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The *Haemophilus ducreyi* trimeric autotransporter adhesin DsrA protects against an experimental infection in the swine model of chancroid

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ABSTRACT

Adherence of pathogens to cellular targets is required to initiate most infections. Defining strategies that interfere with adhesion is therefore important for the development of preventative measures against infectious diseases. As an adhesin to host extracellular matrix proteins and human keratinocytes, the trimeric autotransporter adhesin DsrA, a proven virulence factor of the Gram-negative bacterium *Haemophilus ducreyi*, is a potential target for vaccine development. A recombinant form of the N-terminal passenger domain of DsrA from *H. ducreyi* class I strain 35000HP, termed rNT-DsrA$_I$, was tested as a vaccine immunogen in the experimental swine model of *H. ducreyi* infection. Viable homologous *H. ducreyi* was not recovered from any animal receiving four doses of rNT-DsrA$_I$ administered with Freund's adjuvant at two-week intervals. Control pigs receiving adjuvant only were all infected. All animals receiving the rNT-DsrA$_I$ vaccine developed antibody endpoint titers between 3.5 and 5 logs. All rNT-DsrA$_I$ antisera bound the surface of the two *H. ducreyi* strains used to challenge immunized pigs. Purified anti-rNT-DsrA$_I$ IgG partially blocked binding of fibrinogen at the surface of viable *H. ducreyi*. Overall, immunization with the passenger domain of the trimeric autotransporter adhesin DsrA accelerated clearance of *H. ducreyi* in experimental lesions, possibly by interfering with fibrinogen binding.

1. Introduction

The sexually transmitted pathogen *H. ducreyi* causes chancroid, a genital ulcer disease important for acquisition and transmission of HIV [1–3]. During experimental and natural infection, *H. ducreyi* co-localizes with neutrophils and fibrin in the dermis of the skin [4,5]. *H. ducreyi* binds the precursor of fibrin, fibrinogen (Fg), using the lipoprotein FgbA [6] and the trimeric autotransporter adhesin (TAA) DsrA (Ducreyi serum resistance A) [7], proven virulence factors in experimental models of chancroid [8,9]. DsrA is a multifunctional outer membrane protein also involved in binding fibronectin (Fn) [10], vitronectin (Vn) [9], HaCat keratinocytes [9] and mediating serum resistance in *H. ducreyi* [11,12].

DsrA consists of a functional N-terminal passenger domain variable amongst TAAs and a highly conserved C-terminal translocator domain (Fig. 1A) [13,14]. Using a panel of DsrA proteins truncated only in the passenger domain [15], our laboratory showed that serum resistance and Fg, Fn and Vn binding by DsrA involves amino acids in the C-terminal section of the passenger domain [15] (Fig. 1A). Since DsrA has many attributes of a successful vaccine candidate, including surface expression, involvement in pathogenesis [8,9], immunogenicity [15,16], and eliciting surface-binding antisera [15], we sought to determine if a recombinant preparation of the passenger domain of the *H. ducreyi* DsrA protein from prototypical

Abbreviations: TAA, trimeric autotransporter adhesin; Fg, fibrinogen; Fn, fibronectin; Vn, vitronectin; rNT-DsrA$_I$, passenger domain of the DsrA protein from class I *H. ducreyi* strain 35000HP; Abs, antibodies.

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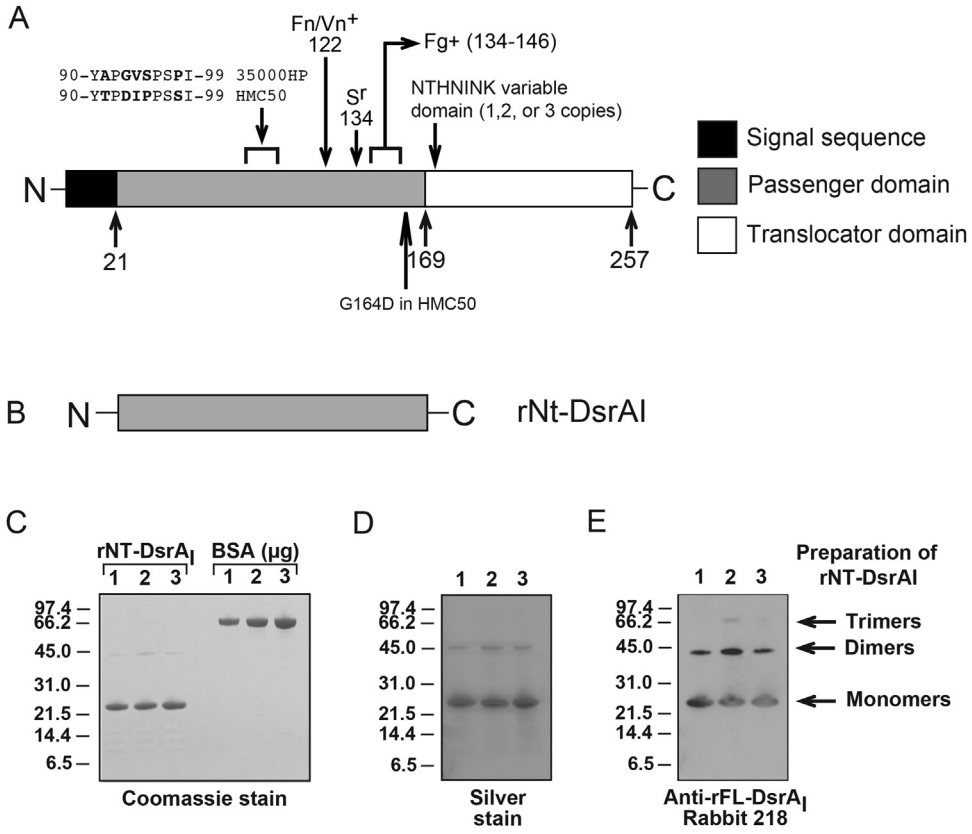

