National Institute of Allergy and Infectious Diseases

VRBPAC Meeting

Staphylococcus aureus – a dangerous and difficult-to-tackle pathogen

November 7th, 2017

NIAID

National Institute of Allergy and Infectious Diseases

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S. aureus causes a multitude of infections

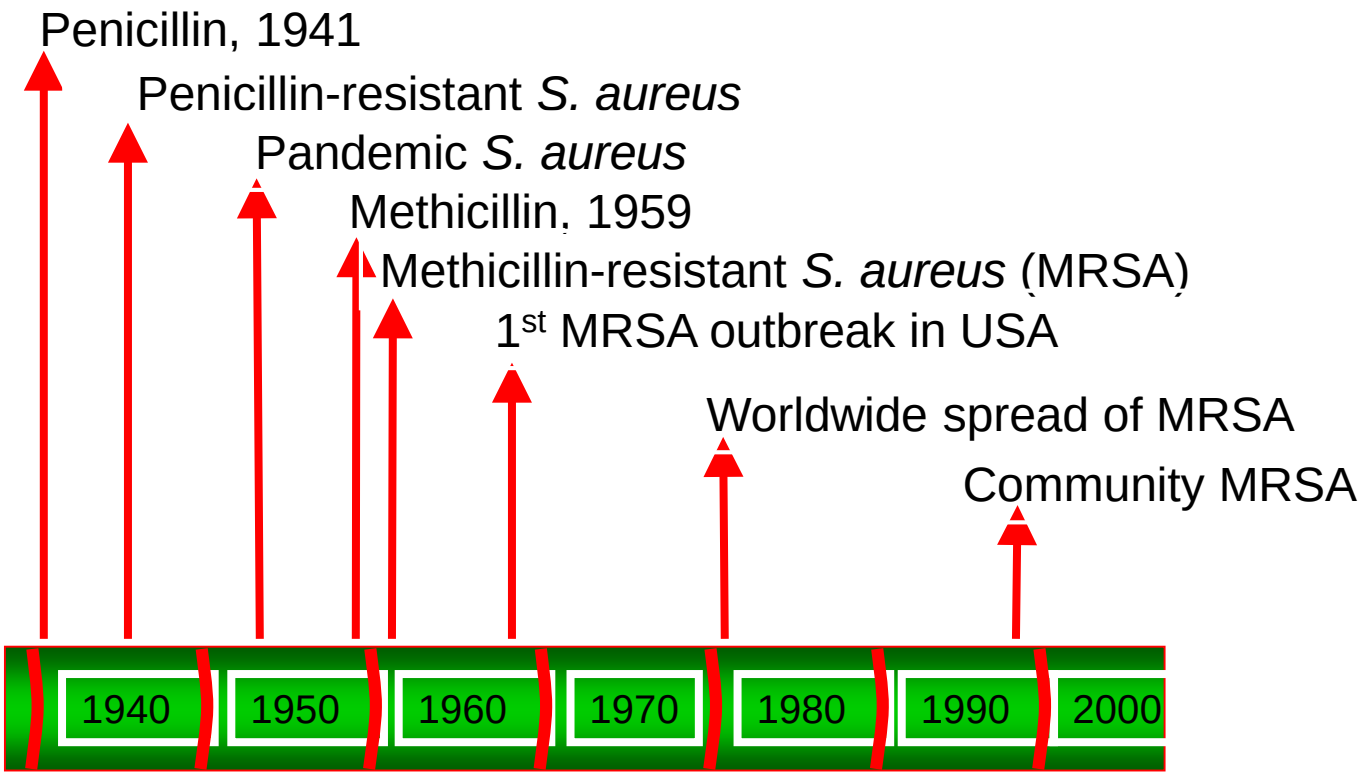
- Lung infections (Pneumonia, influenza-associated)
- Skin infections (mostly moderately severe, rarely necrotizing fasciitis)
- Bone infections (e.g., osteomyelitis)
- Blood infections (bacteremia, sepsis)
- Indwelling medical device associated infections (e.g., endocarditis)
- Specific toxin-related diseases (toxic shock syndrome, food-borne illness)

S. aureus is a leading cause of death in hospitalized patients

- "*Staphylococcus aureus* bacteremia (SAB) is an important infection with an incidence rate ranging from 20 to 50 cases/100,000 population per year. Between 10% and 30% of these patients will die from SAB. Comparatively, this accounts for a greater number of deaths than for AIDS, tuberculosis, and viral hepatitis combined." (van Hal et al. *Clin Microbiol Rev* 2012)
- In the U.S., invasive (serious) MRSA infections occur in ~ 94,000 people each year causing ~ 19,000 deaths. (Klevens et al., *JAMA* 2007)

85% of those are hospital-associated.

