MAJOR ARTICLE

A Serologic Correlate of Protective Immunity Against Community-Onset *Staphylococcus aureus* Infection

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Background. *Staphylococcus aureus* is among the leading causes of human infection. Widespread drug resistance, emergence of highly virulent strains, and the ability of *S. aureus* to colonize >30% of the human population contribute to this organism's pathogenic success. Human serologic responses to *S. aureus* and their relationship to protective immunity remain incompletely defined, challenging the strategic development of efficacious vaccines.

Methods. We measured humoral responses to 2 staphylococcal exotoxins, α-hemolysin (Hla) and Panton-Valentine leukocidin (PVL; LukF-PV/LukS-PV subunits), both premier targets of current vaccine and immunotherapy development. We correlated acute and convalescent serum antibody levels with incidence of recurrent infection over 12 months follow-up in 235 children with *S. aureus* colonization, primary or recurrent skin and soft tissue infection, or invasive disease.

Results. Cutaneous infection elicited transient increases in anti-Hla and anti-PVL antibodies; however, subsequent infection risk was similar between primary and recurrent cutaneous infection cohorts. Patients with invasive infections had the lowest preexisting titers against Hla and LukF but displayed the highest convalescent titers. Across cohorts, convalescent anti-Hla titers correlated with protection against subsequent *S. aureus* infection.

Conclusions. Cutaneous *S. aureus* infection does not reliably provoke durable, protective immune responses. This study provides the first link between protection from disease recurrence and the humoral response to Hla, a virulence factor already implicated in disease pathogenesis. These observations can be utilized to refine ongoing vaccine and immunotherapy efforts and inform the design of clinical trials.

Keywords. *Staphylococcus aureus*; α-hemolysin; humoral immunity; Panton–Valentine leukocidin.

Staphylococcus aureus is a versatile organism that commonly exists as a benign commensal of human skin, seamlessly transitioning to an invasive pathogen that causes primary and recurrent skin and soft tissue infections (SSTI), pneumonia, bone and joint infections, bacteremia, and sepsis. As a leading infectious cause of human morbidity and mortality, *S. aureus* is a premier target for vaccine and immunotherapy development. Success has been elusive owing to the complexity of the organism and a lack of knowledge of the desired immunoprotection signature in the human population. Pressing the need for disease prevention, methicillin-resistant *S. aureus* (MRSA) strains have recently evolved to cause a worldwide epidemic of cutaneous and invasive infections in otherwise healthy individuals, leading to the designation of community-associated MRSA (CA-MRSA) [1–4]. The predominant MRSA clone in the United States is designated USA300 [5–7]. Illustrating the versatility of this pathogen, USA300 isolates are now found worldwide [8], and we and others have reported community-acquired infections caused by methicillin-susceptible *S. aureus* variants of this lineage [9, 10].

The molecular attributes underlying virulence of *S. aureus* isolates causing historic and contemporary

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human disease are incompletely defined. Considerable interest has focused on the staphylococcal pore-forming cytotoxins α-hemolysin (Hla; α-toxin) and Panton–Valentine leukocidin (PVL). Hla is genome encoded by almost all *S. aureus* strains and, through molecular epidemiologic studies, was recently demonstrated to contribute to the success of *S. aureus* phage 80/81 epidemic strains of the 1950s and subsequent infections through the present day in healthy individuals [11]. Hla plays important roles in staphylococcal pneumonia, dermonecrotic infection, and sepsis by co-opting the enzymatic function of its eukaryotic receptor ADAM10 to promote epithelial injury [12–15]. Many contemporary CA-MRSA isolates also carry phage DNA that harbors the *lukF-PV* and *lukS-PV* genes that encode the heterodimeric leukotoxin PVL. These strains are epidemiologically associated with severe SSTI and lethal invasive disease, especially necrotizing pneumonia [1, 16–24]. Studies in experimental animals have demonstrated mixed observations on the role of PVL as a cytolytic and immunomodulatory leukotoxin in disease (eg, SSTI and pneumonia), likely reflecting dissimilarities among model systems and across species [25–27]. Dysregulated exotoxin secretion has been proposed as a mechanism that governs strain-specific virulence properties; this is supported by our prior finding of augmented exotoxin gene expression within human cutaneous MRSA abscesses [28].

