主論文の要約

Analysis of the roles of NrdR and DnaB from *Streptococcus pyogenes* in response to host defense

（生体防御に対するA群レンサ球菌 NrdRとDnaBの役割に関する解析）

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【Introduction】

Toxic shock syndrome caused by *Streptococcus pyogenes* (*S. pyogenes*) is a re-emerging infectious disease. Many virulence-associated proteins play important roles in its pathogenesis and the production of these proteins is controlled by many regulatory factors. CovS is one of the most important two-component sensor proteins in *S. pyogenes*, and it has been analyzed extensively. We clarified the existence of some strains in which a transposon was inserted between *covS* and *nrdR* by analyzing clinically isolated group A and group G streptococcal strains. *covS* and *nrdR* are located adjacently in the *S. pyogenes* genome. *nrdR* is also located immediately upstream of *dnaB* and *dnaI*.

We speculated that this insertion has some biological significance. The function and significance of NrdR and DnaB in the virulence of *S. pyogenes* are completely unknown. Hence, we attempted to reveal their significance in virulence, with special reference to the response to host defense in this study.

【Materials and Methods】

S. pyogenes strain

The *S. pyogenes* strain used in this study was the *emm1* 1529 strain isolated from a Japanese patient with streptococccal toxic shock syndrome (STSS). Bacteria were either cultured in brain heart infusion supplemented with 0.3% yeast extract (BHI-YE) broth without agitation or grown on BHI-YE agar plates at 37°C.

Creation of the knockout and complemented strains

Nonpolar inactivated mutants of *nrdR* and *dnaB* were constructed through a double-crossover allelic replacement in the chromosome of *S. pyogenes* 1529. Successful double-crossover replacement was confirmed by DNA sequencing.

Disk diffusion assays, Lancefield bactericidal assay and mouse model of skin invasion and soft tissue infection

Disk diffusion assay was performed on wild strains and these established knockout strains. Every sterile disk saturated with H_2O_2 was placed on every inoculated plate with strains separately, and the diameter of the zone of inhibition was measured. And then, the Lancefield bactericidal assay was performed on the strains. The strains were added to human whole blood and BHI-YE. Diluted samples of blood and BHI-YE were plated on the BHI-YE agar plates. The killing ratios at specific times were tested

for significance using the Student's *t*-test. At last, we conducted of mouse model of skin invasion and soft tissue infection. The strains diluted in PBS were injected subcutaneously into 3-week-old female Slc:ICR mice. A total of 36 mice were used for the experiment. All animal procedures were approved by the Institutional Animal Care and Use Committee of Nagoya City University.

【Results】

Establishment of *nrdR* and *dnaB* knockout strains

We successfully established both *nrdR* knockout strains that expressed intact DnaB and truncated DnaB (Fig. 1A). Truncated DnaB was considered to lose several amino acids in N-terminal helical domain (NTD), but to retain a linker domain and a RecA-core C-terminal domain (CTD). We unsuccessfully attempted to establish both *nrdR* and complete *dnaB* knockout strain and we did not succeed in establishing the *dnaB*-only knockout strain that carried the intact *nrdR*, either. Although *nrdR* and partial *dnaB* mutant strain exhibited delayed growth, they reached the stationary phase at the same time as the wild type and *nrdR*-only knockout strains (Fig. 2).

Analysis of sensitivities to H_2O_2

We performed disk diffusion analysis to test the difference of the sensitivities of the strains to H_2O_2. The zones of inhibition caused by H_2O_2 were measured, and the significance of the zone of inhibition of each strain relative to that of the wild type and complemented strains was determined by *t*-test. *nrdR* and partial *dnaB* mutant strain was more sensitive to H_2O_2 than the wild type parental strain (Figs. 3A and 3B). *nrdR*- and *dnaB*-complemented strain displayed similar sensitivity as that of the parental strain (Fig. 3A), and *nrdR*-only complemented strain also displayed restored sensitivity (Fig. 3B). The *nrdR*-only knockout strain was a little sensitive, but the complement experiment did not work (Fig. 3C).

In vitro blood bactericidal assay

To determine whether NrdR and DnaB affected the bactericidal function of human leukocytes, we performed bactericidal assays using human blood. The killing ratios suggested that *nrdR* and partial *dnaB* knockout strain was destroyed more efficiently by human blood cells than the parental strain (Figs 4A and 4B). The killing ratio of the *nrdR* and *dnaB* genes complemented strain was restored (Fig. 4A), but not *nrdR*-only complemented strain (Fig. 4B). In addition, there was no significant

difference in the killing ratios between the *nrdR*-only mutant and the wild type strains (Fig. 4C).

Analysis of virulence in a mouse infection model

To examine the virulence *in vivo*, we performed mouse infection model experiments using the parental strain 1529, and *nrdR* and partial *dnaB* knockout strain. As shown in Fig. 5A, the mice injected with the knockout strain survived longer than those injected with the parental strain ($p < 0.001$). The *nrdR*-only knockout strain did not display a significant survival difference (Fig. 5B, $p > 0.05$). These results suggest that DnaB plays a role in *S. pyogenes* infection in the mouse infection model experiments.

【Discussion】

In this study, by the three methods mentioned above, we concluded that NrdR and DnaB from *S. pyogenes* were involved in response to host defense; however, the contribution of NrdR alone was not substantial. Because the function of NrdR as a regulatory factor in *S. pyogenes* is not completely revealed, it is possible that not all the expressions of redundant class Ib RNR genes were changed. On the other hand, as the function of DnaB was insufficient after its truncation in stress condition, its relevance became more apparent. However, as *nrdR* and *dnaB* create an operon judging from the nucleotide sequence (Fig. 1B), both proteins could play important roles in response to host defense.

We did not find the apparent phenotypic differences between the strain with a transposon insertion and the *nrdR*- and *dnaB*-complemented strain,nor did we reveal the significance of transposon insertion (data not shown). Further analyses are needed to elucidate the significance of transposon insertion and the relationship between the proteins, including RNRs regulated by *nrdR*, DnaB, and DnaI.

【Conclusion】

In conclusion, both NrdR protein and DnaB protein could play important roles in response to host defense. In addition, the DnaB protein is more prominent than the NrdR protein.