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A single streptococcal gene can confer innate immune resistance and virulence to a nonpathogenic bacterium

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Abstract. We show here that heterologous expression of a single group B streptococcal (GBS) surface-anchored protein, *sap*B, is sufficient to render the nonpathogenic bacterium *Lactococcus lactis* resistant to innate immune clearance and highly virulent upon animal challenge.

Keywords: Group B *Streptococcus*; *Lactococcus*; Virulence; Macrophage; Neutrophil; Phagocytosis; Intracellular survival; Antimicrobial peptide; Cathelicidin

1. Introduction

Bacterial virulence is increasingly recognized as a complex and transient interaction between factors of both the microbe and a susceptible host [1]. Bacterial genes responsible for pathogenic phenotypes such as host cell adherence, invasion, and resistance to immune clearance are often located together in relatively large blocks of genetic information known as pathogenicity islands (PAIs) or in genetic elements transferred by bacteriophage [2]. The cumulative effect of the encoded phenotypes provides a survival advantage over commensal relatives, and the pathogen's propensity to resist host immune clearance and produce disease [3].

The full range of virulence factors that contribute to the pathogenesis of group B *Streptococcus* (GBS, *Streptococcus agalactiae*), the leading cause of pneumonia, sepsis,

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and meningitis in newborns, is yet to be clarified. As most bacterial virulence factors are expressed on the cell surface, we sought to identify new virulence factors with potential roles in immune resistance based on the presence of a C-terminal surface anchor motif (LPXTG). By genome analysis, we identified two surface-anchored proteins, *sap*A and *sap*B, which are encoded in a locus controlled by a positive transcriptional regulator of virulence, RogB [4]. A related gene encoding a protein sharing 50% homology with SapB has recently been shown to contribute to pili expression in GBS [5].

2. Materials and methods

Isogenic GBS mutants lacking the adjacent genes *sap*A (*sag1408*) and *sap*B (*sag1407*) were generated by precise, in-frame allelic replacement with a chloramphenicol resistance cassette by our established methods [6]. The *sap*B gene cloned in *Escherichia coli*–streptococcal shuttle vector pDCerm for complementation and heterologous expression. Bacterial phagocyte resistance was assessed by co-incubation with human neutrophils or murine macrophages for 2 h before plating dilutions to enumerate cfu; intracellular survival was calculated by adding penicillin/gentamicin to kill extracellular bacteria and harvesting cell lysates over time. Phagocytosis was analyzed by fluorescent microscopy using calcein-labelled bacteria and ethidium bromide to quench extracellular fluorescence. Minimum inhibitor concentrations and for human (LL-37) and murine (mCRAMP) cathelicidins and polymixin were determined as described [7]. The interaction of AMP with bacteria was assessed by co-incubation of strains with purified peptide, centrifugation, and Western blot detection on supernatant and resuspended pellet using anti-LL-37 antibody. In vivo challenges were performed using 6-week-old male CD1 mice infected by intraperitoneal (i.p.) ($n=6$/group) or intravenous (i.v.) injection ($n=10$/group) of bacteria at the specified concentrations with determination of bacterial counts in the blood at 24 h and mortality over a 14-day period.

3. Results

3.1. Loss of sapB renders GBS more susceptible to phagocytic killing in vitro

In assays examining the interaction of these isogenic bacterial mutant strains, GBSΔ*sap*A and GBSΔ*sap*B, with human neutrophils (MOI=5, 2 h), the GBS strain lacking *sap*B was found to be approximately 3.5 times more susceptible to neutrophil killing than wild-type GBS, whereas the *sap*A mutant exhibited similar susceptibility. Similar results were obtained using murine macrophages (data not shown).

3.2. GBS sapB renders Lactococcus lactis resistant to neutrophil and macrophage killing

The role of SapB in immune cell evasion was also apparent in gain of function analysis, as transformation of *Lactococcus lactis* with the GBS *sap*B gene conferred a 7-fold increase survival upon co-culture with human neutrophils (Fig. 1A). The mechanism of SapB-conferred resistance did not involve blockage of phagocytic uptake, as indeed the *L. lactis* expressing SapB were taken up 7-fold more effectively in the early stages of interaction with murine macrophages (Fig. 1B), yet exhibited a 3-log increase in intracellular survival within the macrophages over a 24-h period (Fig. 1C).