Based on these studies and preclinical successes of immunization strategies in animal models of disease [29, 30], Hla and PVL have been considered primary targets for vaccine and monoclonal antibody–based approaches. However, evidence has not convincingly equated toxin-neutralizing human immunologic responses with protection against disease. Further, the field must infer from animal studies the disease states or individuals best targeted by these approaches—a jeopardy in the wake of unsuccessful clinical trials of anti-staphylococcal vaccines. Ongoing vaccine and therapeutics efforts will benefit from an enhanced understanding of exotoxin secretion and cognate host responses in human infection, including identification of immunodominant epitopes and assessment of the protection afforded by antitoxin antibodies.

Here, we report serologic responses to Hla and PVL in children with asymptomatic *S. aureus* colonization as well as cutaneous and invasive *S. aureus* infections, revealing novel features of these responses. Most importantly, we demonstrate that convalescent anti-Hla titers correlate with protection from subsequent infection during 12 months of follow-up, thereby specifying a marker of protective immunity in a defined population.

METHODS

Subject Recruitment, Sample Collection, and Longitudinal Follow-up

The Washington University Human Research Protection Office approved study procedures. Written informed consent (and assent when appropriate) was obtained from each participant. Four cohorts of pediatric patients aged ≥6 months were enrolled between August 2008 and September 2011. The first cohort comprised healthy children identified in prior prevalence studies with MRSA colonization and without history of symptomatic staphylococcal infection [31, 32]. These children completed an outpatient enrollment visit at St. Louis Children's Hospital (SLCH). The second cohort of patients comprised children presenting to the SLCH Emergency Department, acute wound center, or inpatient units with a first-time (primary) SSTI requiring incision and drainage whose cultures yielded *S. aureus*. The third cohort included patients with recurrent *S. aureus* SSTI undergoing drainage procedures at SLCH. The fourth cohort included patients with invasive *S. aureus* infections, including bacteremia, osteomyelitis, septic arthritis, bursitis, pyomyositis, empyema, endocarditis, or septic thrombophlebitis. These patients were enrolled from SLCH inpatient units upon isolation of *S. aureus* from a normally sterile body site. Patients with traditional risk factors for healthcare-associated MRSA infections (eg, indwelling catheter or percutaneous medical device, malignancy, dialysis, cystic fibrosis, immunodeficiency, postoperative infection, residence in a long-term care facility) were excluded from participation.

At enrollment, colonization cultures (CultureSwab Liquid Stuart, Becton Dickinson, Franklin Lakes, NJ) were obtained from the anterior nares, axillae, and inguinal folds of each participant. Culture swabs were processed and *S. aureus* identified by standard methods as previously described [33]. *S. aureus* isolates recovered from sites of acute infection were obtained from the SLCH clinical microbiology laboratory. In addition, acute serum samples were drawn at enrollment. A comprehensive enrollment survey was administered to each participant to collect data regarding patient demographics, symptoms associated with the acute infection, past medical history, prior healthcare exposure, household factors, and activities. Additional clinical data were collected by electronic medical record review using a structured abstraction form.

Participants with SSTI or invasive infection returned as outpatients to SLCH (except for several invasive-disease patients who remained in hospital) 4–8 weeks after their acute infection, at which time convalescent sera were obtained. At that visit, the first follow-up survey was administered to document whether the primary infection had resolved and time to resolution, completion of prescribed antibiotics, and development of any interval infection. All participants were followed longitudinally with surveys administered by mail or telephone every 3 months for 1 year (4 in total) to document interval infection and antimicrobial use.

PVL Gene Detection in *S. aureus* Isolates

Genomic DNA was isolated from *S. aureus* cultures streaked on tryptic soy agar with 5% sheep blood (Becton Dickinson) using

the protocol provided with the BiOstic Bacteremia DNA Isolation Kit (MO-BIO Laboratories, Carlsbad, CA). Genes encoding LukF-PV and LukS-PV were detected by polymerase chain reaction as previously described [28].

Protein Purification

Recombinant Hla and PVL (LukF-PV and LukS-PV) were purified from *Escherichia coli* as glutathione-S-transferase (GST) fusion proteins as previously described [25], then subjected to PreScission protease-mediated removal of the GST partner according to the manufacturer's protocol (GE Healthcare, Pittsburgh, PA).

