EsaD, a Secretion Factor for the Ess Pathway in \textit{Staphylococcus aureus}^{\textsuperscript{\textdagger}}

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\textit{Staphylococcus aureus} encodes the Sec-independent Ess secretion pathway, an ortholog of mycobacterial T7 secretion systems which is required for the virulence of this Gram-positive microbe. The Ess (ESX secretion) pathway was previously defined as a genomic cluster of eight genes, \textit{esxA}, \textit{esxB}, \textit{esxA}, \textit{esxB}, \textit{esxB}, \textit{esxB}, \textit{esxB}, and \textit{esxB}. \textit{esxAABC} encode membrane proteins involved in the stable expression of \textit{esxA}, \textit{esxB}, and \textit{esxA}, genes specifying three secreted polypeptide substrates. \textit{esxB}, which encodes a small cytoplasmic protein, represses the synthesis of \textit{esxC} but not that of \textit{esxA} and \textit{esxB}. Here we investigated a hitherto uncharacterized gene, \textit{esaD}, located downstream of \textit{esxB}. Expression of \textit{esaD} is activated by mutations in \textit{esxB} and \textit{esxA}. \textit{EsaD}, the 617-amino-acid product of \textit{esaD}, is positioned in the membrane and is also accessible to \textit{EsaD}-specific antibodies on the bacterial surface. \textit{S. aureus} mutants lacking \textit{esaD} are defective in the secretion of \textit{esxA}. Following intravenous inoculation of mice, \textit{S. aureus esxD} mutants generate fewer abscesses with a reduced bacterial load compared to wild-type parent strain Newman. The chromosomes of \textit{Listeria} and \textit{Bacillus} species with \textit{EsaD} homologues downstream of \textit{esxB}, suggesting that the contributory role of \textit{EsaD} in \textit{EsaD} secretion may be shared among Gram-positive pathogens.

\textit{Staphylococcus aureus}, a Gram-positive bacterium and commensal of the human skin and nares, is also the most common cause of soft tissue infections (22). Following invasion of deeper tissues, \textit{staphylococcal} secrete proteins that trigger the establishment of abscess lesions and enable this pathogen to persist in host tissues without eliciting protective immune responses (9, 21). During the initial stages of infection, \textit{staphylococci} are surrounded by massive infiltrates of immune cells, predominantly polymorphonuclear leukocytes (PMNs). Within 4 to 5 days, these lesions are programmed for the replication of \textit{staphyloccocal} abscess communities, which are enclosed by a pseudocapsule of fibrin deposits and surrounded by layers of immune cells (9). \textit{Staphylococcal} abscesses are dynamic lesions where immune cells respond to the many secreted products of this pathogen. Eventually, these lesions rupture on the surface of organ epithelia, thereby releasing \textit{staphylococcal} into body fluids and promoting the formation of new abscesses (9). Recent work has shown that the \textit{S. aureus} \textit{EsaD} pathway (ESX or type VII-like secretion system) is an important contributor to this developmental program of abscess formation and \textit{staphyloccocal} persistence in host tissues (7).

\textit{ESAT-6} and its homologue CFP-10 are small, \alpha-helical polypeptides and founding members of the WXG100 motif family (27, 30). Both proteins are secreted by \textit{Mycobacterium tuberculosis}, the causative agent of tuberculosis (18, 29, 32). A hallmark of \textit{M. tuberculosis} infection is the formation of a granuloma, where bacteria replicate within macrophages (11). Following drainage of necrotic debris from a granuloma, \textit{mycobacteria} can disseminate throughout the tissues of an infected host (10). \textit{ESAT-6} and CFP-10 are crucial for the replication of \textit{M. tuberculosis} in macrophages and presumably also for the pathogen’s ability to suppress innate and adaptive immune responses (11, 24, 28, 31, 32). In agreement with this model, the live-attenuated vaccine strain \textit{Mycobacterium bovis} Bacille Calmette-Guérin (BCG) harbors a deletion of the \textit{ESAT-6} secretion gene cluster (23, 28). Upon vaccination, BCG elicits antimycobacterial immune responses that reduce the incidence of military tuberculosis and of \textit{M. tuberculosis} meningitis in children (17).

