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Specific serum IgG at diagnosis of *Staphylococcus aureus* bloodstream invasion is correlated with disease progression

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ABSTRACT

Although *Staphylococcus aureus* is a prominent cause of infections, no vaccine is currently available. Active vaccination relies on immune memory, a core competence of the adaptive immune system.
To elucidate whether adaptive immunity can provide protection from serious complications of *S. aureus* infection, a prospective observational study of 44 patients with *S. aureus* infection complicated by bacteremia was conducted. At diagnosis, serum IgG binding to *S. aureus* extracellular proteins was quantified on immunoblots and with Luminex-based FLEXMAP 3D™ assays comprising 64 recombinant *S. aureus* proteins. Results were correlated with the course of the infection with sepsis as the main outcome variable.
S. aureus-specific serum IgG levels at diagnosis of *S. aureus* infection were lower in patients developing sepsis than in patients without sepsis ($P < 0.05$). The pattern of IgG binding to eight selected *S. aureus* proteins correctly predicted the disease course in 75% of patients.
Robust immune memory of *S. aureus* was associated with protection from serious complications of bacterial invasion. Serum IgG binding to eight conserved *S. aureus* proteins enabled stratification of patients with high and low risk of sepsis early in the course of *S. aureus* infections complicated by bacteremia.
Significance: *S. aureus* is a dangerous pathogen of ever increasing importance both in hospitals and in the community. Due to the crisis of antibiotic resistance, an urgent need exists for new strategies to combat *S. aureus* infections, such as vaccination. To date, however, all vaccine trials have failed in clinical studies. It is therefore unclear whether the adaptive immune system is at all able to control *S. aureus* in humans.
The paper demonstrates the use of proteomics for providing an answer to this crucial question. It describes novel results of a prospective study in patients with *S. aureus* infection complicated by bloodstream invasion. Immune proteomic analysis shows that robust immune memory of *S. aureus* – reflected by strong serum IgG antibody binding to *S. aureus* antigens – is associated with clinical protection from sepsis. This lends support to the notion of a vaccine to protect against the most serious complications of *S. aureus* infection. Hence, the data encourage further efforts in vaccine development.

1. Introduction

Staphylococcus aureus is a serious pathogen in both hospitals and the community, but also a common commensal [1–3]. In spite of intensive efforts, there is no vaccine to protect against *S. aureus* infections [4,5]. Active vaccination strategies rely on immune memory, a core competence of the adaptive immune system, comprised of T cells, B cells and antibodies. This raises the question of what the adaptive immune system can contribute to protection against *S. aureus*.

Both *S. aureus* carriers (around 20% of adults) as well as non-carriers harbor serum antibodies specific for a broad spectrum of *S. aureus* proteins and non-protein antigens [6,7]. Clearly, encounters of *S. aureus* with its human host do not lead to sterile immunity, nor do they prevent

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bacterial invasion, since carriers have an increased risk of *S. aureus* infection mostly with their colonizing strain [1,8].

However, there are indications that the adaptive immune system may confer clinical protection against severe *S. aureus* infection. First, *S. aureus* bloodstream infection has a better outcome in carriers than in non-carriers, which could be due to the generation of strain-specific immune memory during colonization [9,10]. Moreover, functional defects of adaptive immunity increase the risk of severe *S. aureus* infection in humans and animal models. The Th17 T cell subpopulation is currently receiving much attention in this context [11–15]. Finally, good protection has been achieved in animal models with active and passive vaccines targeting a variety of *S. aureus* proteins [16,17].

The natural human antibody response to *S. aureus* is characterized by pronounced heterogeneity [6,18], reflecting the immune memory formed during an individual's encounters with *S. aureus*. We hypothesized that upon bacterial invasion, immune memory of *S. aureus* will confer clinical protection, and the *S. aureus*-specific antibody response might permit patient stratification. To test this hypothesis, we conducted a prospective observational study with 44 patients diagnosed with *S. aureus* infection complicated by bacteremia.

