Infection, Genetics and Evolution xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Staphylococcus aureus toxins – Their functions and genetics

Dorothee Grumann [a], Ulrich Nübel [b], Barbara M. Bröker [a,*]

[a] Institute of Immunology and Transfusion Medicine, University of Greifswald, 17487 Greifswald, Germany
[b] Robert Koch Institute, Wernigerode, Germany

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
S. aureus
Pore-forming toxins
Exfoliative toxins
Superantigens
Evolution
Epidemiology

ABSTRACT

The outcome of encounters between *Staphylococcus (S.) aureus* and its human host ranges from life-threatening infection through allergic reactions to symptom-free colonization. The pan-genome of this bacterial species encodes numerous toxins, known or strongly suspected to cause specific diseases or symptoms. Three toxin families are in the focus of this review, namely (i) pore-forming toxins, (ii) exfoliative toxins and (iii) superantigens. The majority of toxin-encoding genes are located on mobile genetic elements (MGEs), resulting in a pronounced heterogeneity in the endowment with toxin genes of individual *S. aureus* strains. Recent population genomic analysis have provided a framework for an improved understanding of the temporal and spatial scales of the motility of MGEs and their associated toxin genes. The distribution of toxin genes among clonal lineages within the species *S. aureus* is not random, and phylogenetic (sub-)lineages within clonal complexes feature characteristic toxin signatures. When studying pathogenesis, this lineage association, which is caused by the clonal nature of *S. aureus* makes it difficult to discriminate effects of specific toxins from contributions of the genetic background and/or other associated genetic factors.

1. Introduction

Staphylococcus (S.) aureus is notorious as the most common causative agent of hospital-acquired infections, and the spread of antibiotic resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), in hospitals challenges health care systems worldwide. Moreover, *S. aureus* strains of increased virulence, known as community-aquired MRSA (CA-MRSA), can threaten even healthy individuals in the community (Chambers and DeLeo, 2009; David and Daum, 2010; DeLeo et al., 2010). In addition, *S. aureus* is currently being discussed as the trigger and/or enhancer of allergies of the respiratory system and the skin (Bachert and Zhang, 2012; Gould et al., 2007). Nevertheless up to now, no anti-*S. aureus* vaccine has been approved for medical practice (Schaffer and Lee, 2008; Spellberg and Daum, 2012). In spite of the above, the most frequent encounter of *S. aureus* with its human host is peaceful colonization, and around 20% of adults are persistent carriers of the microorganisms, while another 60% are intermittently colonized (van Belkum et al., 2009; Wertheim et al., 2005). What makes the species *S. aureus* so immensely successful?

1.1. Multiple virulence factors encoded in the pan-genome of S. aureus

A salient feature of *S. aureus* is its variability. By indexing nucleotide sequence diversity at seven universally present genetic loci, multilocus-sequence typing (MLST) to date has revealed about 2,400 'sequence types' (ST) for *S. aureus* (see <www.mlst.net>). The vast majority of these diverse STs, however, are clustered in a remarkably limited number of clonal complexes (CC) or lineages, each of which appears to be distributed worldwide (reviewed in (Nübel et al., 2011)). The predominant *S. aureus* lineages are CC1, 5, 8, 15, 22, 30, 45, 59, 80, 97 and 121 (Nübel et al., 2011).

About 75% of the *S. aureus* genes are shared by more than 95% of strains and hence may be considered the 'core genome' of the species. In addition, two kinds of variably present genes can be distinguished: (i) the core variable genes (~10% of genes), which are largely conserved within each of the *S. aureus* clonal complexes and constitute their respective "make up", and (ii) mobile genetic elements (MGEs, ~15% of genes). The core variable genome includes most surface-associated genes (microbial surface components recognizing adhesive matrix molecules, MSCRAMMs) and regulator genes. Core variable genes are encoded on the bacterial chromosome and are, therefore, typically stable and transferred vertically (Lindsay et al., 2006). MGEs include bacteriophages, plasmids, *S. aureus* pathogenicity islands (SaPI), transposons, and staphylococcal chromosomal cassettes (SCC) (Feil et al., 2003; Lindsay, 2010; Lindsay and Holden, 2006; Lindsay et al., 2006). They mainly encode resistance (e.g. methicillin resistance genes) and virulence genes (e.g., Panton-Valentine leukocidin (PVL) genes, superantigen (SAg) genes). MGEs can be distributed either by vertical transmission to daughter cells or by horizontal transfer (Lindsay and Holden, 2006).

The full complement of all genes (also known as the pan-genome) of *S. aureus* encodes a wide array of secreted or cell-sur-

* Corresponding author. Tel.: +49 3834 865595; fax: +49 3834 865490.
E-mail address: broeker@uni-greifswald.de (B.M. Bröker).

1567-1348/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.meegid.2013.03.013

Table 1
Toxin genes in the *S. aureus* pan-genome.