Serologic Testing

Serum samples were retrieved from the Biospecimen Core at Washington University and subjected to enzyme-linked immunosorbent assay (ELISA). Briefly, wells of 96-well high-binding microplates (Nunc Immulon 4 HBX, Thermo Fisher, Waltham, MA) were coated with 1 μg (0.01 mg/mL in phosphate-buffered saline [PBS]) of the target protein overnight at 4°C. Following blockade for 1 hour at room temperature in PBS with 1% bovine serum albumin, 5% sucrose, and 0.05% sodium azide, serum samples were added (100 μL of a 1:100 dilution in PBS) for 2 hours and incubated at room temperature. Detection was with alkaline phosphatase–conjugated anti-human immunoglobulin G (1:5000 in PBS; Sigma, St. Louis, MO) incubated 2 hours at room temperature. Plates were washed between each of the above steps. Reactions were developed by addition of 100 μL p-nitrophenyl phosphate (Sigma) incubated for 30 minutes in the dark and stopped by addition of 25 μL of 3M sodium hydroxide prior to quantification in a Synergy 2 multimode microplate reader (BioTek, Winooski, VT) at 405 nm. Data are presented as the mean of 2 to 3 independent measurements for each sample, each run of which comprised duplicate wells.

Statistical Analysis

Survey responses and ELISA data were analyzed using SPSS for Windows 20 (IBM SPSS, Chicago, IL). $\chi 2$ analysis was performed to compare categorical variables among 3 or more cohorts; Fisher exact test was used for comparison of 2 cohorts. One-way analysis of variance (ANOVA) was performed to compare continuous variables between cohorts. Tukey honestly significant difference analysis was performed to determine which specific cohorts differed after positive ANOVA analyses. To compare mean anti-PVL antibody titers at acute and convalescent time points, paired *t* tests were used. Anti-PVL titers were analyzed only for patients infected with *S. aureus* isolates encoding the *lukF-PV* and *lukS-PV* genes, while anti-Hla titers were analyzed for all patients. Pearson correlation coefficients were computed to examine relationships between antibody titers and leukocyte counts, inflammatory markers, antibiotic duration, and other clinical variables. For longitudinal analyses, mean titers were compared by *t* test between patients with and without recurrent infections over various time intervals. All tests of significance were 2-tailed, and *P* values ≤ .05 were considered significant.

RESULTS

Study Population

A total of 235 patients were enrolled: 12 with colonization only, 99 with primary SSTI, 68 with recurrent SSTI, and 56 with invasive infections. Demographic and other characteristics of the study groups are shown in Table 1. There was a trend for colonized patients to be older and for recurrent SSTI patients to be younger. Subjects in the invasive-disease cohort were more likely to be white and male ($P < .001$ and $P = .002$, respectively). Compared with SSTI, invasive infections were more likely to be caused by methicillin-susceptible strains of *S. aureus* ($P = .002$). Patients with invasive infection were less likely to demonstrate skin colonization with *S. aureus* in general and with MRSA specifically ($P = .001$). Examining only MRSA-infected patients in the invasive cohort, MRSA colonization in this subset was still less common than in SSTI patients ($P = .032$). Only 10 (18%) of 56 invasive-disease patients reported an SSTI in the prior year. Children in the recurrent SSTI cohort were more likely to have visited an emergency department or to have had surgery (including abscess drainage procedures) in the prior year. Presenting signs and symptoms are given in Supplementary Table 1; children with invasive infections more often demonstrated fever, chills, fatigue, headache, and musculoskeletal and pulmonary symptoms compared with children with SSTI.

Acute and Convalescent Seroresponses

Acute ELISA titers were first compared across patients in the 4 cohorts (n = 235 overall and n = 200 with PVL-positive infections). Asymptomatic subjects colonized with *S. aureus* exhibited the highest mean baseline titers against Hla ($P = .002$; Table 2); antibody against LukF in the colonized cohort was also significantly higher than in patients with primary SSTI ($P = .048$; Supplementary Table 2) or invasive infection ($P = .006$; Supplementary Table 2). In contrast, patients with invasive infection had the lowest baseline antibody levels against Hla and LukF ($P = .002$ and $P < .001$, respectively; Table 2). Meanwhile, patients with recurrent SSTI had higher acute titers to both PVL antigens than patients with primary SSTI ($P < .001$; Table 2, Supplementary Figure 1*A* and Supplementary Table 2). The time from symptom onset to the acute serum draw was not different among the cohorts (data not shown).