In addition to the WXG100 proteins \textit{EsxA} and \textit{EsxB}, the \textit{esx} gene clusters of \textit{S. aureus} harbor \textit{esxC}, a homologue of the \textit{PtsK/SpoIIIIE} ATPase superfamily. In \textit{M. tuberculosis}, the products of orthologous genes are thought to fuel protein transport by the type VII secretion system and contribute to substrate recognition (8, 27). The chromosome of \textit{M. tuberculosis} harbors multiple ESX gene clusters, as well as genomic islands that encode non-WXG100 secretion substrates and regulatory factors for these secretion pathways (1, 5, 14). In \textit{S. aureus}, \textit{esx} was initially identified as a single locus flanked by two genes for WXG100 proteins, \textit{esxA esxA esxA esxB esxB esxB esxC esxC esxB esxB esxB esxB esxB esxB esxB esxB esxB}, encode membrane proteins involved in the stable expression of secreted polypeptides; these genes are not conserved in \textit{M. tuberculosis} (8). The same applies to \textit{esxB}, which encodes a small cytoplasmic protein that represses the synthesis of the \textit{staphyloccocal} secreted protein \textit{EsaC} but not the synthesis and secretion of \textit{EsxA} and \textit{EsxB} (7).

We investigated a hitherto uncharacterized gene, \textit{esaD}, which is located downstream of \textit{esxB}. Expression of \textit{esaD} is activated by mutations in \textit{esxB} and \textit{esxA}. \textit{EsaD}, the 617-amino-acid product of \textit{esaD}, is positioned in the membrane and is also accessible to \textit{EsaD}-specific antibodies on the bacterial surface. \textit{S. aureus} mutants lacking \textit{esaD} display a reduction in \textit{EsaD} secretory activity.
secretion. Following intravenous inoculation of mice, *S. aureus* Newman mutants lacking *esaD* display defects in the unique ability of staphylococci to establish persistent abscess lesions. The genomes of *Listeria* and *Bacillus* species also harbor *esaD* homologues downstream of *exdB* in their *esa* cluster, suggesting that the contributory role of EsaD in *Ess* secretion may be shared among Gram-positive pathogens.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and growth conditions.** *S. aureus* cells were grown in tryptic soy broth (TSB) at 37°C. Chloramphenicol and erythromycin were used at 10 mg liter⁻¹ for plasmid and allele selection. *S. aureus* strain USA300 was obtained through the Network on Antimicrobial Resistance in *S. aureus* (NARSA; National Institute of Allergy and Infectious Diseases [NIAID]). All of the mutants used in this study, with the exception of the *exdB* mutant, were obtained from the Phoenix (9NE) library (3) or have been previously described (7). Each Phoenix isolate carries a mapped *bursa aurealis* hprK lgt yvoF yvcD sequence (275 bp upstream of the shuttle vector pOS1, which carries the *Promoter and Shine-Dalgarno* promoter and Shine-Dalgarno sequences of other Gram-positive bacteria identified previously were selected on agar plates. Mice were anesthetized by intraperitoneal injection of 80 to 120 mg/kg ketamine and 3 to 6 mg/kg xylazine and infected by lateral injection of ~10⁻⁷ CFU in 100 μl into the retro-orbital plexus of the right eye. On day 5 or 8 following infection, mice were euthanized by compressed CO₂. Kidneys were removed and homogenized in 1% Triton X-100; aliquots were diluted and plated on agar medium for triplicate determination of CFU. The experiment was reproduced twice. Student’s *t* test was performed for statistical analysis by using the software Analyze-it (Analyze-it Software, Leeds, United Kingdom), and *P* values of less than 0.05 were considered significant. For histology, kidney tissues were incubated at room temperature in 10% formalin for 24 h. Tissues were embedded in paraffin, thin sectioned, hematoxylin-eosin stained, and then examined by microscopy.

