Institut Pasteur
Microbes and Infection 19 (2017) 101−109
www.elsevier.com/locate/micinf

Original article

Pathogenic conversion of coagulase-negative staphylococci

Wenqi Yu, Hwan Keun Kim, Sabine Rauch, Olaf Schneewind, Dominique Missiakas*

Department of Microbiology, University of Chicago, Chicago, IL, USA

Received 6 October 2016; accepted 14 December 2016
Available online 21 December 2016

Abstract

Humans and animals are colonized by members of the genus *Staphylococcus*, however only some of these species evolved to cause invasive disease. The genetic basis for conversion of commensal staphylococci into pathogens is not known. We hypothesized that *Staphylococcus aureus* genes for coagulation and agglutination in vertebrate blood (*coa*, *vwb* and *clfA*) may support pathogenic conversion. Expression of *coa* and *vwb* in *Staphylococcus epidermidis* or *Staphylococcus simulans* supported a coagulase-positive phenotype but not the ability to cause disease in a mouse model of bloodstream infection. However, the simultaneous expression of *coa*, *vwb* and *clfA* in coagulase-negative staphylococci enabled bacterial agglutination in plasma and enhanced survival of *S. simulans* in human whole blood. Agglutination of *S. simulans* in the bloodstream of infected mice upon expression of *coa*, *vwb* and *clfA* provided also a mean for dissemination and replication in distal organs. Thus, the acquisition of genes for bacterial agglutination with fibrin appear sufficient for the conversion of commensal staphylococci into invasive pathogens.

Keywords: Coagulation; Clumping; Agglutination; Staphylococcus; Abscess; Virulence

1. Introduction

Staphylococcus aureus colonizes the nasopharynx, skin and gastrointestinal tract of humans and is also an invasive pathogen, frequently causing skin and soft tissue infection, bacteremia or abscess lesions in any organ tissue [1−3]. The genus *Staphylococcus* is comprised of more than 40 species, which share 16S ribosomal RNA sequence, low genomic DNA G+C content, cell wall composition (pentaglycine cross-bridges, lysostaphin sensitivity), cytochrome and menaquinone profiles as well as susceptibility to erythromycin, bacitracin and furazolidone [4]. Humans and their domesticated animals are colonized by different species of the genus *Staphylococcus*: *S. aureus*, *Staphylococcus auricularis*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, *Staphylococcus simulans* and *Staphylococcus warneri* [5]. Of these, however, only *S. aureus* evolved to consistently cause invasive disease in healthy immuno-competent individuals [5].

Clinical diagnosis of *S. aureus* infection and initiation of appropriate antibiotic therapy requires laboratory identification of bacteria from superficial lesions, drainage of deep-seated abscesses or blood cultures [6]. As clinical samples may be contaminated with commensal *Staphylococcus* species, two laboratory tests, coagulation and clumping, exploit key microbiological traits associated with *S. aureus* to identify the pathogen [7,8].

The coagulation test examines the ability of microbes inoculated into plasma of producing clots [9]. *S. aureus* isolates generate positive test results, owing to the expression of *coa* and *vwb*, whose products are secreted into the extracellular medium [10]. Coagulase (Coa) and von-Willebrand factor binding protein (vWbp) each associate with prothrombin (PT), also designated clotting factor II (FII), of the host coagulation cascade and generate enzymatically active complexes: Coa•PT and vWbp•PT [11]. Unlike thrombin, *i.e.* proteolytically activated FIIa, Coa•PT and vWbp•PT

* Corresponding author.
E-mail address: dmissiak@bsd.uchicago.edu (D. Missiakas).

http://dx.doi.org/10.1016/j.micinf.2016.12.002

complexes cleave fibrinopeptides A and B off fibrinogen without cutting other thrombin substrates (FV, FVIII, FXI, FXIII, protein C, antithrombin and plasmin) [12,13]. In addition, the vWbp•PT complex interacts and activates human FXIII in a non-catalytic manner [13].

The clumping test examines the agglutinating attributes of bacteria immersed in plasma. *S. aureus* isolates test positive in this assay owing to the secretion of coagulases (Coa and vWbp) [14] and to the assembly of clumping factor A (ClfA) in the bacterial envelope [15]. The joint action of coagulases in generating fibrin cables and of ClfA in promoting *S. aureus* association with fibrin protects bacteria from phagocytosis [14,16]. Unlike *S. aureus*, coagulase-negative staphylococcal isolates, for example *S. epidermidis* or *S. simulans*, score negative in both coagulase and clumping tests [4].