We next examined differences between acute and convalescent titers in the subset of infected patients for whom both serum samples were available (n = 162 overall and n = 151 with

Table 1. Comparison of Participant Characteristics by Cohort

Characteristic	Total N = 235 (%)	Cohort				*P*	P^b	P^c
		Colonization Only N = 12 (%)[a]	Primary SSTI N = 99 (%)	Recurrent SSTI N = 68 (%)	Invasive Infection N = 56 (%)			
Age, mean ± SD	8.06 ± 6.03	9.24 ± 5.37	8.68 ± 6.60	6.41 ± 5.76	8.70 ± 5.13	.064	.035	.317
Number of people in primary home, mean ± SD	4.91 ± 6.36	4.00 ± 2.17	5.70 ± 9.62	4.43 ± 1.39	4.32 ± 1.38	.453	.323	.394
Race[d]								
White + other	113 (48)	1 (8)	38 (38)	37 (54)	37 (66)	<.001	.003	.008
African American	122 (52)	11 (92)	61 (62)	31 (46)	19 (34)			
Gender								
Male	112 (48)	7 (58)	44 (44)	23 (34)	38 (68)	.002	.001	<.001
Female	123 (52)	5 (42)	55 (56)	45 (66)	18 (32)			
Health insurance								
Private	79 (34)	5 (42)	23 (23)	28 (41)	23 (41)	.041	.019	.189
Medicaid or no insurance	156 (66)	7 (58)	76 (77)	40 (59)	33 (59)			
Infecting organism								
MRSA	181 (77)	(. . .)	83 (84)	61 (90)	37 (66)	(. . .)	.002	.001
MSSA	42 (18)		16 (16)	7 (10)	19 (34)			
Colonization status[e]								
MRSA	112 (48)	9 (75)	48 (49)	40 (60)	15 (27)	.001	.030	.009
MSSA	45 (19)	0 (0)	20 (20)	10 (15)	13 (24)			
MRSA and MSSA	13 (6)	3 (25)	6 (6)	2 (3)	4 (7)			
Not colonized	63 (27)	0 (0)	25 (25)	15 (22)	23 (42)			
Concurrent symptoms[f]	201 (90)	(. . .)	86 (87)	60 (88)	55 (98)	(. . .)	.062	.018
Skin disorder	77 (33)	3 (25)	36 (36)	24 (35)	14 (25)	.450	.320	.144
Eczema	58 (25)	3 (25)	25 (25)	22 (32)	8 (14)	.143	.066	.048
Acne	17 (7)	1 (8)	9 (9)	1 (2)	6 (11)	.179	.085	.241
Psoriasis	2 (1)	0 (0)	1 (1)	1 (2)	0 (0)	.820	.679	1.000
Other skin disorders[g]	3 (1)	0 (0)	1 (1)	1 (2)	1 (2)	.951	.917	1.000
Antibiotic use in past year	141 (61)	8 (67)	44 (45)	59 (88)	30 (54)	<.001	<.001	.270
Stayed overnight in hospital in past year	23 (10)	2 (17)	7 (7)	10 (15)	4 (7)	.294	.207	.605
Surgery in past year[h]	53 (23)	0 (0)	7 (7)	41 (60)	5 (9)	<.001	<.001	.002
Emergency department visit in past year	116 (50)	9 (75)	43 (43)	47 (69)	17 (31)	<.001	<.001	.003
Household contact with SSTI in past year	99 (43)	7 (58)	48 (50)	32 (47)	12 (21)	.003	.002	<.001
Visited someone in hospital in past 6 mo	45 (19)	3 (25)	13 (13)	19 (28)	10 (18)	.110	.054	1.000
Visited someone in nursing home in past 6 mo	34 (15)	1 (8)	12 (12)	12 (18)	9 (16)	.688	.585	.828
Household contact works in healthcare facility and has contact with patients	60 (26)	5 (42)	19 (19)	18 (27)	18 (32)	.168	.183	.153
Pets in home	162 (69)	5 (42)	59 (60)	57 (84)	41 (75)	.001	.003	.500
Participates in sports	73 (31)	3 (25)	33 (33)	12 (18)	25 (45)	.012	.005	.019

Ten of 56 (18%) participants in the invasive cohort reported an SSTI within the past year.

