Reply to “A Truncation in the Regulator RocA Underlies Heightened Capsule Expression in Serotype M3 Group A Streptococci”

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We read with interest the letter by Lynskey et al. regarding the mutation of the *rocA* gene in serotype M3 isolates of group A *Streptococcus* (GAS). As stated in their letter, we previously identified that serotype M3 GAS isolates produce capsule at a higher level than serotype M1 isolates (1). The data presented by Lynskey et al. are consistent with the enhanced capsule expression of M3 GAS isolates being a consequence of the mutation of the putative kinase-encoding gene *rocA*, a gene previously shown by this group to be mutated in serotype M18 isolates and responsible for the hyper-encapsulation of M18 GAS (2). We agree with the findings presented in the letter of Lynskey et al., not only because of the data that they present, but also because we separately identified *rocA* as being mutated in serotype M3 isolates and the source of enhanced capsule expression by this serotype.

We became interested in RocA after observing that M3 isolates have five nonsynonymous single nucleotide polymorphisms (SNPs) and a frame-shifting 1-bp deletion within *rocA*, relative to *rocA* from M1 and most other GAS serotypes (data not shown). As RocA represses capsule expression in other serotypes (2, 3), we tested the hypothesis that this is also true for M3 GAS isolates and that the natural mutations present in the M3 *rocA* allele disrupt activity. To facilitate testing our hypothesis, we created a derivative of our parental serotype M3 isolate, MGAS10870, in which the M3 *rocA* allele had been replaced with the M1 allele, creating strain 10870::*rocA*^{M1}. To ensure that any phenotype observed with the *rocA*-complemented strain was a consequence of the *rocA* allele and not of spurious mutations generated during strain construction, we reintroduced the M3 *rocA* allele into the *rocA*-complemented strain via homologous recombination, creating strain 10870::*rocA*^{M1-RV-M3}. Finally, as the M3 GAS RocA protein may retain some regulatory activity, we also created a *rocA* null mutant strain, 10870Δ*rocA*, in which the entire *rocA* gene was deleted.

The parental serotype M3 isolate MGAS10870, *rocA*-complemented derivative 10870::*rocA*^{M1}, *rocA* revertant strain 10870::*rocA*^{M1-RV-M3}, *rocA* deletion mutant strain 10870Δ*rocA*, and parental serotype M1 strain MGAS2221 were compared via hyaluronic acid capsule assays as previously described (4). The *rocA*-complemented M3 strain had a 150-fold reduction in capsule expression relative to the parental M3 isolate, the *rocA* revertant, and the *rocA* deletion mutant strains (Fig. 1). Our data, like those presented in the letter of Lynskey et al., show that complementation of the *rocA* mutation in serotype M3 isolates reduces capsule expression. In addition, by including revertant strain 10870::*rocA*^{M1-RV-M3}, we confirm that this phenotype is a consequence of the *rocA* alleles and not of spurious mutations. Furthermore, by including strain 10870Δ*rocA*, we show that the M3 RocA protein does not have partial regulatory activity. In summation, serotype M3 isolates show increased capsule expression due the presence of one or more naturally occurring mutations in *rocA*. Currently, we are investigating whether regulatory rewiring is a contributing factor in the epidemiologically identified association of M3 isolates with severe invasive infections (5).

FIG 1 Complementation of the *rocA* mutation in serotype M3 GAS reduces capsule expression. Exponential-phase cultures of the indicated GAS strains were analyzed for levels of the hyaluronic acid capsule. The experiment was performed twice, using duplicate cultures in each experiment, with mean ($\pm$ standard deviation) values shown. Asterisks indicate those samples that were statistically significantly different, via *t* test ($P < 0.01$), from the parental M1 strain MGAS2221.

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