Journal of Proteomics 128 (2015) 1–7

Contents lists available at ScienceDirect

Journal of Proteomics

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

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

Sebastian Stentzel [a,*], Nandakumar Sundaramoorthy [b,c], Stephan Michalik [b,c], Maria Nordengrün [a], Sarah Schulz [a], Julia Kolata [a], Peggy Kloppot [d], Susanne Engelmann [d,e,f], Leif Steil [b], Michael Hecker [d], Frank Schmidt [b,c], Uwe Völker [b], Mary-Claire Roghmann [g], Barbara M. Bröker [a,*]

[a] Institute of Immunology and Transfusion Medicine, Department of Immunology, University Medicine Greifswald, 17475 Greifswald, Germany
[b] Interfaculty Institute of Genetics and Functional Genomics, University Medicine Greifswald, 17475 Greifswald, Germany
[c] ZIK-FunGene Junior Research Group "Applied Proteomics", University Medicine Greifswald, 17475 Greifswald, Germany
[d] Institute for Microbiology, University of Greifswald, 17489 Greifswald, Germany
[e] Microbial Proteomics Research Group, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany
[f] Institute for Microbiology, Technical University of Braunschweig, 38106 Braunschweig, Germany
[g] Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD 21201, USA

ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 11 May 2015
Accepted 30 June 2015
Available online 5 July 2015

Keywords:
Bacteremia
Human
IgG
Protection
Serum antibodies
Staphylococcus aureus

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

* Corresponding authors at: Institut für Immunologie und Transfusionsmedizin, Universitätsmedizin Greifswald, Ferdinand-Sauerbruchstraße/DZ7, 17475 Greifswald, Germany.
E-mail address: broeker@uni-greifswald.de (B.M. Bröker).

http://dx.doi.org/10.1016/j.jprot.2015.06.018
1874-3919/

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

or could not be clustered. None of the isolates were positive for the exfoliative toxins encoding genes *eta* or *etd* (Table S1).

3.2. Serum IgG binding to S. aureus cells or extracellular proteins

USA300, the most frequent cause of bloodstream invasion in this cohort, was selected for quantification of *S. aureus*-specific serum IgG. A protein-A gene deletion mutant, USA300Δ*spa*, was generated to avoid non-specific antibody binding. Patient sera were obtained at diagnosis, no later than four days after onset of symptoms, and IgG binding to extracellular bacterial proteins was measured (n = 46). Total IgG binding varied by a factor of 27 (range: 65140–1763988 AUC, Fig. 1A). Patients who subsequently developed sepsis – uncomplicated sepsis, severe sepsis or septic shock – showed lower IgG binding to *S. aureus* extracellular proteins ($P = 0.0481$, Fig. 1B). Moreover, IgG binding to extracellular *S. aureus* proteins decreased with increasing sepsis severity (Fig. 1D). On the other hand, 7 out of 11 patients in the lower quartile of the antibody binding values developed sepsis, but only 3 out of 11 in the upper quartile did so (Fig. 1E). Similarly, IgG binding to the bacterial surface was lower in the sepsis group than in patients who remained sepsis-free ($P = 0.0409$, Fig. 1C).

3.3. Serum IgG binding to selected S. aureus proteins

To identify antibody specificities associated with protection against sepsis, IgG binding to 64 recombinant *S. aureus* proteins was measured, comprising extra-cellular, surface bound and cytoplasmic *S. aureus* proteins (Table S2). 44 sera were available for this analysis (sera obtained before or at onset of symptoms). At first, patients were grouped into (i) no sepsis, (ii) uncomplicated sepsis, and (iii) severe sepsis or septic shock and results were subjected to a partial least squares analysis (PLS) (Fig. 2A). Patients with uncomplicated sepsis and severe sepsis/septic shock clustered together in one area and were hence handled as one group, "sepsis", in a second PLS analysis. This resulted in a separation from patients without sepsis, as shown in Fig. 2B.

