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Impaired T-Lymphocyte Responses During Childhood *Staphylococcus aureus* Infection

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Background. *Staphylococcus aureus* infections are common throughout the lifespan, with recurrent infections occurring in nearly half of infected children. There is no licensed vaccine, underscoring the need to better understand how *S. aureus* evades protective immunity. Despite much study, the relative contributions of antibodies and T cells to protection against *S. aureus* infections in humans are not fully understood.

Methods. We prospectively quantified *S. aureus*-specific antibody levels by ELISA and T-cell responses by ELISpot in *S. aureus*-infected and healthy children.

Results. *S. aureus*-specific antibody levels and T-cell responses increased with age in healthy children, suggesting a coordinated development of anti-staphylococcal immunity. Antibody levels against leukotoxin E (LukE) and Panton-Valentine leukocidin (LukS-PV), but not α-hemolysin (Hla), were higher in younger infected children, compared with healthy children; these differences disappeared in older children. We observed a striking impairment of global and *S. aureus*-specific T-cell function in children with invasive and noninvasive infection, suggesting that *S. aureus*-specific immune responses are dysregulated during childhood infection regardless of the infection phenotype.

Conclusions. These findings identify a potential mechanism by which *S. aureus* infection actively evades adaptive immune responses, thereby preventing the development of protective immunity and maintaining susceptibility to recurrent infection.

Keywords. *Staphylococcus aureus*; MRSA; immunity; T cells; antibody.

Staphylococcus aureus frequently causes infections in community and health care settings in children and adults, ranging from mild, such as skin and soft tissue infections (SSTI), to severe, including osteoarticular infections, pneumonia, and bacteremia [1]. The emergence of virulent methicillin-resistant *S. aureus* (MRSA) USA300 isolates heralded the onset of an epidemic in the United States [2]. *S. aureus* infections are a leading cause of hospitalization during childhood [3]. Recurrent infections occur in nearly half of *S. aureus*-infected children within a year [4]. These observations have prompted an emerging paradigm that *S. aureus* infections do not reliably elicit protective immunity, highlighting the need for a vaccine [4–6].

Unfortunately, there is no licensed vaccine to prevent *S. aureus* infections, underscoring the importance of understanding the mechanisms of immune-mediated evasion and protection [6–9]. These efforts are complicated by a nascent understanding of how immunity against *S. aureus* develops during childhood. Most studies in children have focused on antibody responses against specific staphylococcal antigens, most notably toxins such as leukotoxin ED (LukED), Panton-Valentine leukocidin (PVL, LukSF-PV), and α-hemolysin (α-toxin, Hla), revealing that antibody levels are variable and increase with age in healthy children [10, 11]. Less is understood about T-cell responses to *S. aureus* and whether they contribute to protection. Rodent models of *S. aureus* infection demonstrate the importance of antibodies in protection, especially those directed at Hla, while the importance of T cells is more nuanced [12–16]. In contrast, defects in T cell-mediated immunity, but not antibodies, result in human susceptibility to recurrent *S. aureus* infections [17, 18]. These observations mandate the prioritization of better understanding how T-cell responses develop. Although there have been recent reports of *S. aureus*-specific T lymphocyte responses in healthy adults and those with *S. aureus* infection [16, 19, 20], these have not been applied to the pediatric population.

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The goals of this study were to quantify T-cell responses in healthy and *S. aureus*-infected children and to document their relationship to antibody levels and the severity of infection. We observed an age-dependent development of antibodies against LukE and LukS-PV in healthy children, while these antibody levels were already high in the youngest infected children. Despite high antibody levels, effector T-cell responses were markedly impaired in *S. aureus*-infected children, regardless of whether the infection was superficial or invasive. Together, these findings suggest that *S. aureus* infection suppresses T-cell responses, potentially precluding the development of protective immunity.