Continuous variables analyzed by 1-way analysis of variance unless otherwise specified.

Categorical variables analyzed by $\chi 2$ test unless otherwise specified.

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*; SD, standard deviation; SSTI, skin or soft tissue infection.

[a] Variables with (. . .) in the colonization only cohort were not applicable for this cohort. Total N is reduced to 223.

[b] Comparison excludes colonization only cohort.

[c] Comparison of invasive cohort to combined SSTI cohorts (primary + recurrent): continuous variables analyzed by *t* test; categorical variables analyzed by Fisher exact test.

[d] Other race includes 2 Asian participants. African American race includes 10 biracial participants.

[e] Reflects all available historical colonization data for the colonization only group.

[f] Complete list of symptoms reported in Supplementary Table 1.

[g] Other skin disorders include molluscum contagiosum, keratosis pilaris, and dermographism.

[h] Includes incision and drainage procedures.

Table 2. Comparison of Mean Antitoxin ELISA Titers Within Time Points and Between Cohorts

Time Point	Cohort				P^a
	Colonization Only, mean ± SD	Primary SSTI, mean ± SD	Recurrent SSTI, mean ± SD	Invasive Infection, mean ± SD	
Acute					
Total subjects, n	12	99	68	56	
Hla titer	1.82 ± 0.66	1.28 ± 0.51	1.40 ± 0.55	1.22 ± 0.48	.002
PVL+, n	10	96	68	46	
LukS titer	1.51 ± 0.43	1.26 ± 0.47	1.67 ± 0.46	1.60 ± 0.49	<.001
LukF titer	2.02 ± 0.55	1.54 ± 0.62	1.98 ± 0.49	1.39 ± 0.47	<.001
Convalescent					
Total subjects, n	(. . .)	67	50	45	
Hla titer	(. . .)	0.95 ± 0.27	0.89 ± 0.30	1.65 ± 0.61	<.001
PVL+, n	(. . .)	64	50	37	
LukS titer	(. . .)	1.15 ± 0.28	1.18 ± 0.19	1.64 ± 0.34	<.001
LukF titer	(. . .)	1.36 ± 0.30	1.32 ± 0.21	1.84 ± 0.41	<.001

LukS and LukF titers were analyzed only for patients harboring a *Staphylococcus aureus* isolate possessing the PVL genes. Tukey honestly significant difference analyses are shown in Supplementary Table 2.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PVL, Panton–Valentine leukocidin; SD, standard deviation; SSTI, skin or soft tissue infection.

[a] One-way analysis of variance.

PVL-positive infections). Convalescent titers to Hla and PVL were significantly lower than acute titers in both SSTI groups (Table 3). In contrast, a rise in titers to Hla and LukF was observed in subjects following invasive disease ($P < .001$), while LukS antibody levels were not augmented (Table 3). Consistent with this observation, convalescent titers were significantly higher in the overall invasive cohort than in the SSTI groups (Table 2 and Supplementary Figure 1*B*). Of note, the mean and median intervals between the acute and convalescent draws were shorter in the invasive cohort (mean 37.6 ± 11.5 days, median 35 days) than in the SSTI cohorts (primary SSTI, mean 47.2 ± 17.5 days, median 42 days; recurrent SSTI, mean 48.7 ± 10.9 days, median 46 days). Consistent relationships between acute or convalescent antibody titers and leukocyte counts, inflammatory markers (erythrocyte sedimentation rate and C-reactive protein), or duration of antibiotic therapy were not observed (data not shown).

Longitudinal Follow-up and Recurrence Risk

All participants were followed for 12 months with quarterly surveys to ascertain the incidence of recurrent infection, defined here to include abscess, boil, cellulitis, bacteremia, bone or joint infections, and "spider bites" (CA-MRSA abscesses are often mistaken for spider bites [7]). Overall, recurrences were reported by 75 (50%) of 151 patients who completed 12 months of follow-up (Table 4). Infections were reported by 2 (20%) of 10 colonized children, consistent with our published observations [34]. The incidence of subsequent infection was identical in both SSTI cohorts (62%; Table 4), despite the temporary relative elevation in acute antitoxin antibody titers in the recurrent SSTI cohort (Table 2). Patients with invasive infection demonstrated the lowest overall rate of recurrent infection over 1 year (22%; $P < .001$; Table 4).