The top 20 *S. aureus* proteins responsible for the discrimination are listed in Table 2. Almost all of these proteins belonged to the extracellular proteome, which elicited the strongest antibody binding, as expected (Fig. 3). They included known toxins and virulence factors of *S. aureus* and also proteins of unknown function. Further analysis with a support vector machine revealed the best discrimination between patients with and without sepsis by applying Fisher linear discriminant analysis to the intensities of IgG binding to the top 8 proteins: phospholipase C (Plc), staphopain B (SspB), the immunodominant staphylococcal protein A (IsaA), the staphylococcal exotoxin M (SEM), glycerophosphoryl diester phophodiesterase (GlpQ), the C component of γ-hemolysin (HlgC) and two proteins of unknown function, SACOL0444 and SACOL0985. To validate this result, a principal component analysis was performed with serum IgG binding to the top 8 proteins upon diagnosis. The degree of separation of patients with and without subsequent sepsis is depicted in Fig. 2C. Sepsis was correctly predicted by Fisher linear discriminant analysis in 16 of 21 cases and no sepsis in 17 out of 23 cases; thus, the prediction was correct in 75% of the cases (sensitivity 76%; specificity 74%). IgG binding to the top 8 proteins differed significantly between patients who subsequently developed sepsis or no sepsis as depicted in Fig. 2D. However, the two groups could not be separated on the basis of antibody binding to single *S. aureus* proteins.

Details of the data analysis are provided in the supporting material.

4. Discussion

At diagnosis of *S. aureus* bloodstream infection, serum IgG binding to the bacterial extracellular proteome was inversely correlated with the risk of sepsis development. We observed an almost 30-fold variation in total serum IgG binding to extracellular *S. aureus* proteins. Very high *S. aureus*-specific IgG serum levels characterized a subgroup of patients who almost never experienced sepsis. The fate of patients with medium to low intensity IgG binding was non-uniform. Antibody binding to extracellular *S. aureus* proteins and *S. aureus* cells discriminated both groups equally well.

An immune proteome signature of eight *S. aureus* proteins predicted the disease course of bacteremia patients with 75% accuracy (76% sensitivity and 74% specificity). This proteomic signature was mainly

