS survival in blood, but is also associated with disease outbreaks involving transmission by a respiratory route [28]. Escape from NETs may also play a role at the epithelium. An M1 triple mutant inactivated in chromosomal and phage genes encoding DNases is attenuated for pharyngitis in macaques [29]. Conceivably, DNase mediates GAS escape from NETs formed at the epithelium, providing a long term evolutionary advantage if bacterial transmission is concomitantly enhanced.

The extent to which the impact of *covS* mutations on invasive GAS disease are specific to M1 strains remains to be established [30,31]. M1 organisms may be unique among throat strains in their high levels of SpeB activity because several throat isolates of other M-types display only very low levels of SpeB activity [32]. Nevertheless, the important finding on the role of *covS* mutations in GAS invasive disease may be broadly applicable to numerous strains of GAS, and also to other streptococcal pathogens having CovR/S regulator homologues, such as β-hemolytic group B streptococci.

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