Antibiotic resistance in *S. aureus*: MRSA



Modified from Fitzgerald & Musser, *MRSA: Current Perspectives*, 2003

Community-associated MRSA

- “Resistance and virulence converge” (H. F. Chambers)
- Characteristically as virulent as many MSSA and more virulent than hospital-associated MRSA
- 15% of invasive MRSA infections
- Pulsed-field type USA300 in the U.S.
- Increasing on a global scale with a multitude of genetically divergent strains
- USA300 now also frequent in hospitals

Strategies to overcome the problem of AMR in *S. aureus*

- Novel antibiotics
- Anti-virulence drugs
- Probiotics
- mAbs (toxin-directed)
- Vaccines

Who could benefit from an *S. aureus* vaccine?

Table 1
Target populations for a *Staphylococcus aureus* vaccine

Active immunisation	Passive immunisation
Haemodialysis patients	Patients undergoing emergency surgery
Residents of nursing homes and other long-term care facilities	Patients implanted with intravascular or prosthetic devices
Men who have sex with men	Trauma victims
Military personnel	Immunocompromised individuals
Prisoners	Low birthweight neonates
Patients undergoing elective surgery	Patients in intensive care units
Individuals with diabetes	
Patients with HIV	
Intravenous drug users	
Healthcare providers	
Athletes	
School children	

HIV, human immunodeficiency virus.

Immunocompromised
Time constraint

Schaffer & Lee *Int J Antimicrob Agents* 2008

S. aureus infections are mainly controlled by innate host defense

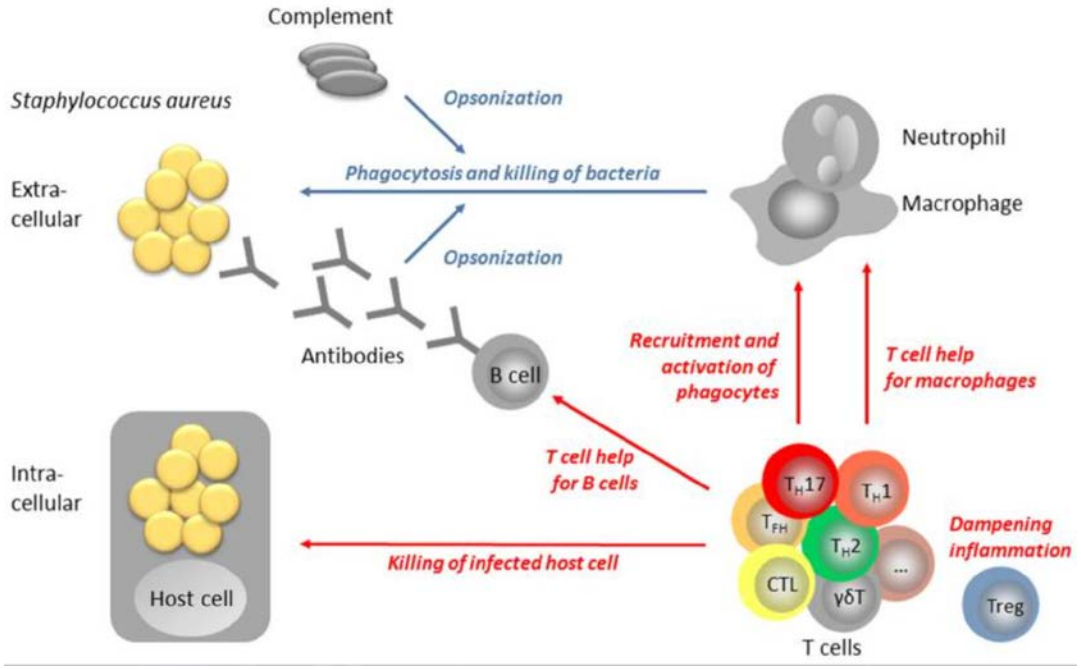
- Previous *S. aureus* infection does not protect from re-infection.
- *S. aureus* infection is controlled by innate host defense (mostly neutrophils).
- Patients with defects in innate host defenses (e.g., CGD patients) are much more prone to develop *S. aureus* infection.
- The role of protective immunity in staphylococcal infections, if any, is poorly understood.