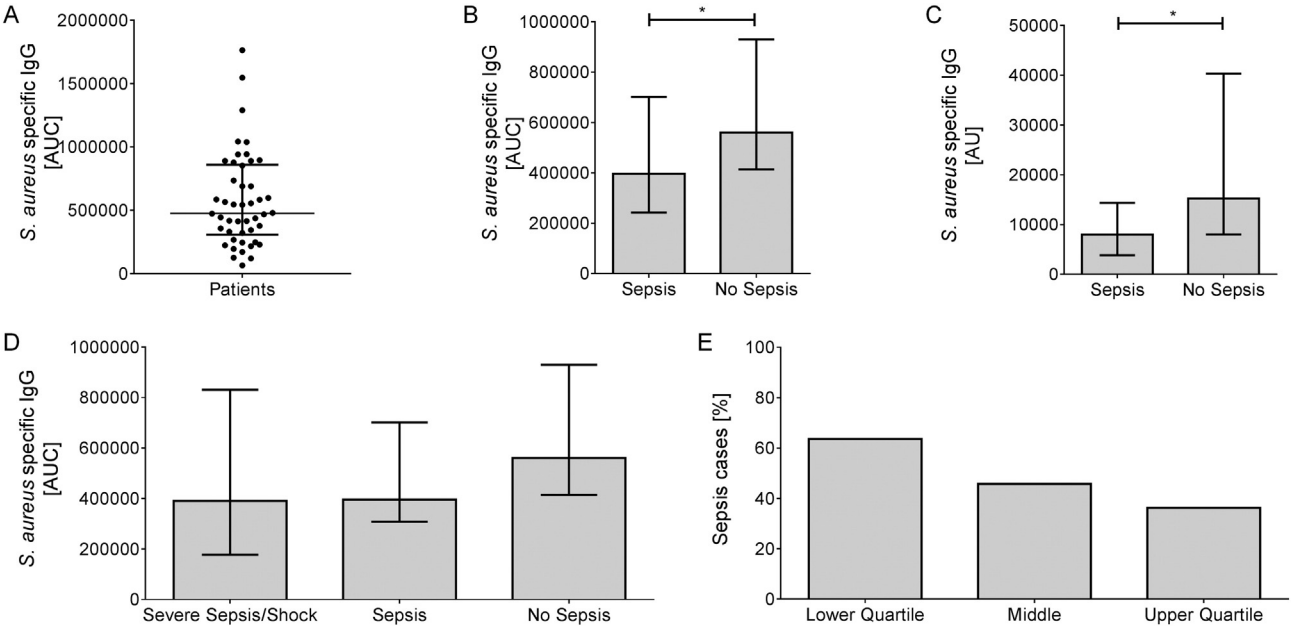

Fig. 1. Association between *S. aureus* specific serum IgG and outcome of bacteremia. Binding of IgG to extracellular *S. aureus* proteins (A, B, D and E) and whole *S. aureus* cells (C) was determined by immunoblot – Peggy Simple Western Assay – and ELISA, respectively. At diagnosis, binding of IgG to extracellular *S. aureus* proteins varied strongly between patients (A). Patients with high amounts of *S. aureus*-specific IgG had a significantly better disease outcome (B [$P = 0.0481$; Mann–Whitney], D and E). This could also been shown for IgG binding to *S. aureus* cells (C [$P = 0.0409$; Mann–Whitney]). Figures show medians with interquartile ranges. Abbreviations: AUC, area under curve; AU, arbitrary units.

