Genetic and Molecular Studies of Virulence Factors of *Staphylococcus aureus*

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Contents

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The papers have been divided into sections according to particular themes. Within each section the papers are presented in chronological order.

Section 1

Site-specific mutations in chromosomal genes.

The construction of site-specific mutations by allelic exchange in genes encoding putative virulence factors (1) paved the way for testing the role of such factors in pathogenesis using animal infection models – called testing Koch's Postulates at the molecular level. This was dependent on the isolation of site-specific mutations in targeted genes and showing that changes in phenotype were due to the mutation and not to alterations in expression of other genes.

After seminal work in Dublin with alpha-toxin and protein A (1) animal infection studies were subsequently performed by collaborators eg alpha-toxin and beta-toxin in mastitis (2), clumping factor A (ClfA, section 2) in endocarditis (3) and septic arthritis (4 and 5).

Specific mutations were employed in the studying the role of clumping factor B in nasal colonization (section 3) and fibronectin binding proteins in host cell invasion (section 4). Recent advances in genetic manipulation are described in section 6.

Section 2.

Clumping factor A, the archetypal MSCRAMM of *Staphylococcus aureus*

Genetic studies showed that clumping factor is distinct from coagulase, an important discovery to make at the time (6). Cloning and sequencing the *clfA* gene revealed the structural organization of ClfA and showed that it has features of other cell wall-anchored surface proteins and that the fibrinogen binding domain is located in the N-terminal 580 residues (7, 8). In collaboration with Magnus Hook biochemical and biophysical analysis investigated the mechanism of ligand binding (9-11). ClfA is a founder member of the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) family of surface proteins. The X-ray crystal structure of ClfA prompted formulation of the dock-lock-latch mechanism of ligand binding by Hook. This was supported by comparative analysis of ClfA and Fbl from *S. lugdunensis* (12). A function-blocking monoclonal antibody defined a second binding site for fibrinogen on ClfA and revealed a two-step ligand binding mechanism that is more complex than the original dock-lock-latch model (13). The definition of

MSCRAMMs was refined (14, 15). ClfA was also found to be a protective antigen in a rodent infection models and a non-fibrinogen binding variant of recombinant ClfA protein was a superior protective antigen compared to the wild type (5). This discovery was patented and subsequently licensed by GlaxoSmithKline.

Section 3.

Clumping factor B and nasal colonization

Southern blot hybridization analysis of chromosomal DNA using a *clfA* gene probe suggested the presence of genes encoding proteins related to ClfA (7). The first to be cloned, sequenced and analysed at the molecular level was clumping factor B (ClfB) (16). This protein had similar structure and sequence organization to ClfA and was shown to bind fibrinogen thus contributing to adhesion and clumping.

ClfB was shown to bind to cytokeratin 10 (CK10), a protein that occurs in desquamated epithelial cells (squames) in human skin and the anterior nares (17). The binding site in CK10 was localized to the C-terminal region. This is composed of omega loops rich in Gly-Ser repeats (18). The binding site in the α-chain of fibrinogen is also a Gly-Ser rich sequence. X-ray crystal structure analysis of ClfB in the apo form and complexed with fibrinogen and CK10 peptides revealed that ligands bound by the dock-lock-latch mechanism (14, 15, 19).

The ability of ClfB to bind CK10 is important in bacterial adherence to nasal squames (17). Using genetically manipulated strains ClfB was found to promote colonization of the nares of mice (20) and human volunteers (21). Active immunization with recombinant ClfB protein and passive immunization with a function-blocking monoclonal antibody reduced nasal carriage (20). ClfB also bound loricrin, a major protein component of the cornified envelope of squames. This interaction is also important in mouse nasal colonization, a conclusion reinforced by studying loricrin knockout mice (22). ClfB is also important in bacterial binding to deformed corneocytes in the inflamed skin of eczema sufferers (23).

Section 4

The multifunctional fibronectin-binding proteins

Binding of *S. aureus* to fibronectin was shown to be mediated by two related fibronectin binding proteins (FnBPs) (24). This is important for adherence to and invasion of host epithelial and endothelial cells (25), a process that is now known to occur by endocytosis with fibronectin acting as a bridge between the fibronectin-binding repeats of FnBPs and an integrin.

Staphylococcal binding to elastin was originally thought to be mediated by the elastin binding protein EbpS (26). While EpbS promoted binding to soluble elastin peptides, bacterial adherence to elastin in the solid phase was found to be a property of FnBPs (27). The N-terminal A domain of FnBPs are related to ClfA and ClfB. They promote binding to elastin (as well as fibrinogen). Modelling the A domain of FnBP indicated that the same residues interact with both ligands most likely by the dock-lock-latch mechanism (14, 15, 28).

Some *S. aureus* clinical isolates form biofilm mediated by homophilic interactions between FnBPs. For FnBPA homophilic binding occurs at the N2 and N3 subdomains of region A by a mechanism that

is distinct from dock-lock-latch (29). Force microscopy studies by Dufrene revealed that cell-cell adhesion occurs by multiple low affinity homophilic bonds (30).

Another FnBPA A domain ligand is plasminogen as shown by Speziale. Bound plasminogen can be activated to plasmin which likely helps bacteria survive in the infected host. Binding occurs via patches of lysine residues on subdomain N3 and kringle 4 of the host protein (31)

Section 5

Evasion of innate immunity promoted by surface proteins

In the early 2000s there was growing evidence that *S. aureus* elaborates a plethora of factors that promote innate immune evasion (reviewed in reference 32). Early work focussed on secreted proteins and apart from protein A there was no known role for surface proteins. Then we showed that ClfA contributes to evasion of neutrophil phagocytosis by both fibrinogen-dependent and fibrinogen-independent mechanisms (33). Cunnion suggested that ClfA bound and activated the host C3-degrading protease factor I (34). The iron-regulated surface determinant protein IsdH and the second immunoglobulin binding protein Sbi both promoted evasion of neutrophil phagocytosis and survival in human blood, the former by accelerating C3b degradation (35, 36). Unlike classical wall-anchored proteins Sbi was shown to be attached non-covalently by binding to lipoteichoic acid and it was also found to be present extracellularly. Both forms contributed to immune evasion (36, 37).

Section 6.

Improved genetic manipulation

The ability to manipulate *S. aureus* genetically had been limited to a few laboratory strains because of extensive restriction barriers. The major barrier to transfer of plasmid DNA from *Escherichia coli* to *S. aureus* was found to be a type I restriction system (SauI) that cleaves cytosine methylated DNA (38). Preparation of plasmid DNA in the *E. coli* cloning host (DC10B) that lacked the ability to methylate cytosine bases allowed SauI to be by-passed. However the efficiency of transformation was still impaired by type IV restriction-modification systems. Access to all clinical isolates of *S. aureus* irrespective of the number of or type of type IV system was accomplished by constructing DC10B variants that could modify plasmid DNA to carry the type IV adenine methylation profiles of key lineages (39). A streamlined method for isolating mutations by allelic exchange was devised (38). Advances in staphylococcal genetics were reviewed (40).

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