S. aureus has multiple factors to subvert innate host defenses

- Many proteins that block pathogen recognition receptors (PRRs) and complement cascade
- Toxins that attack white blood cells: Leukotoxins [AB (GH), ED, SF (PVL)], PSMalpha
- Toxins that attack a wide variety of cell types including leukocytes: alpha-toxin, PSMalpha, gamma-toxin, delta-toxin
- Toxins that destroy leukocytes after phagocytosis: LukAB (GH), PSMalpha: a particular problem for drug and vaccine development
- Systems to sense and evade the activity of antimicrobial peptides (AMPs): Dlt, MprF, surface polysaccharides, sensor Aps (Gra)

The role of adaptive immunity in *S. aureus* infections

- Humans produce antibodies to staphylococcal antigens, but they do not seem to offer significant protection.
- Protective immunity seen in animal infection models is not completely understood mechanistically, but appears to be mediated by T-cells.
- Role of T-cell mediated protection is being unraveled.



Broeker et al.
Pathogens 2016

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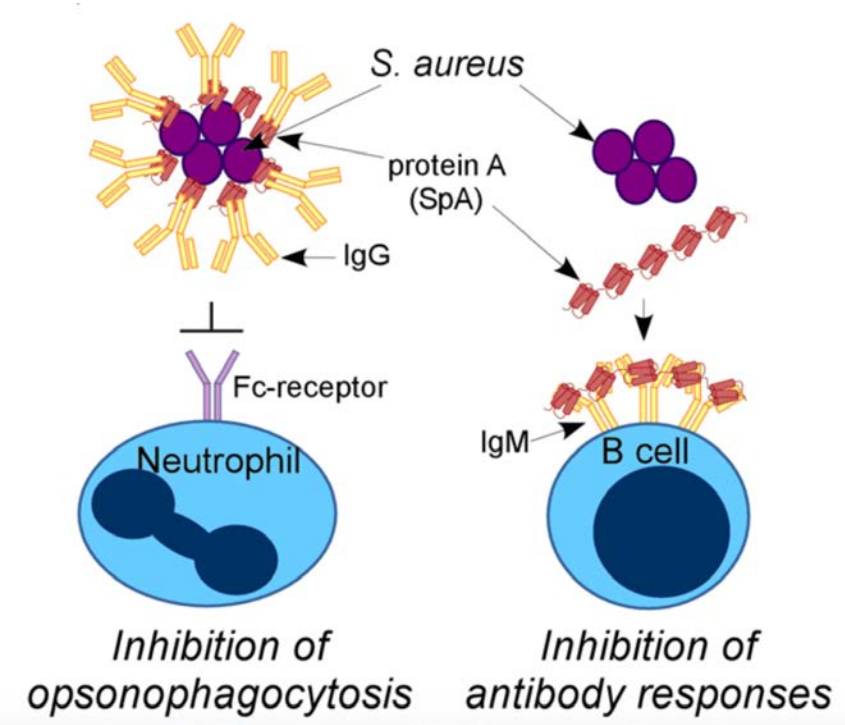
Dual mechanisms of Protein A to subvert antibody-mediated opsonophagocytosis

- SpA present on the surface of *S. aureus* binds the Fc region of antibody, thereby inhibiting opsonophagocytosis.
- Alternatively, SpA binds the Fab regions of the B-cell receptor, which induces B-cell death and prevents the production of antibody specific for *S. aureus*.



O. Schneewind
http://www.who.int/immunization/research/forums_and_initiatives/4_OSchneewind_Staphylococcal_Vaccines_gvirf16.pdf?ua=1

S. aureus circumvents killing after phagocytosis

S. aureus is opsonized by bacteria-specific antibody and serum complement, which promote rapid binding and uptake of the bacteria by neutrophils. After uptake, S. aureus uses multiple mechanisms to survive and cause the death of the cell, allowing the escape of sequestered bacteria.