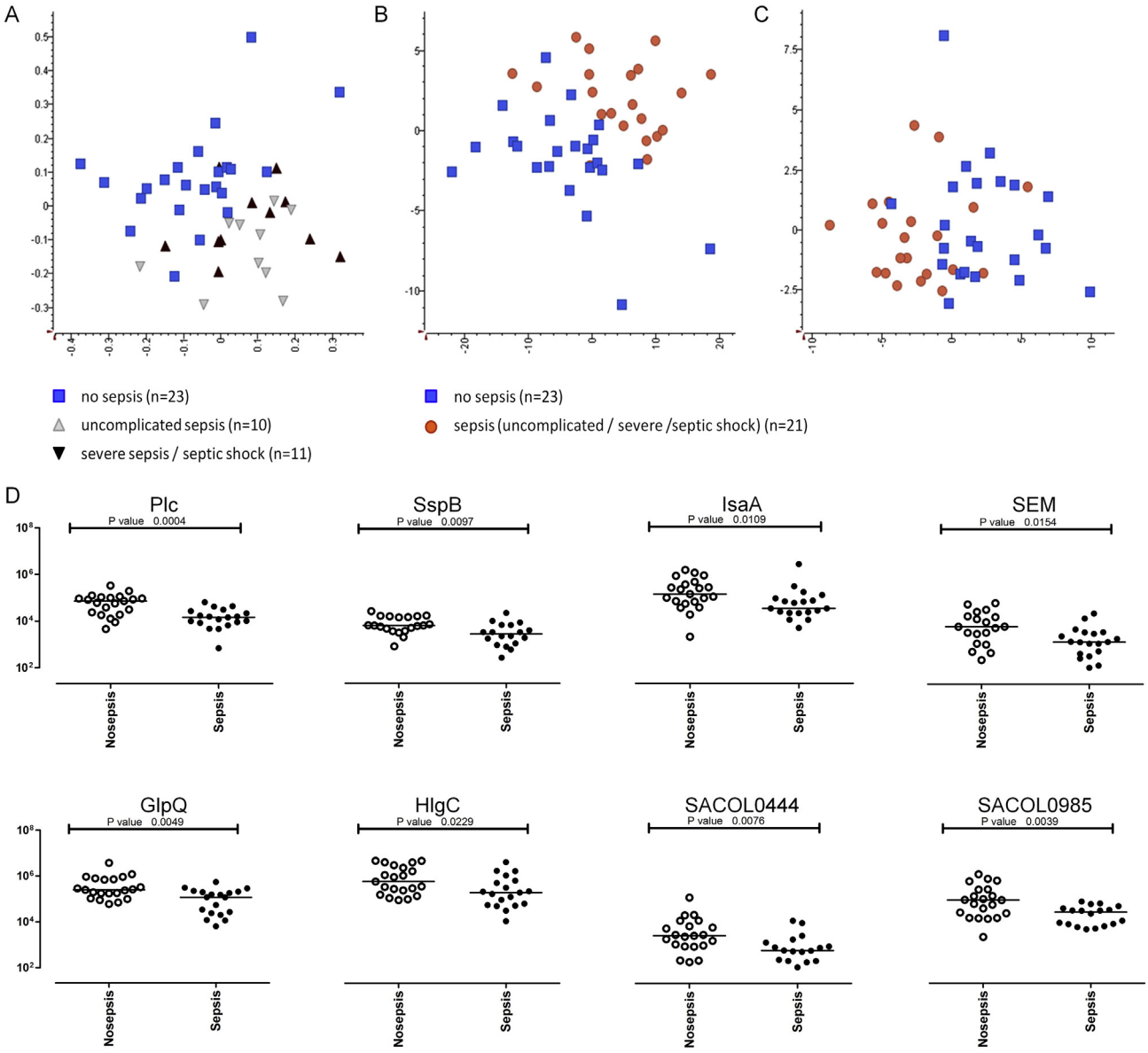

Fig. 2. Stratification of patients according to disease progression (sepsis or uncomplicated recovery) based on *S. aureus*-specific antibodies. Quantification of IgG specific to 64 *S. aureus* proteins was performed with FLEXMAP 3D™ technology. Patients with uncomplicated sepsis and severe sepsis or septic shock grouped together (as sepsis) in partial least squares analysis (PLS) (A). PLS showed good separation of patients with sepsis – including uncomplicated sepsis, severe sepsis and septic shock – from patients with no sepsis (B). Using the eight most discriminating *S. aureus* proteins for a principal component analysis (see also Table 2) enabled stratification of septic and non-septic patients at diagnosis of *S. aureus* bloodstream invasion (C). Differences of quantified median IgG binding to these eight proteins between septic and non-septic patients are shown in D.

comprised of conserved extracellular *S. aureus* proteins: with the exception of the superantigen SEM, all belong to the *S. aureus* core genome [23]. This was expected because patients were not stratified according to the type of the infecting *S. aureus* isolate, and many *S. aureus* toxin genes – such as those encoding superantigens and other toxins – are highly variable in the species *S. aureus*, as are serum antibodies specific to them [10,24]. Keeping in mind that the infecting *S. aureus* isolates in the present study reflect the epidemiological situation in the US, the results should be regarded as a proof of concept. While the predictive value of the described *S. aureus* signature antigens may not justify clinical application, the results do provide impetus for the investigation of larger patient cohorts from different geographical areas to either define a universal immune proteome signature of protection against *S. aureus* sepsis or to develop more specialized tests capturing the antigen repertoire of invasive *S. aureus* in different regions of the world.

Although vaccine trials have not prevented *S. aureus* infection [4,5,25], the results of this prospective study strengthen the case of a protective role of the adaptive immune system in ameliorating *S. aureus* infections. Specific serum IgG indicates immune memory of *S. aureus*, a core competence of the adaptive immune system, which is the basis of vaccination effects. It was clearly associated with protection from septic complications of *S. aureus* bloodstream invasion. Remarkably, the association of *S. aureus*-specific serum IgG with a favorable disease course was lost if sera were obtained later than 4 days after infection presentation (data not shown). This suggests that the protection from septic complications of *S. aureus* invasion was mainly provided by pre-existing specific IgG or immune memory of *S. aureus* rather than by the IgG response triggered by the current infection.