METHODS

Study Design

This single-site prospective observational study was approved by the institutional review board at Nationwide Children's Hospital. Hospitalized children aged 6 months to 21 years old were identified by positive *S. aureus* cultures or by clinical criteria with subsequent culture confirmation. Healthy children were enrolled in an outpatient setting. Children who had an infection that was caused by a bacterium other than *S. aureus* were also included. Exclusion criteria included documented immunodeficiency, receipt of immunosuppressive medications within 2 months, or receipt of an immunoglobulin-based product within 6 months. Following informed consent and assent when applicable, clinical information was entered into a REDCap database. Blood samples were collected within 3 days of culture collection. Blood was separated into mononuclear cell preparation tubes (CPT) and serum separation (SST) tubes (Becton Dickinson). Peripheral blood mononuclear cells (PBMCs) and serum were isolated; PBMCs were frozen in liquid nitrogen and serum at −80°C.

Antibody Quantification

Antibody quantification was modified from our previous study [21]. First, a standardized serum stock was prepared by pooling serum from healthy adults with high levels of *S. aureus*-specific antibodies. For quantification of antibody levels by enzyme-linked immunosorbent assay (ELISA), 96-well plates (Costar, Corning) were coated with 4 μg/mL of purified Hla, LukE, or LukS-PV. Serial dilutions of the standard were included on each plate to minimize plate-to-plate variability. Serial dilutions of subject sera were added in duplicate. Quantification of immunoglobulin G (IgG) was performed using alkaline phosphatase (AP)-conjugated goat anti-human IgG (1:5000; Kirkegaard and Perry Labs) and AP substrate *p*-nitrophenyl phosphate (Sigma-Aldrich). Absorbance (OD_{405}) was measured using a GENios spectrophotometer (Tecan). Based on the standards, IgG levels were quantified as arbitrary ELISA units. Results were discarded and repeated if (1) the r^2 of the standard curve was <0.995, (2) the coefficient of variance between duplicates was > 20%, (3) the OD_{405} was <0.5 or >2.5 or outside of the standard range, or (4) blank wells had an OD_{405} >0.10.

Quantification of Immune Cells

PBMCs were thawed and an aliquot separated for cell counting. Following filtering and washing with phosphate-buffered saline, cells were stained with Live/Dead stain, washed with fluorescence-activated cell sorting (FACS) buffer, and blocked with Human FcR Block. Surface staining was performed with anti-CD3 (UCHT1), anti-CD4 (L200), anti-CD8 (SK1), anti-CD19 (HIB19), anti-CD45 (HI30), anti-CD16 (3G8), anti HLA-DR (L243), anti-CD56 (NCAM1), anti-CD11c (Bu15), and anti-CD14 (M5E2) in Brilliant stain buffer at 4°C for 30 minutes. Except for antibodies for live/dead staining, CD56, and CD4 (Becton Dickinson), all antibodies were purchased from BioLegend. Cells were washed with FACS buffer and fixed in 4% paraformaldehyde overnight at 4°C. The following day, cells were washed and reconstituted with FACS buffer and counting beads added. Flow cytometry was performed on a BD LSRFortessa flow cytometer; data were analyzed using Flowjo.

Quantification of Effector T-Cell Responses

Human interleukin 17 (IL-17) and interferon- γ (IFN-γ) T-cell ELISpot kits (U-Cytech biosciences) were used to quantify T-cell responses. Plates (96-well) were coated with anti-IL-17 or anti-IFN-γ antibody. PBMCs were thawed and added to each well ($0.5–4 \times 10^5$ cells/well, depending on the conditions). PBMCs were then incubated with phorbal myristate acetate (PMA) and ionomycin, heat-killed *S. aureus* (5×10^5 colony-forming units/well), or purified antigen (Hla, LukE, or LukS-PV, 20 μg/mL) for 40 hours at 37°C. Following washing, biotin-labeled detection antibodies were added to the wells, followed by avidin-horseradish peroxidase (eBioscience). Spots were counted following addition of the 3-Amino-9-ethylcarbazole (AEC) substrate solution (BD Biosciences) using an Immunospot series 1 analyzer (Cellular Technology).