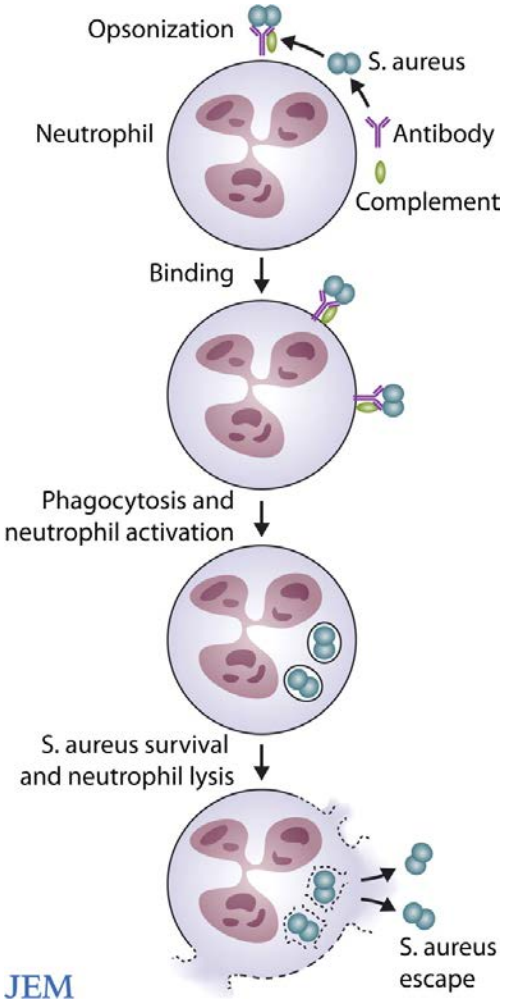
Mechanisms of Resistance:

Resistance to ROS-, AMP- mediated killing

Neutrophil-lytic toxins:

PSMalpha

LukAB (GH)



DeLeo & Otto
J Exp Med 2008 JEM

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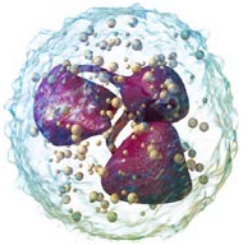
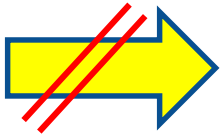
S. aureus survives intracellularly

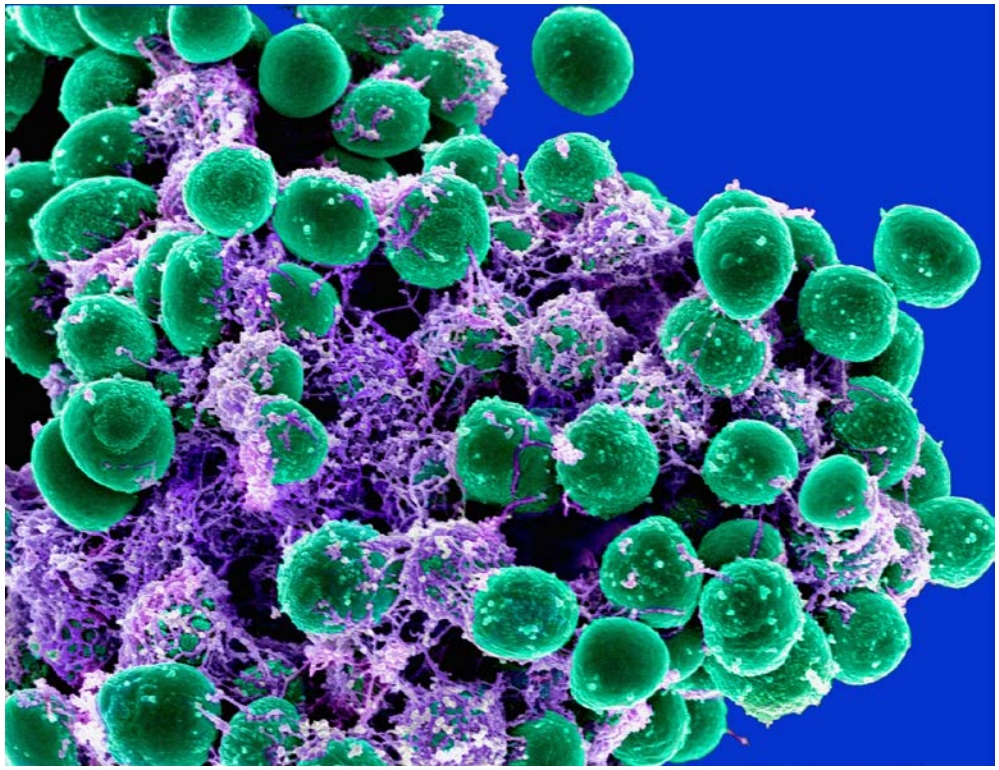
- Instead of killing leukocytes after phagocytosis, *S. aureus* may persist intracellullarly.
- Intracellular *S. aureus* may provide for immune evasion and recurrent, persistent infection.
- Role of small colony variants (SCVs).