The findings corroborate previous observations: *S. aureus* carriers have higher levels of specific antibodies than do non-carriers [6,18], but a lower mortality rate in the case of *S. aureus* bacteremia [9]. Similarly, patients with uncomplicated recovery from bloodstream infection had higher antibody titers specific to *S. aureus* exotoxins at diagnosis than did patients who developed sepsis [19]. Epidermolysis bullosa

Table 2
Top 20 *S. aureus* proteins for patient stratification.
The eight most discriminating *S. aureus* proteins used in the principal component analysis are highlighted (see also Fig. 2C).

Abbreviation	Description	Gene locus	GI–Number	Protein localization[a]			
				Psortb	LocateP	SignalP	TM domain
Plc	1–Phosphatidylinositol phosphodiesterase	SAUSA300_0099	87128097	Extracellular	N–terminally anchored	Yes	1
SspB	Staphopain B	SA0900	15926634	Extracellular	Secretory	Yes	1
IsaA	Probable transglycosylase	SAOUHSC_02887	88196515	Extracellular	N–terminally anchored	Yes	0
	Uncharacterized protein	SACOL0444	57652631	Cytoplasmic Membrane	Lipid anchored	Yes	0
SEM	Staphylococcal enterotoxin M	SA1647	15927403	Extracellular	N–terminally anchored	Yes	0
	Surface protein, putative	SACOL0985	57650173	Cytoplasmic Membrane	N–terminally anchored	Yes	0
GlpQ	Glycerophosphoryl diester phosphodiesterase	SAUSA300_0862	87126873	Extracellular	N–terminally anchored	Yes	0
HlgC	Gamma–hemolysin component C	SACOL2421	57650965	Extracellular	Secretory	Yes	0
SplB	Serine protease SplB	SAOUHSC_01941	88195635	Extracellular	N–terminally anchored	Yes	1
Sak	Staphylokinase, putative	SAOUHSC_02171	88195848	Extracellular	Secretory	Yes	0
Atl	Bifunctional autolysin	SAOUHSC_00994[b]	88194750	Extracellular	Secretory	Yes	0
Efb–c	Fibrinogen–binding protein	SAOUHSC_01114	88194860	Extracellular	N–terminally anchored	Yes	0
	Uncharacterized protein	SACOL0480	57651321	Cytoplasmic Membrane	N–terminally anchored	Yes	1
GreA	Transcription elongation factor	SA1438	15927190	Cytoplasmic	Intracellular	No	0
LukF–PV	LukF–PV	SAUSA300_1381	87126598	–	–	–	–
TSST–1	Toxic shock syndrome toxin–1	SA1819	15927587	Extracellular	Secretory	Yes	1
HlgB	Gamma–hemolysin component B	SACOL2422	57650966	Extracellular	Secretory	Yes	0
Coa	Coagulase	SAOUHSC_00192	88194002	Extracellular	Secretory	Yes	0
Lip	Triacylglycerol lipase 1	SAUSA300_2603	87126156	Extracellular	N–terminally anchored	Yes	1
	Uncharacterized protein	SACOL0908	57651598	Cytoplasmic Membrane	N–terminally anchored	Yes	0

Abbreviations: TM, transmembrane.
[a]Protein localization was predicted with different algorithms using the Aureowiki database and *S. aureus* COL as reference strain (http://www.protecs.uni-greifswald.de/aureowiki/Main_Page; November 2014).
[b]N-terminal part of the protein.

patients, suffering from chronic wounds heavily colonized by *S. aureus*, show high serum concentrations of anti-staphylococcal antibodies and do not usually develop systemic *S. aureus* infection [26]. Conversely, children with low pre-existing IgG titers to *S. aureus* α-hemolysin and Panton–Valentine leukocidin were more prone to invasive *S. aureus* disease [27]. In summary, the literature agrees about a negative correlation between specific antibodies and severity of *S. aureus* disease.

The present work extends this knowledge and indicates for the first time that patient stratification based on *S. aureus*-specific antibodies may be possible at diagnosis of *S. aureus* bloodstream invasion. In particular, a subgroup of bacteremia patients with very strong *S. aureus*-specific antibody binding rarely developed septic complications. Such information may also be useful in clinical intervention studies, such as active or passive *S. aureus* vaccination trials. For this, a panel of eight *S. aureus* antigens identified among 64 tested recombinant *S. aureus* antigens was instrumental. Encouragingly – in contrast to earlier data reported from the same patient cohort [19] – seven of these eight signature proteins belong to the conserved *S. aureus* core genome. This made it unnecessary to filter patients according to the infecting *S. aureus* strain and will make it easier to translate the findings into a diagnostic tool.