**RESULTS**

**Identification of EsaD.** BLAST searches using *S. aureus* Newman *essa* and *exxAB* sequences as queries against the genomic sequences of other Gram-positive bacteria identified *essa* clusters, as well as their flanking genes. *S. aureus* Newman WNMM_0228 is located downstream of *exdB*, and its homologues assume similar positions in *Listeria* and *Bacillus* species with *Ess* pathways (Fig. 1A). In agreement with the possibility that WNMM_0228 is a component of the *S. aureus* type VII-like secretion pathway, Gram-positive organisms that lack *essa* gene clusters also do not harbor a homologue of WNMM_0228. The product of WNMM_0228, a 617-residue polypeptide, or of its homologues, harbors the ancient conserved domain COG5444, which is also found in EsxL, a putative WXG motif secretion substrate of *Bacillus anthracis* (Fig. 1B). Some homologues of WNMM_0228 harbor a C-terminal domain, either DUF600 (a domain of unknown function) or VIP2 (*Bacillus*-produced vegetative insecticidal proteins related to a family of actin-ADP-ribosylating toxins) (Fig.)
FIG. 1. Schematic of Ess loci and EsaD-like proteins in Gram-positive bacteria. (A) Comparison of the S. aureus Ess (type VII-like) secretion loci with L. monocytogenes and “Bacillus cytotoxicus” ess loci, as well as the B. anthracis ess gene cluster (16). Genes showing sequence homology are depicted in the same color. (B) Domain organization of S. aureus EsaD and protein homologues showing the conserved COG5444 domain. With the exception of B. subtilis YeeE, all COG5444 proteins lie within an Esa-like locus. The colors of some conserved proteins and domains indicate WXG100 proteins (red), FtsK SpoIIIE-like ATPases (yellow), EsaD and COG5444 (dark blue), proteins with a DUF600 domain (light blue), and VIP2 (pink).

1B). Thus, depending on the host genome and unique attributes of each Ess pathway, the homologues of NWMN_0228 may have acquired specific functions that are not shared by all of the members of this gene family. Here we designated NWMN_0228 esaD for Ess-associated gene D.

To characterize esaD and its product, we expressed codons 1 to 100 of its open reading frame as a translational hybrid of the members of this gene family. Here we designated may have acquired specific functions that are not shared by all of the members of this gene family. Here we designated

FIG. 2. EsaB and EssB regulate EsaD production in the staphylococcal Ess pathway. (A) Whole-culture lysates of S. aureus Newman or its variants with bursa aurealis insertions (exsA, exsA, esaB, esaB, essC, essC, essC, essD, and esaD) were examined for production of EsaD. Staphylococci were grown in TSB to an A600 of 1.0 and treated with lysostaphin. Proteins in whole-culture lysates were precipitated with methanol-chloroform, separated by SDS-PAGE, and detected by immunoblotting with specific antibodies (anti-EsaD antibodies, as well as anti-SrtA antibodies as a loading control). As a control, an extract of S. aureus Newman carrying pOS-esaD, a plasmid expressing esaD under the control of the constitutive hprK promoter, was examined for EsaD production. (B) Plasmid complementation analysis of esaB and essB mutants via immunoblotting of total cell extracts derived from S. aureus Newman or esaB and essB mutants harboring the vector alone (pOS) or plasmids pOS-esaB and pOS-essB. (C) Whole-culture lysates of S. aureus USA300 and its esaD variant were examined for EsaD production. Extracts of USA300 cultures were compared to strain Newman extracts. Samples were prepared and analyzed as described for panel A. (D) Whole-culture lysates of S. aureus Newman and its esaD variant were prepared and analyzed as described for panel A, except that cultures were grown in TSB with (+) or without (−) 5% horse serum.

in mutants with a bursa aurealis insertion at nucleotide 23 of the esaD open reading frame (Fig. 2A). Mutations in exsA, esaB, esaA, esaC, or essC did not affect esaD expression. Surprisingly, a bursa aurealis insertion in essB, which encodes a membrane protein that is required for Ess secretion, also induced the expression of esaD (Fig. 2A). The esaD regulatory phenotypes of esaB and essB mutants could be complemented in trans by plasmids expressing either wild-type esaB or essB, respectively (Fig. 2B). Thus, the regulatory phenotypes of esaB and essB mutants are due to the corresponding genetic lesions of their bursa aurealis insertions and are not caused by polar effects on other ess or esa genes.

