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DltABCD-mediated D-alanylation of teichoic acids in Group A *Streptococcus* confers innate immune resistance

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Abstract. Group A *Streptococcus* (GAS) is a major cause of both mucosal and invasive human infections. Epithelial and leukocyte production of cationic antimicrobial peptides (AMPs) is an important aspect of mammalian innate immune defense against bacterial infection. In this study, we identify a specific GAS phenotype that confers resistance to host AMPs. Inactivation of the *dlt*A gene in an invasive serotype M1 GAS isolate led to loss of teichoic acid D-alanylation. Compared to the wild-type strain, the GAS *dlt*A mutant was found to be more susceptible to AMP and lysozyme killing. Killing of the *dlt*A mutant by human PMN, which produce AMPs in large amounts, was greatly accelerated. Thus, teichoic acid D-alanylation may contribute to the ability of invasive GAS to bypass mucosal defenses and produce systemic infection. © 2006 Published by Elsevier B.V.

Keywords: Group A *Streptococcus*; Teichoic acid; Antimicrobial peptide; Neutrophil killing

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1. Introduction

One key component of mammalian innate immunity that contributes to defense against invasive bacterial infection is epithelial and phagocyte production of cationic antimicrobial peptides (AMPs). Some bacterial pathogens are inherently resistant to AMPs [1], e.g. modifications of bacterial cell surface constituents to incorporate positively charged residues decreases the ability of AMPs to reach their cell wall target of action, affording the organism relative protection. An example of this phenomenon is *dltABCD*-mediated D-alanylation of cell envelope teichoic acids in *Staphylococcus aureus* [1]. In this study [2], we applied targeted mutagenesis to a Group A *Streptococcus* (GAS) serotype M1T1 isolate from a patient with necrotizing fasciitis to determine the potential contribution(s) of GAS teichoic acid D-alanylation to (1) AMP and lysozyme resistance and (2) impairment of human neutrophil killing.

2. Materials and methods

The applied materials and methods for bacterial culture, allelic replacement mutagenesis, antimicrobial testing, and neutrophil killing assays have been described in detail elsewhere [2].

3. Results

3.1. Teichoic acid D-alanylation protects GAS from AMP, lysozyme and neutrophil killing

Inactivation of the *dlt*A gene in GAS led to a loss of teichoic acid D-alanylation and an increase in the negative surface charge compared to the wild-type strain (not shown). Next, we tested if the decreased positive surface charge of the *dlt*A mutant was associated with an enhanced susceptibility to lysozyme and cationic AMPs. The *dlt*A mutant exhibited clearly decreased MICs for the murine cathelicidin mCRAMP and for lysozyme (MICs = 3.5 μM and 4 mg/ml, respectively) compared to wild-type GAS (MICs = 14 μM and > 12 mg/ml, respectively). As both lysozyme and AMP production are components of effective bactericidal activity of human phagocytes, we hypothesized that teichoic acid D-alanylation could help GAS to survive neutrophil killing. Comparing the killing kinetics of

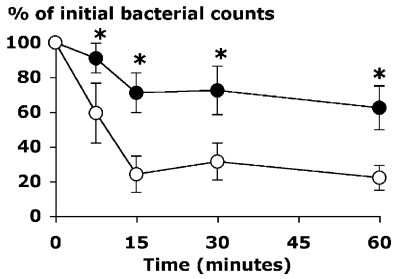

Fig. 1. Killing of GAS wild-type (closed circles) and *dltA* mutant (open circles) by human neutrophils. Samples were run in triplicate and the mean percentages ± S.E. of surviving bacteria of three experiments are shown (*$p < 0.05$, two-tailed *t*-test).

preopsonized GAS wild-type and *dlt*A mutant bacteria by human blood neutrophils, the mutant strain was killed considerably faster and more efficiently (Fig. 1).

4. Discussion

GAS is a major human pathogen increasingly associated with deep-seated invasive infections. The ability of GAS to produce invasive infection reflects the organism's ability to resist innate immune defenses and penetrate mucosal epithelial barriers. In this study, we demonstrate that the *dlt* operon of GAS functions to incorporate D-alanine into the teichoic acids expressed on the bacterial cell surface. This modification results in an increase in positive surface charge, increased resistance to antimicrobial peptide, lysozyme and neutrophil killing. Thus teichoic acid D-alanylation represents a potential virulence phenotype that could contribute in multiple fashions to the pathogenesis of invasive GAS infection.

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An extended version of the work described herein is published in the Journal of Bacteriology [2].

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