2. Patients, materials and methods

2.1. Clinical study design

In a prospective observational study, sera from a convenience sample of patients with *S. aureus* infection complicated by bacteremia were collected at diagnosis as previously described [19]. Human experimentation guidelines of the United States Department of Health and Human Services and those of the authors' institution(s) were followed while performing of clinical research. The study was approved by the University of Maryland Baltimore Institutional Review Board and was granted a waiver of informed consent. Patients with AIDS, severe immune suppression other than AIDS and symptoms of infection more than 4 days prior to infection presentation were excluded. Patients were monitored for three days after the first positive blood culture for the presence or development of sepsis. The outcome criteria for uncomplicated sepsis, severe sepsis and septic shock were adapted from the American College of Chest Physicians and Society for Critical Care Medicine definition [20]. Other variables shown in Table 1 were abstracted from the medical record.

2.2. S. aureus isolates, protein extracts and bacterial cell preparations

Infecting *S. aureus* isolates were molecularly typed based on their *spa*-sequences, and virulence genes encoding superantigens, exfoliative toxins, as well as PVL were identified by multiplex-PCR [21]. Extracellular protein extracts were obtained from a protein A gene (*spa*) deletion mutant of *S. aureus* USA300 (USA300Δ*spa*) cultivated to stationary phase under iron-restricted conditions [22]. Whole bacterial cells were washed and UV-inactivated. Recombinant *S. aureus* proteins were obtained from Protagen AG (Dortmund, Germany).

2.3. Quantification of serum IgG binding to S. aureus

Serum IgG binding to extracellular bacterial protein extracts was quantified by semi-automated immunoblotting (Peggy Simple Western Assay). Binding to the *S. aureus* cell surface was determined by ELISA. Finally, serum IgG specific to 64 recombinant *S. aureus* proteins was measured using FLEXMAP 3D™ technology, and normalized mean fluorescent intensities were calculated as measures of antibody binding intensity.

2.4. Data analysis and statistics

Binding data obtained with immunoblot or ELISA were compared using the Mann–Whitney test (two groups) or the Kruskal–Wallis test with Dunn's post-test (more than two groups). *P*-values below 0.05 were considered statistically significant. Principle component analysis (PCA), partial least square analysis (PLS) and prediction analysis of the FLEXMAP 3D™ data were performed using the Analyst Software (Genedata, Basel, Switzerland).

The supporting information contains additional details about patients, materials and methods.

Table 1
Comparison of the characteristics of immune-competent, hospitalized adults with a maximum of 4 days of symptoms at the time of *S. aureus* bacteremia by the development of sepsis.

	Sepsis (n = 19)	No sepsis (n = 21)	Odds ratio[a] (CI)	*P*-value
Demographics				
Mean age (±SD)	59 ± 13	57 ± 18		0.75
Gender			0.99 (0.29–3.43)	1.0
Female	9	10		
Male	10	10		
Race				0.63
African American	11	15		
White	7	6		
Unanswered	1	2		
Prior *S. aureus* infection			1.52 (CI 0.34 to 6.76)	0.71
Yes	5	4		
No	14	17		
Prior MRSA infection or colonization				0.71
Yes	7	6		
No	10	11		
No data	2	4		
Dialysis patient			0.48 (CI 0.12 to 1.81)	0.33
Yes	5	9		
No	14	12		
Diabetes mellitus patient			0.53 (CI 0.13 to 2.23)	0.49
Yes	4	7		
No	15	14		
Infection Characteristics				
Bacteremia type (primary?)			5.23 (CI 0.95 to 28.9)	0.069
Primary	17	13		
Secondary	2	8		
If secondary bacteremia, type			1.0 (CI 0.03 to 33.4)	1.0
SSTI	2	7		
UTI	0	1		
Nosocomial infection			2.31 (CI 0.56 to 9.47)	0.31
Yes	15	13		
No	4	8		
Current MRSA[b]			0.46 (CI 0.12 to 1.79)	0.32
Yes	11	15		
No	8	5		
Days since presentation of symptoms	0.9 ± 0.3 (n = 18)	1.3 ± 0.3		0.35

Abbreviations: CI, confidence interval; MRSA, methicillin-resistant *S. aureus*; SD, standard deviation; SSTI skin and soft tissue infection; UTI, urinary tract infection.
[a] Odds ratio for sepsis development.
[b] Reflects the current infection isolate. Data of one non-septic patient were missing.

3. Results

3.1. Infecting S. aureus isolates

Of the 44 infecting *S. aureus* isolates, 14 (35%) belonged to the *spa* type t008 and most of these were further characterized by *agr* type 1, *pvl* and the superantigen gene *seq*, indicating that these strains represent USA300 strains. The remaining isolates were of mixed *spa* types