Biofilms broadly inhibit immune defenses

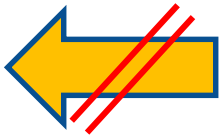


innate





adaptive



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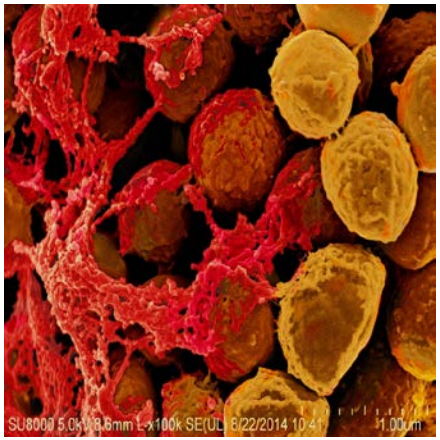
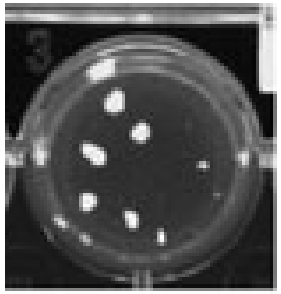
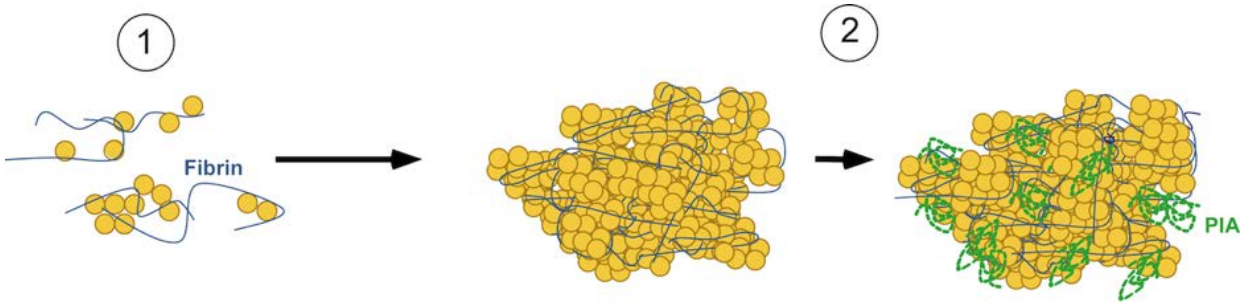
S. aureus forms large biofilm-like aggregates in synovial fluid



After 20 min in SF, fibers cover bacterial clumps and macroscopic aggregates develop.



Likely a common phenotype in all orthopedic infections



Initial binding to fibrin and fibronectin mediated by ClfA, ClfB, FnbA, FnbB

Low activity of Agr causes low PSM production, resulting in increased cell-cell attachment and surface attachment of the biofilm matrix molecule PIA

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Dastgheyb et al. *J Infect Dis* 2015
Dastgheyb et al. *Infect Immun* 2015

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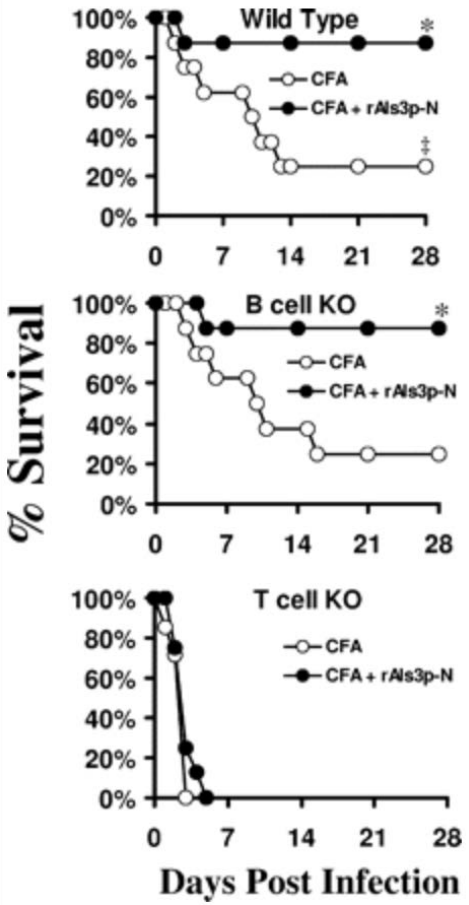
Evidence for the role of immune mechanisms in *S. aureus* systemic infection from knockout mice

B-cell-deficient: no difference

Hypersusceptibility:
T-cell-deficient
IFN-gamma-deficient
TNF-deficient
IL 17A/F-deficient

There is T-cell and possibly innate immunity-mediated protective memory against *S. aureus* infections in mice.
In humans?