Statistical Analysis

Differences between cohorts were evaluated with χ^2 or Fisher exact tests, Mann-Whitney tests, or 1-way ANOVA with the Kruskal-Willis post test for multiple comparisons. Multivariable linear regression was used to evaluate differences between groups while accounting for age; in cases where the association between variables differed by age group, we stratified results. To determine appropriate age groupings, the relationship between age and antibody levels was evaluated graphically with a loess curve and statistically with polynomial regression and restricted cubic spline regression. All linear regression analyses were based on $\log_{10}$-transformed antibody levels. The Mann-Whitney test was used to compare immune responses between

groups. Differences were considered significant if $P < .05$. All analyses were performed using R for Statistical Computing or GraphPad Prism.

RESULTS

Study Subjects

Seventy-six hospitalized children with *S. aureus* infection and 79 healthy children were enrolled between September 2018 and February 2020 at Nationwide Children's Hospital (Table 1). Infections were classified as invasive (40%) or noninvasive (60%); the most common sites of infection were SSTI (73% of noninvasive infections) and bacteremia (87% of invasive infections). *S. aureus*-infected children were older compared with healthy children, but there were no differences in sex or race/ethnicity between the groups (Table 1).

Antibody Levels in Healthy and *S. aureus*-Infected Children

IgG levels against Hla, LukE, and LukS-PV were quantified by ELISA. There was a linear association, albeit weak, between age and the $\log_{10}$-transformed antibody levels against each of the antigens in healthy children, demonstrating an age-dependent increase in antibody levels (Figure 1A). In contrast, only anti-Hla IgG levels correlated with increased age in *S. aureus*-infected children; there was no association between age and anti-LukE or anti-LukS-PV antibody levels (Figure 1B). This raised the possibility that antibodies against LukE and LukS-PV, but not Hla, increased during infection of younger children.

To account for differences in age between healthy and infected children, the cohorts were divided into 3 age groups for further analysis (<5, 5–10, >10 years). There were no differences in anti-Hla antibody levels in any group between healthy

Table 1. Characteristics of the Study Subjects

Characteristic	Healthy Control	Staphylococcus *aureus* Infected			*P* Value[a]	*P* Value[b]
		All	Invasive	Noninvasive		
Subjects, No.	79	76	31	45		
Age, y, median (IQR)	4.8 (2.4–7.7)	8.4 (5.5–12.7)	9.5 (6.4–11.7)	7.6 (3.2–13.7)	<.001	.453
Age group, y					<.001	.186
<5	42 (53.2)	18 (23.7)	4 (12.9)	13 (28.9)		
5–10	28 (35.4)	30 (39.5)	14 (45.2)	16 (35.6)		
>10	9 (11.4)	28 (36.8)	13 (41.9)	16 (35.6)		
Sex					1.000	.985
Male	45 (56.8)	43 (56.6)	17 (54.8)	26 (57.8)		
Female	34 (43.2)	33 (43.4)	14 (45.2)	19 (42.2)		
Race/ethnicity					.333	.672
White	63 (79.7)	61 (80.3)	25 (80.6)	36 (80.0)		
Black	5 (6.3)	8 (10.5)	3 (9.7)	5 (11.1)		
Hispanic	4 (5.1)	5 (6.6)	3 (9.7)	2 (4.44)		
Other	7 (8.9)	2 (2.6)	0 (0.0)	2 (4.44)		
Complex condition	NA	21 (28.0)	11 (35.5)	10 (22.7)	NA	.342
Prior *S. aureus* infection	NA	15 (20.0)	3 (9.7)	12 (27.3)	NA	.113
Susceptibility					NA	.261
MRSA	NA	39 (51.3)	13 (41.9)	26 (57.8)		
MSSA	NA	37 (48.7)	18 (58.1)	19 (42.2)		
PVL status						
Negative	NA	23 (30.3)	15 (48.4)	8 (17.8)	NA	.013
Positive	NA	43 (56.6)	12 (38.7)	31 (68.9)		
Unknown	NA	10 (13.2)	4 (12.9)	6 (13.3)		
Fever	NA	44 (60.3)	25 (83.3)	19 (44.2)	NA	.002
Site of infection[c]					NA	<.001
SSTI	NA	40 (52.6)	7 (22.6)	33 (73.3)		
Blood	NA	27 (35.5)	27 (87.0)	0 (0.0)		
Wound	NA	15 (19.7)	0 (0.0)	15 (33.3)		
Bone/joint	NA	5 (6.6)	5 (16.1)	0 (0.0)		
Respiratory	NA	3 (3.9)	3 (9.7)	0 (0.0)		
Other	NA	3 (3.9)	1 (3.2)	2 (4.4)		