Finally, we examined the rate of subsequent infection during follow-up in relation to absolute convalescent antibody titers against Hla and PVL. Elevated titers to Hla, LukF, and LukS were significantly correlated with protection from recurrent infection through 3 months of follow-up (Table 5). While titers against LukF and LukS did not correlate with sustained protection, anti-Hla titers were significantly associated with lower incidence of subsequent *S. aureus* infection throughout the 12 months of follow-up (all $P \leq .042$; Table 5).

DISCUSSION

In spite of the tremendous historic and current burden of *S. aureus* infection, we lack sufficient data on human immunologic correlates of protection to inform preventive approaches, including vaccine development. Prior *S. aureus* clinical immunization trials have targeted candidate antigens with defined or suspected roles in disease pathogenesis, aiming to augment absent or suboptimal preexisting antibody responses. A number of studies have documented apparent shortcomings of the humoral response to *S. aureus* in the human population. Low antibody titers to an array of staphylococcal toxins were observed in individuals susceptible to sepsis during hospitalization [35]. Further, anti-PVL responses have been reported following contemporary

Table 3. Comparison of Mean Antitoxin ELISA Titers Within Cohorts and Between Time Points

Group	n	Time Point: Acute, mean ± SD	Time Point: Convalescent, mean ± SD	P^a
Primary SSTI				
Hla	67	1.26 ± 0.46	0.95 ± 0.27	<.001
LukS	64	1.23 ± 0.46	1.15 ± 0.28	.116
LukF	64	1.57 ± 0.58	1.36 ± 0.30	<.001
Recurrent SSTI				
Hla	50	1.46 ± 0.57	0.89 ± 0.30	<.001
LukS	50	1.71 ± 0.45	1.18 ± 0.19	<.001
LukF	50	2.02 ± 0.47	1.32 ± 0.21	<.001
Invasive infection				
Hla	45	1.21 ± 0.49	1.65 ± 0.61	<.001
LukS	37	1.62 ± 0.49	1.64 ± 0.34	.784
LukF	37	1.38 ± 0.41	1.84 ± 0.41	<.001

Only patients with available paired sera are included in these analyses. LukS and LukF titers were analyzed only for patients harboring a *Staphylococcus aureus* isolate possessing the Panton–Valentine leukocidin genes.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; SSTI, skin or soft tissue infection.

[a] Paired *t* test.

infection with PVL-positive *S. aureus* [36–38]; in one study, single titers were similar in children with primary vs nonprimary SSTI [38]. In contrast to these earlier studies of human seroresponses to contemporary *S. aureus* infections, a distinguishing feature of our study is the coupling of paired serum analysis with long-term follow-up to assess the impact of seropositivity on subsequent disease—a true measure of the development of protective immunity in response to prior infection.

Several observations from our study provide insight into the development of natural immunity to *S. aureus* infection. First, invasive *S. aureus* infections elicited an amplified, more durable response to both Hla and PVL compared with cutaneous infections. As the route of antigen exposure, the total antigenic burden, and duration of exposure are likely important in patterning adaptive immunity, invasive disease may simply meet an immunostimulation threshold that is not attained in cutaneous disease. Alternatively, *S. aureus* may dampen host immune responses during cutaneous infection as a means to ensure continued coexistence with the host in a commensal state from which transmission may occur. Second, baseline anti-Hla and anti-PVL titers were lowest among invasive disease patients. Consistent with prior reports [35, 39], these findings suggest that a unique susceptibility to severe disease may exist among individuals upon first encounter with the pathogen or in whom prior exposure elicited only a limited immunologic response. In contrast, initial antitoxin titers were highest in colonized individuals without a known history of staphylococcal infection. This cohort may represent those individuals in the population who express natural immunity to *S. aureus*, having generated a productive response to prior antigenic exposure in the absence of overt infection.

Most importantly, this study defines a correlation between anti-Hla titers and protection against subsequent *S. aureus* disease. Murine models have demonstrated that an effective humoral response to Hla affords protection against pneumonia, severe skin infection, and sepsis [29]; however, these models do not permit the study of protection against the recurrent infections that characterize the ongoing human epidemic. While the present study does not define a precise mechanism of protection in humans, it is plausible to suggest that protection directly relates to neutralization of Hla activity, with clear implications for vaccine design and efficacy studies. Alternatively, the observed anti-Hla responses may represent a marker for a second, protective, yet unmeasured, host immune response.