Fig. 1. rNT-DsrA$_I$ as a vaccine immunogen against experimental chancroid. (A) Schematic representation of the DsrA protein showings its different domains, the sections involved in its different functions and the amino acids that vary between challenge strains. The arrows and numbers at the bottom of the cartoon indicate the amino acid at the end of each domain. Removal of the passenger domain of DsrA abrogates Fg, Vn and Fg binding and renders *H. ducreyi* serum sensitive in the presence of 50% normal human serum [7,15]. These functions are regained (Fg+, Fn/Vn+, S^r) when the indicated C-terminal portions of the passenger domain of DsrA are expressed along with the translocator domain in an isogenic *dsrA* mutant strain [7,15]. (B) The passenger domain of rDsrA$_I$, termed rNT-DsrA$_I$, was used as the immunogen. (C) Coomassie stained gel of the three preparations of rNT-DsrA$_I$ used in vaccination studies. Bovine serum albumin (BSA) was used to confirm concentration of the purified protein, in addition to determination of protein concentration using a commercially available kit. (D) Silver stain of the same preparations in C. (E) Western blot analysis of rNT-DsrA$_I$ preparations with a polyclonal Ab against full-length DsrA (rFL-DsrA$_I$) [16]. Sr, serum resistance; Fn, fibronectin; Fg, fibrinogen; Vn, vitronectin; + indicates region of DsrA involved in binding to the indicated proteins.

class I strain 35000HP (rNT-DsrA$_I$) is protective against homologous and heterologous challenges in the experimental swine model of chancroid.

2. Materials and methods

2.1. Bacterial strains and culture conditions

H. ducreyi strains are grouped in classes, class I or class II, based on variant outer membrane determinants [16–18]. Prototypical class I strain 35000HP, a human-passage isolate [19] of strain 35000 [20], is the source of the *dsrA* gene used for preparation of rNT-DsrA$_I$. 35000HPΔ*dsrA* (FX517) is an isogenic *dsrA* mutant of strain 35000HP [11]. Strain HMC50 is a class I *H. ducreyi* strain isolated in Jackson, MS [12]; the isogenic *dsrA* mutant of this strain is termed FX530 [12]. Strain HMC 112 is a class II *H. ducreyi* Bangladesh isolate [16,21,22]. Strain BE3145 was isolated from a cutaneous, non-genital chancroid lesion in a Samoan patient [23] and is a class I *H. ducreyi* strain, based on the amino acid sequence of DsrA. All strains were routinely sub-cultured on chocolate agar plates (CAP) supplemented with 5% FetalPlex (Gemini Bio-Products, West Sacramento, CA) and 1× GGC (0.1% glucose, 0.001% glutamine, 0.026% cysteine) [24] at 34.5 °C with 5% CO_2. *Escherichia coli* strain BL21(DE3)pLysS (Life Technologies, Grand Island, NY [25]) was grown at 37 °C in LB broth supplemented with ampicillin (100 μg/mL) and chloramphenicol (30 μg/mL) (Sigma–Aldrich, St. Louis, MO).

2.2. Expression and purification of rNT-DsrA$_I$

The nucleotide sequence encoding the passenger domain of *dsrA*$_I$ was amplified using PCR as previously described [16] and cloned into the pCRT7/CT-TOPO expression vector (Invitrogen, Carlsbad, CA), which contains a 6-histidine fusion tag. rNT-DsrA$_I$ was purified from a culture of BL21(DE3)pLysS expressing pUNCH1293 using nickel affinity chromatography as previously described [9,16,26], using the Prep Ease His-Tagged Protein Purification Maxi kit (Affimetrix, Cleveland, OH).

2.3. SDS-PAGE and Western blotting

Purified rNT-DsrA$_I$ was subjected to SDS-PAGE, followed by Coomassie, silver staining or Western blotting to determine purity of the protein, formation of multimers and to confirm its concentration and the absence of lipopolysaccharides (LPS), as previously described [16,27]. Images were captured using the FluorChemE imager (Protein Simple, Santa Clara, CA). LPS content of rNT-DsrA$_I$ preparations was also determined using the Pyrogent 5000 LAL Assay kit (Lonza Inc., Allendale, NJ) following the manufacturer's instructions and was found to be under detectable limits.

2.4. Animals studies

Three immunization experiments, approved by The Institutional Animal Care and Use Committee (IACUC), were performed at the