Data are No. (%) except where indicated.

Abbreviations: IQR, interquartile range; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; NA, not applicable; PVL, Panton Valentine leucocidin; SSTI, skin and soft tissue infection.

[a] *P* values comparing the differences between healthy controls and *S. aureus*-infected children.

[b] *P* values comparing the differences between *S. aureus* invasive and noninvasive infection.

[c] Children could have more than 1 site of infection.

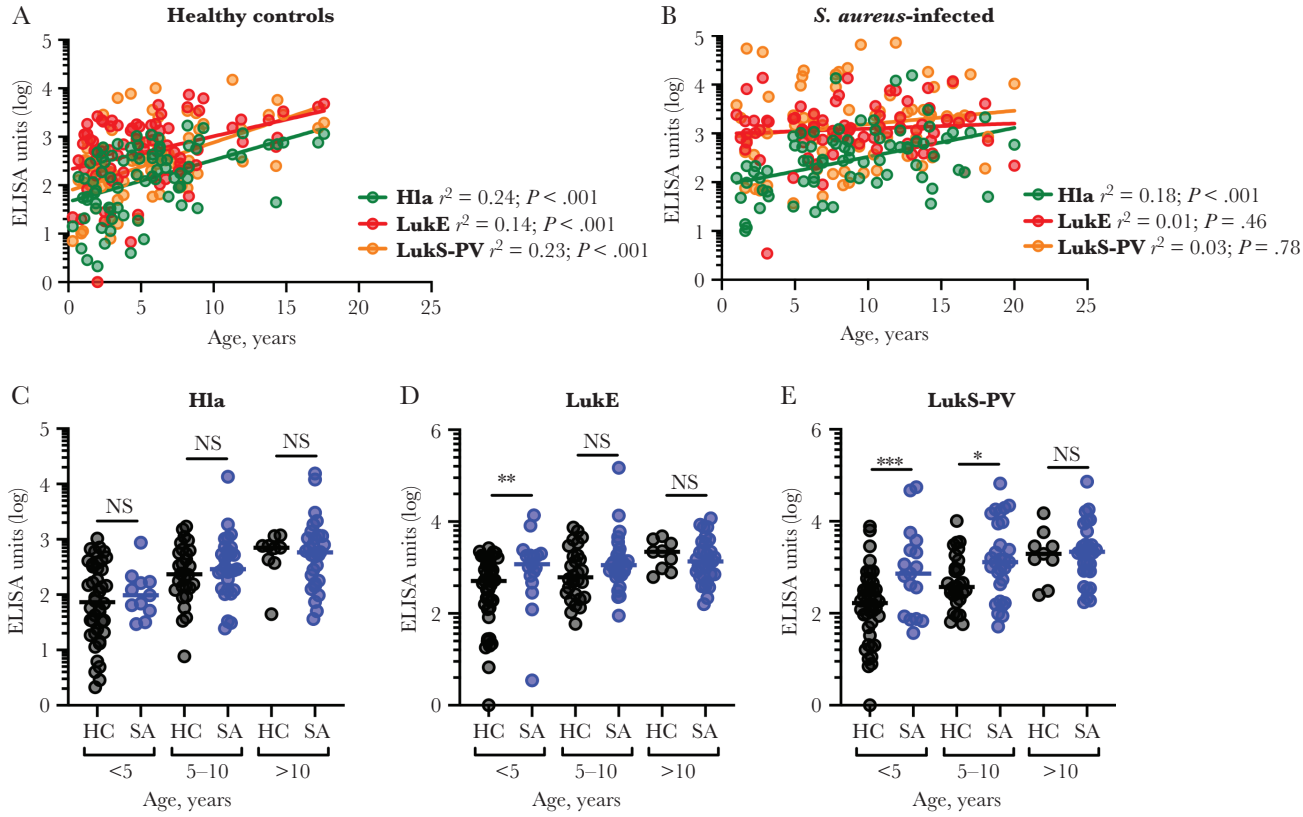

Figure 1. Antibody levels in healthy and Staphylococcus *aureus*-infected children. Antibody levels against Hla, LukE, and LukS-PV were quantified by ELISA in 79 healthy children and 76 children with *S. aureus* infection. *A*, Antibody levels against Hla, LukE, and LukS-PV were positively associated with age in healthy children. *B*, Antibody levels against Hla, but not LukE or LukS-PV, were positively associated with age in children with *S. aureus* infections. *C–E*, Antibody levels against Hla, LukE, and LukS-PV in healthy children and children with *S. aureus* infection aged <5, 5–10, or >10 years. Antibody levels are presented as ELISA units and were $\log_{10}$-transformed for analysis. Correlations were determined by linear regression analysis and antibody levels were compared using the Mann-Whitney test, with lines indicating the median values. * P < .05, ** P < .01, *** P < .001. Abbreviations: ELISA, enzyme-linked immunosorbent assay; HC, healthy children; Hla, α-hemolysin; LukE, leukotoxin E; LukS-PV, Panton-Valentine leucocidin; NS, indicates not significant; SA, *S. aureus* infection.

and infected children (Figure 1C), consistent with Hla-specific IgG increasing with age and being unaffected by infection. In contrast, there were higher levels of anti-LukE (Figure 1D) and anti-LukS-PV (Figure 1E) IgG in younger infected children compared with healthy children, although these differences did not persist in the older children in whom antibody levels were already