Regarding the mechanisms of protection provided by the adaptive immune system, there are several mutually non-exclusive possibilities [28]. On the one hand, antibodies may opsonize the bacteria or neutralize potent *S. aureus* virulence factors such as the pore-forming toxins α-toxin, PVL and LukE/LukF, as well as superantigens. On the other hand, the antibodies might also be considered as a marker of *S. aureus*-specific T cell memory, because most B cells require cognate (antigen-specific) T cell help to generate antibodies, especially for performing a class switch from IgM to other Ig subclasses. Besides help for B cells, memory T cells could fulfill numerous functions in anti-bacterial defense, e.g., they promote the maturation of neutrophils, their release from the bone marrow and recruitment to the site of infection [28–30].

In summary, besides indicating the possibility of patient stratification according to their sepsis risk, our data lend support to the idea of a vaccine to protect against the most serious complications of *S. aureus* infection and encourage further efforts in this direction.

Transparency document

The Transparency document associated with this article can be found, in online version.

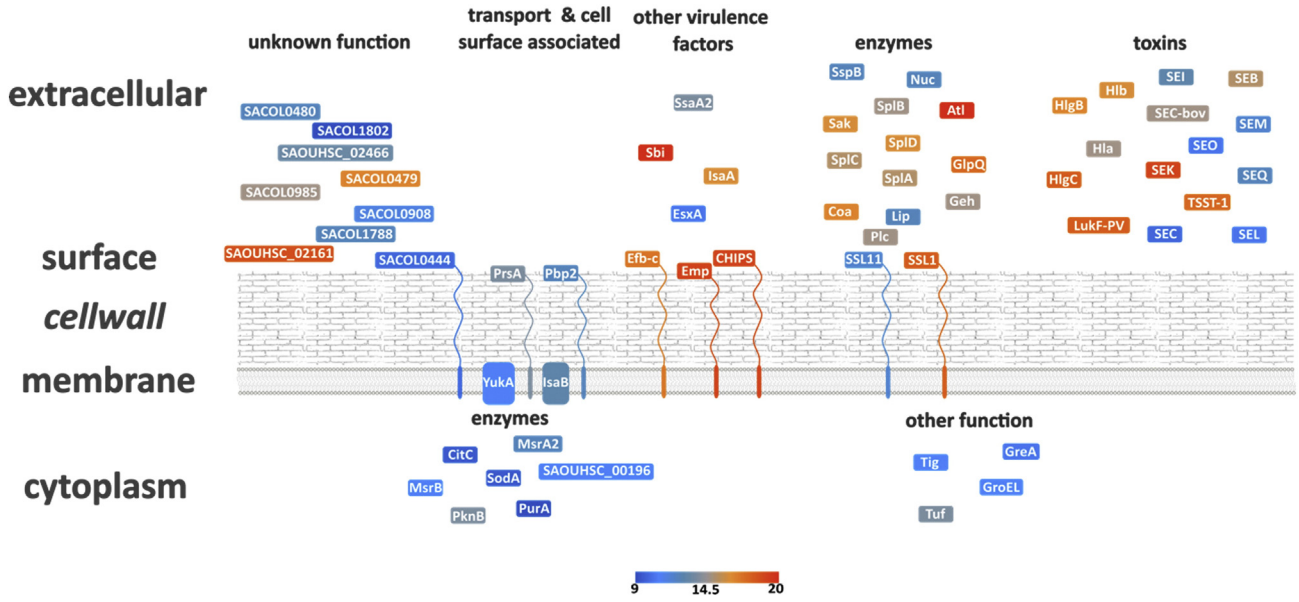

Fig. 3. IgG binding to 64 recombinant *S. aureus* proteins. Proteins are grouped according to their predicted cellular location (see also Table S2) and function. Normalized median serum IgG binding intensity of all patients is expressed by a color code (Log2 median of antibody binding intensity). Extra-cellular and surface-bound proteins were preferentially recognized by the serum antibodies.