We wondered whether other S. aureus isolates express EsaD and examined USA300, a strain responsible for the current North American epidemic of community-acquired methicillin-resistant S. aureus infections. Earlier work on the secretion substrate EsaC showed that, when grown in laboratory medium, S. aureus USA300 produces machine components and transport reactions of the Ess pathway. In agreement with this, an anti-EsaD antibody-immunoreactive protein was detected in lysostaphin extracts of USA300 but not in those of S. aureus Newman (Fig. 2C). EsaD was not detectable by immunoblotting in extracts of the USA300 esaD variant with bursa aurealis inserted at nucleotide 23 of its open reading frame (Fig. 2C).

To test whether S. aureus Newman can express esaD under...
growth conditions that more closely resemble infected host tissues, we inoculated bacteria into TSB broth supplemented with fetal calf serum. When grown under these conditions, *S. aureus* Newman, but not its isogenic *esaD* mutant, produced EsaD polypeptide (Fig. 2D). Taken together, these results indicate that *esaD* is expressed in clinical isolates of methicillin-sensitive and methicillin-resistant *S. aureus* strains.

**EsaD is displayed on the staphylococcal surface.** When analyzed with the Kyte-Doolittle hydrophobicity plot, EsaD is predicted to harbor an α-helical hydrophobic transmembrane segment at amino acids 217 to 250 of the 617 residue polypeptide. As positively charged amino acids (three Lys residues and one Arg residue) are positioned near the C-terminal end of the transmembrane segment, we predicted that the N-terminal portion of EsaD (residues 1 to 216) may be positioned on the staphylococcal surface, whereas the C-terminal domain (residues 251 to 617) may be located in the cytoplasm. To test this, affinity-purified rabbit anti-EsaD antibodies were added to *S. aureus* Newman Δspa mutants, which are unable to bind immunoglobulin by nonimmune mechanisms. When analyzed by immunoblotting, lysostaphin extracts of Δspa mutants harboring plasmid pesaD revealed the presence of EsaD, whereas Δspa mutants with the corresponding pOS1 vector control did not (Fig. 3A and B). Antibody binding to the staphylococcal surface was detected with goat anti-rabbit secondary antibodies conjugated to Alexa Fluor 488 (green) and fluorescence microscopy. Anti-EsaD antibodies bound to staphylococci harboring pesaD but not to cells with the pOS1 vector control. Further, irrelevant anti-V10 antibodies, which recognize the plague-protective antigen LcrV, failed to generate fluorescent signals on the staphylococcal surface. Taken together, these data indicate that EsaD is displayed on the staphylococcal surface.

**EsaD fractionates with staphylococcal membranes.** Membrane proteins of *S. aureus*, for example, sortase A, are generally not accessible to antibodies on the surface of these bacteria. We wonder whether the surface display of EsaD requires the posttranslational processing of this polypeptide, for example, cleavage of the transmembrane segment so that the N-terminal domain of EsaD may be transported to the surface. To examine this, *S. aureus* Newman, as well as USA300, cells were sedimented by centrifugation, peptidoglycan was removed by lysostaphin, and the resulting protoplasts were lysed in hypotonic buffer. Protoplast lysates were subjected to ultracentrifugation at 100,000 ×g, and membrane sediment was separated from soluble proteins in the supernatant. As a control, immunoblotting of fractionation samples demonstrated the sedimentation of sortase A with staphylococcal membranes (Fig. 3C). Immunoblotting of samples derived from *S. aureus* strain USA300 or Newman carrying either pesaD or the pOS1 vector control revealed that most of the EsaD products sedimented with staphylococcal membrane proteins. Further, immunoblotting with anti-EsaD antibodies failed to detect soluble EsaD products with altered mobility on SDS-PAGE. These results suggest that the surface display of EsaD is a unique feature of this membrane protein and is likely not due to the generation of soluble EsaD fragments.