high. Surprisingly, there were no major differences in antibody levels between children with invasive or noninvasive infection (Supplementary Figure 1A–1C). Antibody levels against LukS-PV, but not Hla or LukE, were higher in children who were infected with PVL-expressing isolates, consistent with the fact that not all *S. aureus* isolates express PVL (Supplementary Figure 1D–1F). Together, these findings suggest that infection elicited higher levels of LukE- and LukS-PV-specific antibodies only in younger children, but anti-Hla antibodies were not elicited in children of any age. Because antibodies specific for Hla, but not LukE or LukS-PV, are associated with protection [4, 22], this observation underscores the discordance between immunogenicity and protective immunity elicited by *S. aureus* infection.

Impaired Effector T-Cell Responses in Children With *S. aureus* Infection

We next quantified functional T-cell responses in an age-matched cohort of 25 healthy children and 39 children with acute *S. aureus* infection (20 with an invasive infection, 19 with a noninvasive infection) (Supplementary Table 1). First, PBMCs were incubated with PMA and ionomycin to quantify the capacity for T cells to respond to a potent nonspecific stimulus. Following PMA/ionomycin incubation, there were significantly reduced (median 2 to 3-fold) IL-17A and IFN-γ responses among *S. aureus*-infected children compared with healthy children (Figure 2A and 2D). These findings are consistent with strongly impaired global T-cell function in children with *S. aureus* infection. To assess whether this impairment was specific to children with *S. aureus* infection, 12 children with an infection caused by another bacterial pathogen were enrolled (Supplementary Table 1). In contrast to *S. aureus*-infected children, children with other infections did not have impaired IL-17A or IFN-γ responses compared with healthy children (Figure 2A and 2D). Surprisingly, impairment of T-cell function was independent of the infection phenotype; even children