Spellberg et al. *Infect Immun* 2008
Lin et al. *PloS Pathog* 2009
Hultgren et al. *J Immunol* 1998
Ishigame et al. *Immunity* 2009

Previous active vaccination attempts

Drug	Company	Target	Status
StaphVAX	**NABI**	**CP5/CP8**	**Failed phase 3**
Pentastaph	NABI (=> GSK)	CP5/CP8/WTA/PVL/ alpha-toxin	Phase 1 (PVL/alpha-toxin) ended
V710	**Merck**	**IsdB**	**Failed phase 3**
NVD3	Novadigm	Als3	Phase 1/2
STEBVax	IBT	SEB	Phase 1
SA3Ag	Pfizer	CP5/CP8/ClfA	Phase 1
SA4Ag	**Pfizer**	**CP5/CP8/ClfA/MntC**	**Phase 2b**

S. aureus vaccines: previous phase-III attempts

- StaphVax (Nabi)
- Antigen: Capsular polysaccharides CP5 and CP8
- Failed phase III (Hemodialysis patients)
- Likely problems: poor protective efficacy, modest contribution to virulence
- not present in important clinical strains (e.g., USA300)
- V710 (Merck)
- Antigen: IsdB (surface protein involved in iron uptake)
- Failed phase II/III in patients undergoing cardiothoracic surgery with median sternotomy (no efficacy; safety concerns)

S. aureus vaccines: optimal antigen characteristics

- Immunogenicity in humans
- Surface location
- Essential for in-vivo survival (growth, virulence)
- Widespread in epidemiologically important strains

Pfizer SA4Ag vaccine targets

- Recombinant mature form of ClfA
- Conjugated CP5 & CP8
- Recombinant MntC

Pfizer vaccine targets: CP5/CP8

- No efficacy in StaphVax trial
- Non-capsulating strains occur in ~ 20%, includes USA300. Cocchiaro et al. *Mol Microbiol* 2006
- Orthopedic/surgical site infections in the U.S. appear to be predominantly due to USA300. Kourbatova et al. *Am J Infect Control* 2005; Patel et al. *J Clin Microbiol* 2007
- Only modest contribution to virulence O'Riordan & Lee *Clin Microbiol Rev* 2004
- Capsule-negative mutants are more virulent than the parental isolates in catheter-induced endocarditis model. Baddour et al. *J Infect Dis* 1992

Pfizer vaccine targets: ClfA

- Surface protein, fibrinogen/fibrin-binding
- Nasal colonization factor
- Virulence factor in bloodstream infection, septic arthritis, skin infection Tkaczyk et al. *MBio* 2016; Malachowa et al. *PLoS ONE* 2016; Joseffson et al. *J Infect Dis* 2001
- Anti-ClfA Abs efficacious in mouse models for prevention of sepsis, in combination with alpha-toxin Ab or thrombin inhibitors

  Tkaczyk et al. *MBio* 2016, McAdow *PLoS Pathog* 2011
- passive immunization with human anti-ClfA-enriched intravenous (i.v.) immunoglobulin (Ig) (Veronate) did not result in significantly changed rates of late-onset sepsis. De Jonge et al. *J Pediatr* 2007; an anti-ClfA mAb (Aurexis) failed to treat *S. aureus* bacteremia Weems et al. *Antimicrob Agents Chemother* 2006
- Conflicting results of targeting ClfA alone by active immunization Li et al. *MBio* 2016 (mouse abscess, surgical wound, bacteremia); Joseffson et al. *J Infect Dis* 2001 (mouse arthritis); Narita et al. *Infect Immun* 2010 (mouse bacteremia)

Pfizer vaccine targets: MntC

- Manganese transporter; conserved in staphylococci
- Active vaccination with MntC reduced bacterial load (~ 1 log) in murine bacteremia model (*S. aureus* & *S. epidermidis*)
- Anti-MntC mAbs reduced CFUs in rat intraperitoneal infection model (~ 1 log).

  Anderson et al. *J Infect Dis* 2012

- Membrane-inserted protein (lipoprotein): accessibility?