This study has several limitations. First, it is impossible to devise a "true negative" cohort in which investigators are confident that

Table 4. Cumulative Recurrent Infection Rates Over 12-Month Follow-Up, by Cohort

Cumulative Recurrent Infection Over 12 mo	Total, N (%)	Cohort: Colonization Only N (%)	Cohort: Primary SSTI N (%)	Cohort: Recurrent SSTI N (%)	Cohort: Invasive Infection N (%)	P^a	$P^{a,b}$	P^c
Yes[d]	75 (50)	2 (20)	37 (62)	28 (62)	8 (22)	<.001	<.001	<.001
No	76 (50)	8 (80)	23 (38)	17 (38)	28 (78)			

Recurrent infection includes abscess, cellulitis, joint or bone infection, bacteremia, and spider bite.

Abbreviation: SSTI, skin or soft tissue infection.

[a] χ^2 analysis across all cohorts.

[b] Comparison excludes colonization only cohort.

[c] Comparison of invasive cohort to combined SSTI cohorts (primary + recurrent): Analyzed using Fisher exact test.

[d] For the colonization only, primary SSTI, and recurrent SSTI cohorts, all recurrences were SSTI; among patients with invasive infection at enrollment, 3 experienced invasive recurrent infection, while the episodes of recurrence for 5 patients were SSTI.

Table 5. Convalescent Titers of Patients With and Without Recurrent Infections Between Enrollment and Follow-up Surveys

Interval	Recurrent Infection	HLA, mean ± SD	*P*	LukS, mean ± SD	*P*	LukF, mean ± SD	*P*
Enrollment →Convalescent titer	Yes	0.90 ± 0.31	<.001	1.18 ± 0.23	.013	1.38 ± 0.24	.083
	No	1.19 ± 0.54		1.31 ± 0.36		1.48 ± 0.40	
Enrollment →3-month follow-up	Yes	0.91 ± 0.37	<.001	1.19 ± 0.23	.006	1.38 ± 0.27	.039
	No	1.25 ± 0.55		1.33 ± 0.38		1.50 ± 0.42	
Enrollment →6-month follow-up	Yes	0.97 ± 0.40	.001	1.23 ± 0.26	.097	1.43 ± 0.29	.332
	No	1.24 ± 0.57		1.32 ± 0.37		1.49 ± 0.43	
Enrollment →9-month follow-up	Yes	1.01 ± 0.44	.007	1.23 ± 0.27	.116	1.43 ± 0.30	.540
	No	1.25 ± 0.58		1.31 ± 0.36		1.47 ± 0.43	
Enrollment →12-month follow-up	Yes	1.04 ± 0.47	.042	1.23 ± 0.28	.202	1.44 ± 0.33	.937
	No	1.21 ± 0.54		1.30 ± 0.36		1.45 ± 0.41	

Abbreviation: SD, standard deviation.

subjects have never been exposed to *S. aureus*, though our selection of a pediatric population would tend to reduce the likelihood of prior subclinical *S. aureus* infection compared with adult cohorts. Second, we analyzed anti-PVL titers only in patients with current PVL-positive isolates in order to minimize potential bias that could arise from the possibility that a patient enrolled with PVL-negative infection encountered a PVL-positive strain in the past that was not detected at enrollment. Finally, referral bias likely influenced our observation that the invasive cohort (arriving at our referral center from a wider geographic area) was more predominantly white than the SSTI groups (derived mainly from our nearby urban population).

Identification of optimal vaccine targets and well-defined humoral and cellular correlates of protective immunity will facilitate the strategic development of vaccines against complex human pathogens like *S. aureus*. While Hla has been a target for microbiologists and vaccinologists for almost a century, this study provides the first clear evidence that anti-Hla antibodies provide durable protection from human disease. Given that *S. aureus* remains a prominent challenge in populations not included in our study (eg, hospitalized adults), ongoing discovery of additional correlates of protective anti-staphylococcal immunity in these groups is of critical importance. While prior *S. aureus* vaccine trials have focused on "at-risk" adult populations predisposed to life-threatening infection, our data suggest that otherwise healthy children may represent a target population in which considerable benefit from an effective *S. aureus* vaccine can be realized.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. J.B.W. has the potential to receive royalties from Novartis Vaccines and Diagnostics in relation to patents owned by the University of Chicago. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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