Conflicts of interest

J. K., S. E., L. S., M. H., U. V. and B. M. B. have filed a patent on an immune proteomic signature of *S. aureus* bacteremia (Patent No. 10194983.2-2401). All other authors report no potential conflicts.

Acknowledgments

The authors wish to thank Marc Burian and Silva Holtfreter for the support with the generation of USA300Δ*spa*. They are also grateful to Martina Selle, Knut Ohlsen, Devika Bhagwat, Barbara Kahl and Christian Kohler for providing recombinant bacterial proteins.

This work was supported by the German Research Council (GRK840, CRC-TRR34) and the German Ministry of Education and Research (Infection Genomics 0315829C, ZIK-FunGene 03Z1CN22, HICARE 01KQ1001E).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jprot.2015.06.018.

References

[1] H.F.L. Wertheim, D.C. Melles, M.C. Vos, W. van Leeuwen, A. van Belkum, H.A. Verbrugh, et al., The role of nasal carriage in *Staphylococcus aureus* infections, Lancet Infect. 5 (2005) 751–762.
[2] F.D. Lowy, *Staphylococcus aureus* infections, N. Engl. J. Med. 339 (1998) 520–532.
[3] M.Z. David, R.S. Daum, Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic, Clin. Microbiol. Rev. 23 (2010) 616–687.
[4] B. Spellberg, R. Daum, Development of a vaccine against *Staphylococcus aureus*, Semin. Immunopathol. 34 (2012) 335–348.
[5] V.G. Fowler, K.B. Allen, E.D. Moreira, M. Moustafa, F. Isgro, H.W. Boucher, et al., Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial, JAMA 309 (2013) 1368–1378.
[6] N.J. Verkaik, C.P. de Vogel, H.A. Boelens, D. Grumann, T. Hoogenboezem, C. Vink, et al., Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of *Staphylococcus aureus*, J. Infect. Dis. 199 (2009) 625–632.
[7] S. Holtfreter, J. Kolata, B.M. Bröker, Towards the immune proteome of *Staphylococcus aureus* — the anti-*S. aureus* antibody response, Int. J. Med. Microbiol. 300 (2010) 176–192.
[8] C. von Eiff, K. Becker, K. Machka, H. Stammer, G. Peters, Nasal carriage as a source of *Staphylococcus aureus* bacteremia, N. Engl. J. Med. 344 (2001) 11–16.
[9] H.F. Wertheim, M.C. Vos, A. Ott, A. van Belkum, A. Voss, J.A. Kluytmans, et al., Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers, Lancet 364 (2004) 703–705.
[10] S. Holtfreter, K. Roschack, P. Eichler, K. Eske, B. Holtfreter, C. Kohler, et al., *Staphylococcus aureus* carriers neutralize superantigens by antibodies specific for their colonizing strain: a potential explanation for their improved prognosis in severe sepsis, J. Infect. Dis. 193 (2006) 1275–1278.
[11] M.C. Cook, S.G. Tangye, Primary immune deficiencies affecting lymphocyte differentiation: lessons from the spectrum of resulting infections, Int. Immunol. 21 (2009) 1003–1011.
[12] E.D. Renner, S. Rylaarsdam, S. Anover-Sombke, A.L. Rack, J. Reichenbach, J.C. Carey, et al., Novel signal transducer and activator of transcription 3 (STAT3) mutations, reduced T(H)17 cell numbers, and variably defective STAT3 phosphorylation in hyper-IgE syndrome, J. Allergy Clin. Immunol. 122 (2008) 181–187.
[13] J.D. Milner, J.M. Brenchley, A. Laurence, A.F. Freeman, B.J. Hill, K.M. Elias, et al., Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome, Nature 452 (2008) 773–776.
[14] J.S. Cho, E.M. Pietras, N.C. Garcia, R.I. Ramos, D.M. Farzam, H.R. Monroe, et al., IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice, J. Clin. Invest. 120 (2010) 1762–1773.
[15] H. Ishigame, S. Kakuta, T. Nagai, M. Kadoki, A. Nambu, Y. Komiyama, et al., Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses, Immunity 30 (2009) 108–119.
[16] N.J. Verkaik, W.J. van Wamel, A. van Belkum, Immunotherapeutic approaches against *Staphylococcus aureus*, Immunotherapy 3 (2011) 1063–1073.
[17] K. Ohlsen, U. Lorenz, Immunotherapeutic strategies to combat staphylococcal infections, Int. J. Med. Microbiol. 300 (2010) 402–410.
[18] J. Kolata, L.G. Bode, S. Holtfreter, L. Steil, H. Kusch, B. Holtfreter, et al., Distinctive patterns in the human antibody response to *Staphylococcus aureus* bacteremia in carriers and non-carriers, Proteomics 11 (2011) 3914–3927.
[19] R.P. Adhikari, A.O. Ajao, M.J. Aman, H. Karauzum, J. Sarwar, A.D. Lydecker, et al., Lower antibody levels to *Staphylococcus aureus* exotoxins are associated with sepsis in hospitalized adults with invasive *S. aureus* infections, J. Infect. Dis. 206 (2012) 915–923.
[20] R.C. Bone, R.A. Balk, F.B. Cerra, R.P. Dellinger, A.M. Fein, W.A. Knaus, et al., Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine, Chest 101 (1992) 1644–1655.
[21] S. Holtfreter, D. Grumann, M. Schmudde, H.T. Nguyen, P. Eichler, B. Strommenger, et al., Clonal distribution of superantigen genes in clinical *Staphylococcus aureus* isolates, J. Clin. Microbiol. 45 (2007) 2669–2680.
[22] S. Stentzel, H.C. Vu, A.M. Weyrich, N. Jehmlich, F. Schmidt, M.G. Salazar, et al., Altered immune proteome of *Staphylococcus aureus* under iron-restricted growth conditions, Proteomics 14 (2014) 1857–1867.
[23] J.A. Lindsay, C.E. Moore, N.P. Day, S.J. Peacock, A.A. Witney, R.A. Stabler, et al., Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes, J. Bacteriol. 188 (2006) 669–676.
[24] D. Grumann, U. Nubel, B.M. Broker, Staphylococcus aureus toxins — their functions and genetics, Infect. Genet. Evol. 21 (2014) 583–592.