**EsaD is required for the efficient secretion of EssA.** *esaD* expression is controlled by *esaB* and *essB*, two genes that are involved in regulating either the expression of specific Ess secretion substrates or the transport of EssA, EssB, and EssC by this pathway. We therefore wondered whether EsaD is also involved in Ess secretion. To test this, we analyzed isogenic variants of *S. aureus* USA300 for the ability to express the EssB machine component or secrete EssA into the extracellular medium. Staphylococcal cultures were grown to mid-log phase and then centrifuged to separate proteins secreted into the culture medium with the supernatant from proteins in the bacterial sediment. Following lysis of staphylococci with lysostaphin, proteins in both fractions were precipitated with methanol-chloroform, separated by SDS-PAGE, and detected by immunoblotting with specific antibodies (anti-EsaD antibodies and anti-SttA antibodies as a control for a membrane protein).
infection caused an average of 3.8 surface abscesses per kidney. Vol. 193, 2011 Wgx100 protein secretion 1587

P. CFU on day 5 (\textit{Fig. 6}). One of the two kidneys was fixed in formalin, embedded for the presence or absence of surface abscess lesions (stage I). Following the entry of \textit{S. aureus} into the bloodstream, the microbes escape innate immune mechanisms and are disseminated via the lymph and blood circulation and the formation of new infectious lesions. The establishment of abscesses, the pathological-anatomical substrates of staphylococcal disease, can be thought of as a developmental program with four discernible stages (9). Following the entry of \textit{S. aureus} into the bloodstream, the microbes escape innate immune mechanisms and are disseminated via the vasculature to peripheral organ tissues (stage I). Once within organ tissue, staphylococci attract massive infiltrates of PMNs, as well as other immune cells accumulated in the cytoplasm of mutant staphylococci. This defect in EssA secretion was restored by transformation of \textit{esaD} mutant staphylococci with the plasmid encoding wild-type \textit{esaD} (p\textit{esaD}) (\textit{Fig. 4A} and B).

\textbf{Virulence defects of \textit{esaD} mutant staphylococci.} Previous experiments suggested that Ess secreted products EssA, EssB, and EssC are important for \textit{S. aureus} Newman to replicate in organ tissues of infected mice (7, 8). Further, Ess secretion is required for the ability of staphylococci to establish abscess lesions wherein this pathogen can persist and evade host immune responses. To ascertain the contribution of \textit{esaD} to staphylococcal pathogenesis, cohorts of 10 BALB/c mice (6-week-old female animals) were infected by retro-orbital injection of \(1 \times 10^7\) CFU \textit{S. aureus} Newman or its isogenic \textit{esaD} mutant into the bloodstream. At 5 or 15 days following infection, animals in each cohort were euthanized and necropsied to remove both kidneys. One kidney from each animal was homogenized and spread on agar plates. Following incubation and enumeration of staphylococcal colonies, the bacterial load was calculated as the number of CFU per organ (\textit{Fig. 5A}). As expected, the average load of \textit{S. aureus} Newman in kidney tissues was \(6.8 \log_{10}\) CFU on day 5 and \(6.3 \log_{10}\) CFU on day 15. The average load of the isogenic \textit{esaD} mutant was reduced by \(1.5 \log_{10}\) CFU on day 5 (\(P < 0.02\)) and \(2.3 \log_{10}\) CFU on day 15 (\(P < 0.01\)), revealing defects in the ability of the \textit{esaD} variants to replicate and persist in mouse organ tissues. This ability was restored to wild-type levels when \textit{esaD} mutants were transformed with a plasmid carrying the wild-type \textit{esaD} allele (p\textit{pos}\textit{esaD}) but not with the plasmid vector alone (p\textit{OS}) (\textit{Fig. 5C}).

Following necropsy, the kidneys of infected mice were analyzed for the presence or absence of surface abscess lesions (\textit{Fig. 6}). One of the two kidneys was fixed in formalin, embedded in paraffin, thin sectioned (four sagittal sections 200 \(\mu\)m apart), and tissue stained with hematoxylin-eosin. Renal tissue slides were analyzed by microscopy for immune cell infiltrates and abscess formation, which allowed us to assess the number of internal lesions on four slides per kidney. \textit{S. aureus} Newman infection caused an average of 3.8 surface abscesses per kidney (\textit{Fig. 6A} and B), as well as an average of 2.2 internal abscesses (\textit{Fig. 6C} and D). By comparison, \textit{esaD} mutant staphylococci produced an average of 1.8 surface abscesses (\textit{Fig. 6A} and B; \(P = 0.03\), wild type versus \textit{esaD} mutant) and 0.7 internal abscess per infected organ (\textit{Fig. 6C} and D; \(P = 0.002\), wild type versus \textit{esaD} mutant).