Several *Staphylococcus* species produce coagulases, however these microbes (*Staphylococcus delphini*, *Staphylococcus intermedius* and *Staphylococcus pseudintermedius*) adapted to causing invasive disease in other hosts: mink, fox, pigeon, cats or dogs [17,18]. Genome sequence analysis of pathogenic and non-pathogenic *Staphylococcus* species suggested that horizontal gene transfer may be responsible for the evolution of pathogenic staphylococci [19]. However, it is not clear what genes may be sufficient for the conversion of commensal staphylococci into an invasive pathogen. This question is addressed here and we show that transfer of the *S. aureus* genes for coagulation and agglutination (*coa*, *vwb* and *clfA*) is sufficient to convert the coagulase-negative species *S. simulans* into a pathogen that coagulates vertebrate blood, agglutinates in human and mouse plasma and disseminates from the vasculature of infected mice to replicate in distal organs.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Wild-type isolate *S. aureus* Newman and its isogenic Δ*coa/vwb/clfA* variant were described previously [14,20]. *S. epidermidis* ATCC 12228 was obtained from American Type Culture Collection (ATCC.org). *S. simulans* MK148 (ATCC 27848) was a gift from Prof. Friedrich Götz. All staphylococcal strains were grown in Brain Heart Infusion (BHI) broth at 30 °C. Strains harboring plasmids pOS1 and its derivatives were grown in BHI supplemented with 5 μg chloramphenicol/ml. *Escherichia coli* strain DC10B was cultivated in Luria broth with 100 μg ampicillin/ml at 30 °C.

2.2. Cloning procedures and plasmids

The shuttle vector pOS1 (also referred to as vector) was used for all cloning procedures [21]. Plasmid p*coa-vwb* expressing the *coa* and *vwb* genes under their respective promoters was described previously [10]. The *clfA* gene with its native promoter was cloned by amplification with the polymerase chain reaction (PCR) using genomic DNA from *S. aureus* Newman as template and the primer pair 5′-CGGGGATCCAAGCTTTTTCAAGCTAGGATTACATTAGGTA-3′ and 5′-GCGGAATTCGAATCATATGATTAATTTAATATCA-3′. The ends of the PCR product were cut with BamHI and EcoRI restriction enzymes and ligated into pOS1 cut with the same enzymes, thereby generating p*clfA*. The *clfA* PCR product was also cut and ligated into the BamHI and SmaI restriction sites of p*coa-vwb* to generate p*coa-vwb-clfA*. All cloning steps were performed in *E. coli* DC10B [22]. Plasmid clones were verified by DNA sequencing prior to electroporation into *S. epidermidis* ATCC 12228 and *S. simulans* MK148.

2.3. Culture fractionation and immunoblot analysis

Overnight cultures were diluted 1:100 into BHI and grown for 6 h. Samples were normalized to the same absorbance at 600 nm of 4 units (A_{600} 4) and one milliliter of culture was centrifuged at 8000 × *g* for 5 min. Supernatant containing coagulases was transferred to another tube. Bacterial cells containing cell wall bound ClfA were suspended in 1 ml 50 mM Tris–HCl (pH 7.5), 150 mM NaCl and incubated with 10 μg lysostaphin/ml at 37 °C for 30 min. Ice-cold trichloroacetic acid (150 μl TCA) was added to each sample, mixed and incubated for 30 min on ice; TCA precipitated proteins were sedimented by centrifugation (10,000 × *g* for 15 min), washed with cold acetone, air-dried and solubilized in 100 μl SDS sample buffer (62.5 mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.01% bromophenol blue). Protein samples were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked for 30 min with 10 ml TBS-T (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.1% Tween 20) containing 5% milk and 50 μl human IgG (Sigma) prior to the addition of affinity-purified rabbit antibodies against Coa (α-Coa), vWbp (α-vWbp) or ClfA (α-ClfA) for 1 h at room temperature. PVDF membranes were washed three times for 10 min with TBS-T and incubated with secondary anti-rabbit HRP-linked IgG for 1 h at room temperature, washed, and developed using enhanced chemiluminescence substrates.

2.4. Coagulation and clumping assays

Overnight cultures were diluted 1:100 into 10 ml BHI, grown for 6 h and normalized to A_{600} 4. For the coagulation assay, 10 μl bacterial suspensions were mixed with 90 μl anticoagulated mouse plasma (sodium citrate) or rabbit plasma (EDTA) and incubated at room temperature for 24 h prior to tilting the tubes. Rabbit plasma anti-coagulated with EDTA was obtained from BBL™ (Coagulase Plasma). Mouse plasma was obtained by drawing blood into 10 mM sodium citrate via cardiac puncture. Mouse blood was incubated at room temperature for 10 min, centrifuged at 2000 × *g* for 10 min and plasma was retrieved. For the clumping assay, bacteria from 10 ml staphylococcal culture were centrifuged, diluted in 1 ml PBS to A_{600} 4 and incubated with 10 μl of 3 mg human fibrinogen (Sigma)/ml PBS. Clumping of staphylococci was observed by briefly inverting the tubes.