[25] R.A. Proctor, Challenges for a universal *Staphylococcus aureus* vaccine, Clin. Infect. Dis. 54 (2012) 1179–1186.
[26] M.M. van der Kooi-Pol, C.P. de Vogel, G.N. Westerhout-Pluister, Y.K. Veenstra-Kyuchukova, J.C. Duipmans, C. Glasner, et al., High anti-staphylococcal antibody titers in patients with epidermolysis bullosa relate to long-term colonization with alternating types of *Staphylococcus aureus*, J. Invest. Dermatol. 133 (2013) 847–850.
[27] S.A. Fritz, K.M. Tiemann, P.G. Hogan, E.K. Epplin, M. Rodriguez, D.N. Al-Zubeidi, et al., A serologic correlate of protective immunity against community-onset *Staphylococcus aureus* infection, Clin. Infect. Dis. 56 (2013) 1554–1561.
[28] B.M. Bröker, S. Holtfreter, I. Bekeredjian-Ding, Immune control of *Staphylocuccus aureus* — regulation and counter-regulation of the adaptive immune response, Int. J. Med. Microbiol. 304 (2014) 204–214.
[29] T. Korn, E. Bettelli, M. Oukka, V.K. Kuchroo, IL-17 and Th17 cells, Annu. Rev. Immunol. 27 (2009) 485–517.
[30] M.J. McGeachy, S.J. McSorley, Microbial-induced Th17: superhero or supervillain? J. Immunol. 189 (2012) 3285–3291.