Although considered sublethal, an intravenous injection of \(1 \times 10^7\) staphylococci leads to some mortality in 6-week-old BALB/c mice. We noticed that infection with the isogenic \textit{esaD} mutant does not cause mortality, unlike infection with wild-type strain Newman. To assess the significance of this finding, we examined animal survival with Kaplan-Meier plots (\textit{Fig. 5B}) for three independent experiments and found a significant difference for mice challenged with either wild-type or \textit{esaD} mutant staphylococci (\(P = 0.037\)). Taken together, these data provide strong support for the role of EsaD and the Ess secretion pathway during \textit{S. aureus} infection of mice.

\textbf{Discussion}

In mice, as in humans, \textit{S. aureus} causes infections that persist with reiterative cycles of bacterial deposition in multiple organ tissues, abscess formation, and maturation of purulent lesions. Abscesses rupture, thereby permitting staphylococcal dissemination via lymph and blood circulation and the formation of new infectious lesions. The establishment of abscesses, the pathological-anatomical substrates of staphylococcal disease, can be thought of as a developmental program with four discernible stages (9). Following the entry of \textit{S. aureus} into the bloodstream, the microbes escape innate immune mechanisms and are disseminated via the vasculature to peripheral organ tissues (stage I). Once within organ tissue, staphylococci attract massive infiltrates of PMNs, as well as other immune cells...
volves the *esaB essB* regulatory arm of this pathway. A similar trait has been reported for *EsaC* (7). In *S. aureus* Newman, very little *EsaC* is expressed; however, expression is induced by the addition of serum to growth medium or by a genetic lesion in *esaB* (7). Highly virulent strains such as *S. aureus* USA300 synthesize greater amounts of *EsaC* and *EsaD* than the less virulent isolates do (7) (Fig. 2). Together, these findings indicate that *EsaD* is part of the Ess pathway and that the polypeptide supports the secretion of *EsaA* and is thereby involved in the pathogenesis of staphylococcal infections.

Bioinformatic searches revealed that *EsaD* belongs to the cluster of orthologous group of proteins COG5444 without known function (25). These proteins are typically associated with *ess- or ess-like genes in Firmicutes* (Fig. 1A). Some COG5444 proteins carry additional domains; for example, *Listeria monocytogenes* *lmo0066* carries a VIP2 domain otherwise found in the family of toxins that ADP-ribosylate actin. In bacilli, the YeeE and YeeF proteins (COG5444 cluster) are annotated as uncharacterized transposase. However, an alignment of COG5444 and transposase_30 failed to identify overlaps for the transposase catalytic residues. The YeeE and YeeF proteins carry a partial COG5444 domain followed by the sequence for a domain of unknown function named DUF600. Of note, genes located downstream of *S. aureus esaD* also harbor DUF600 domains. Depending on the strain examined, staphylococci carry 10 to 12 tandem repeats of DUF600 genes. The significance of a Rosetta stone association between the *esaD* and DUF600 genes in bacilli is still unclear (26). Intriguingly, mycobacterial chromosomes carry tandem repeats of PE and PPE repeat genes whose products are secreted via the Ess-5 pathway (2). Thus, it seems plausible that DUF600 polypeptides could also represent *S. aureus* substrates for the Ess pathway, analogously to PE/PPE in *M. tuberculosis*. Finally, BLAST searches identified homology between the last 140 amino acids of *EsaD* and the C-terminal end of filamentous-hemagglutinin-type outer membrane proteins in Gram-negative bacteria (expect = 1e-26; identities = 59/145 = 40% between NM_0228 and HSM_1651, for example). These very large proteins, often exceeding 2,500 amino acids, are part of a two-partner secretion system that promotes the transport of polypeptides across the outer membrane (19, 20). It is interesting that *EsaD* shares some sequence similarity with these proteins as both represent components of Sec-independent secretion systems.

While mycobacterial ESX (type VII) secretion systems share little in sequence homology with the Ess pathway of *S. aureus*, both organisms reside in abscesses and granulomas in a manner that appears to prevent the acquisition of protective immunity in infected hosts (9, 34). Thus, these secretion systems may have evolved to transport their immunosuppressive factors to unique locations during infection, thereby enabling the establishment of characteristic infectious lesions and disease pathologies.

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REFERENCES