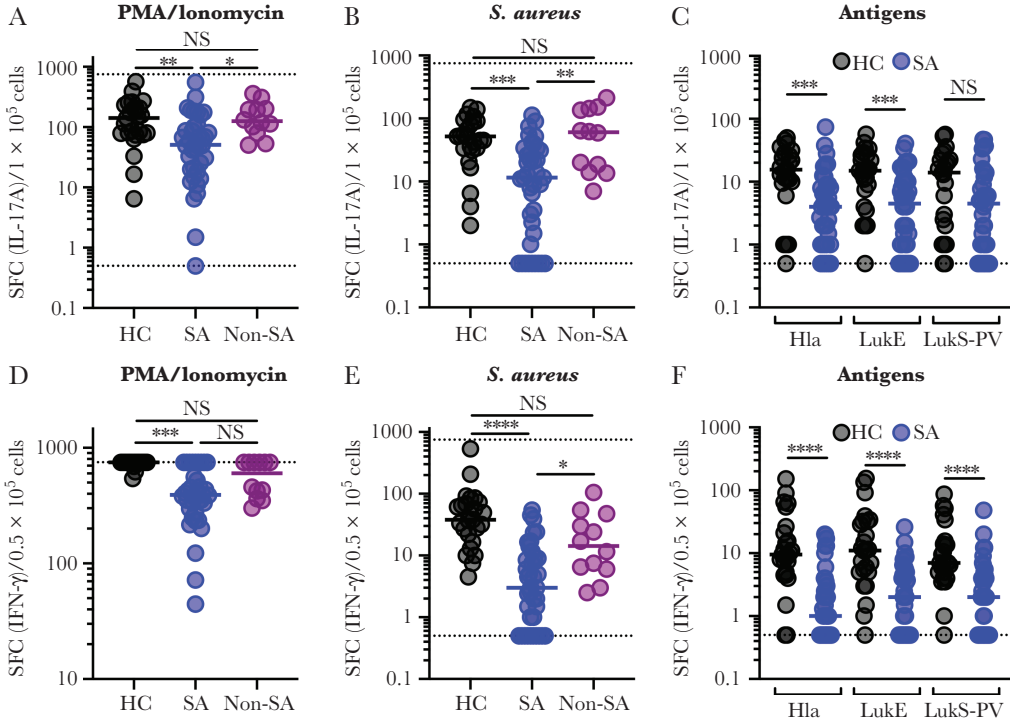

Figure 2. Effector T-cell responses in healthy and *Staphylococcus aureus*-infected children. Functional effector T-cell responses were quantified by IL-17A (*A–C*) or IFN-γ (*D–F*) ELISpot in thawed PBMCs from 25 healthy children, 39 children with *S. aureus* infection, and 12 children with an infection not caused by *S. aureus*. Following culture with PMA and ionomycin, there were impaired IL-17A (*A*) and IFN-γ (*D*) responses in children with *S. aureus* infection compared with healthy controls or children with other infections. Following culture with heat-killed *S. aureus*, there were markedly impaired IL-17A (*B*) and IFN-γ (*E*) responses in infected children compared with healthy children or children with other infections. Following culture with purified Hla, LukE, or LukS-PV, there were markedly impaired IL-17A (*C*) and IFN-γ (*F*) responses in infected children. Individual data points are plotted with medians overlaid and distributions were compared using 1-way ANOVA with the Kruskal-Willis post test for multiple comparisons (*A, B, D, E*) or the Mann-Whitney test (*C, F*). ** $P < .01$, *** $P < .001$, **** $P < .0001$. The dashed lines indicate the upper (750) and lower (0.5) detectable limits of the assay. Abbreviations: ANOVA, analysis of variance; HC, healthy children; Hla, α-hemolysin; IFN-γ, interferon-γ; IL-17A, interleukin 17A; LukE, leukotoxin E; LukS-PV, Panton-Valentine leucocidin; Non-SA, infection not caused by *S. aureus*; NS, indicates not significant; PBMCs, peripheral blood mononuclear cells; PMA, phorbal myristate acetate; SA, *S. aureus* infection; SFC, spot-forming colonies.

with localized infections had strongly impaired responses (Supplementary Figure 2A and 2D).