	Toxin genes	References
Core genome		
	hla	Bhakdi and Tranum-Jensen (1991)
	hld	Janzon et al. (1989)
	hlg gene cluster	Kaneko and Kamio (2004)
	psmα, psmβ	Wang et al. (2007)
	selx	Wilson et al. (2011)
Genomic islands		
νSaβ	enterotoxin gene cluster (*egc*): *seg, sei, selm, seln, selo,* (*selu, selu2, selv*)	Baba et al. (2002), Letertre et al. (2003) , Thomas et al. (2006)
	lukDE	Baba et al. (2002), Barrio et al. (2006), Lindsay and Holden (2006)
νSaγ	*etd*	Highlander et al. (2007), Yamaguchi et al. (2002)
Plasmids		
pIB485	*sed, sej, ser*	Bayles and Iandolo (1989), Omoe et al. (2003), Zhang et al. (1998)
pF5	*sej, ser, ses, set*	Omoe et al. (2003), Ono et al. (2008)
$pGSA_{18}$ *rep32* (pETB)	*etb*	McCarthy and Lindsay (2012), Yamaguchi et al. (2001)
$pGSA_{11}$ *rep22* (SAP057A)	*etb*	McCarthy and Lindsay (2012)
Staphylococcal cassette chromosomes		
SSC*mec* types II, III, VIII	*psm-mec*	Chatterjee et al. (2011), Queck et al. (2009)
Pathogenicity islands		
SaPIn1 (N315)/SaPIm1 (Mu50) (νSa4 type I)	*sell, sec, tst*	Novick and Subedi (2007)
SaPI3 (COL νSa1)	*seb, selk, selq*	Novick and Subedi (2007)
SaPImw2	*sell, sec*	Baba et al. (2002), Lindsay and Holden (2006)
Bacteriophages		
ϕSa1	*lukFM* (φPV83)	Choorit et al. (1995), Kaneko and Kamio (2004), McCarthy et al. (2012), Zou et al. (2000)
	eta (φETA)	Kuroda et al. (2001), McCarthy et al. (2012), Yamaguchi et al. (2000)
ϕSa2	*lukFS-PV*	Baba et al. (2002), Kaneko et al. (1998), Narita et al. (2001)
ϕSa3	*sea*; *selp*; *sea, selq, selk*	Baba et al. (2002), McCarthy et al. (2012)

face-associated virulence factors (Foster, 2005). These include proteins that

(1) mediate adherence to damaged tissue, extra-cellular matrix and the surface of host cells (Foster and Hook, 1998),
(2) facilitate tissue destruction and spreading,
(3) promote iron uptake (Skaar and Schneewind, 2004),
(4) bind to proteins in the bodily fluids to help evade antibody- and complement-mediated immune responses, including the action of phagocytes,
(5) lyse host cells and
(6) manipulate the innate and adaptive immune responses.

However, a clear association between virulence genes and disease symptoms has been established or is strongly suspected only for some potent *S. aureus* toxins causing, for example, toxic shock syndrome (TSS), staphylococcal scalded skin syndrome (SSSS), necrotizing pneumonia, or deep-seated skin infections (Dinges et al., 2000; Holtfreter and Bröker, 2005; Jarraud et al., 1999, 2002; Ladhani, 2003). This review focuses on such toxins, including pore-forming toxins, like PVL and hemolysin-α (Hla, α-toxin,), exfoliative toxins (ET) and the SAgs. They damage the membranes of host cells, degrade inter-cellular junctions, or modulate the immune response by aberrant activation of immune cells. Only a few *S. aureus* toxins, such as Hla and the phenol-soluble modulins (PSMs), are core genome-encoded, while most of the other toxin genes are localized on MGEs (Table 1). Hence, the species *S. aureus* is characterized by extraordinary heterogeneity regarding the toxin gene equipment of individual clinical isolates.

2. Pore-forming toxins

S. aureus can produce several toxins that damage the membranes of host cells, which can ultimately lead to cell lysis. At sublytic concentrations, these pore-forming toxins are potent cell stressors. In synergy with other danger signals such as lipoproteins that activate the toll-like receptor 2 the toxins trigger the NALP3-inflammasome response resulting in release of cytokines IL1, IL18 and IL33 (Franchi et al., 2012). Hla, hemolysin-γ (Hlg) and PVL have been shown to exert strong pro-inflammatory effects in this manner (Holzinger et al., 2012; Kebaier et al., 2012; Munoz-Planillo et al., 2009; Perret et al., 2012).

2.1. Hemolysin-α (Hla, α-toxin)

Hla is released by 95% of *S. aureus* strains as a water-soluble monomer of 33 kDa with pore-forming and pro-inflammatory properties. The *hla* gene is not mobile. Its expression is regulated by at least three global regulatory systems including the accessory gene regulator (*agr*) (Xiong et al., 2006), it is therefore not surprising that Hla is produced in varying amounts by *S. aureus* strains. Upon binding to a membrane receptor, Hla forms heptameric pores, thereby destroying a variety of host cells, including epithelial cells, erythrocytes, fibroblasts, monocytes, macrophages, and lymphocytes, but not neutrophils. The Hla receptor has long remained elusive and only recently ADAM10 (A disintegrin and metalloproteinase 10) has been identified as a binding partner of Hla (Inoshima et al., 2011; Wilke and Bubeck Wardenburg, 2010). Binding of Hla and pore formation activates the enzyme, which degrades E-cadherin in the epithelial adherens junctions (Inoshima et al., 2011). Moreover, the ADAM10-Hla complex interferes with focal adhesion complexes (Wilke and Bubeck Wardenburg, 2010). Both mechanisms would be able to disrupt the integrity of the epithelial and endothelial layers, thereby paving the way for *S. aureus* invasion. The group of Julie Bubeck Wardenburg has used murine infection models to demonstrate that Hla strongly contributes to the pathogenesis of skin infections and pneumonia induced by *S. aureus*-USA300, which produces the toxin in abundance (Bubeck Wardenburg et al., 2007a,b; Bubeck Wardenburg and