Next, we compared *S. aureus*-specific T-cell responses following incubation with heat-killed *S. aureus*. Strikingly, *S. aureus*-specific IL-17A responses in infected children were dramatically lower (median 5-fold) compared with healthy children, with a fourth of infected children having responses below the limit of detection (Figure 2B). *S. aureus*-specific IFN-γ responses were even more strongly impaired in infected children (median 12-fold); a third of infected children had responses that were below the detection limit (Figure 2E). Similar to nonspecific responses, there were no differences in IL-17A and IFN-γ responses in children with invasive or noninvasive infection (Supplementary Figure 2B and 2E), underscoring the potency of even a mild *S. aureus* infection in impairing *S. aureus*-specific T-cell function. Again, the impairment of *S. aureus*-specific T-cell responses was limited to children with *S. aureus* infection (Figure 2B and 2E). These findings were recapitulated following stimulation with *S. aureus* toxins, as infected children had significantly decreased Hla- and LukE-specific IL-17A responses (Figure 2C). Similarly, there were markedly reduced IFN-γ responses against all 3 toxins in infected children (Figure 2F). Again, there were no significant differences in antigen-specific IL-17A or IFN-γ responses between children with an invasive or noninvasive infection (Supplementary Figure 2C and 2F). Therefore, as we observed with global T-cell function, *S. aureus*-specific T-cell responses are strongly impaired during infection, regardless of the infection phenotype.

To test the possibility that T-cell impairment might be due to fewer T cells and/or antigen-presenting cells (APC) in children with *S. aureus* infection, we quantified immune cell populations in thawed PBMCs by flow cytometry (Supplementary Figure 3A). Although there was a lower percentage of $CD3^+$ T cells in infected children (Supplementary Figure 3B), the differences were modest, and there was a higher percentage of $CD4^+$ T cells in infected children (Supplementary Figure 3C). This decrease in T cells was accompanied by a higher percentage of B cells in infected children (Supplementary Figure 3B), consistent with the higher antibody levels we observed. There were also more

monocytes, but fewer dendritic cells (DCs) and natural killer cells in infected children compared with healthy children (Supplementary Figure 3D). These modest differences in lymphocyte and APC populations seem unlikely to explain the severely impaired T-cell responses we observed.

We next tested whether the reduced *S. aureus*-specific T-cell responses in infected children were dependent on age or antibody levels. As we observed with antibody levels, *S. aureus*-specific IL-17A responses correlated with age in healthy children, but IL-17A responses were low even in older infected children (Figure 3A). IFN-γ responses were not correlated with age in healthy children and only weakly associated with age in infected children (Supplementary Figure 4A). Similarly, LukE- and LukS-PV–specific IL-17A responses and antibody levels were correlated in healthy, but not infected, children (Figure 3B and 3C). Surprisingly, Hla-specific antibody and IL-17A responses were not correlated for either group (Figure 3D), suggesting a disconnect between these arms of adaptive immunity for Hla-specific responses. While there was a weak association between LukS-PV–specific antibody and IFN-γ responses in healthy children (Supplementary Figure 4C), there were no associations between LukE- and Hla-specific antibody levels and IFN-γ responses in either group (Supplementary Figure 4B and 4D). Together, these findings suggest that antigen-specific antibody and T-cell–mediated immunity develops throughout childhood, but these responses are dysregulated in children with *S. aureus* infection.

DISCUSSION

The immune response during *S. aureus* infection in childhood remains poorly understood. Herein, we found higher antibody levels against the *S. aureus* toxins LukE and LukS-PV, but not Hla, during infection, compared with healthy children. Despite higher antibody levels, global and *S. aureus*-specific IL-17A and IFN-γ responses were markedly impaired in infected children, regardless of the severity of infection. These findings suggest a coordinated development of *S. aureus*-specific antibody and T-cell responses in healthy children, but this is uncoupled in children who have active infection. Our findings additionally suggest a failure to elicit Hla-specific antibodies in children with *S. aureus* infection. Taken together, our study identifies 2 potential mechanisms, both at the level of T-cell responses and toxin-specific antibody levels, by which *S. aureus* infection might result in

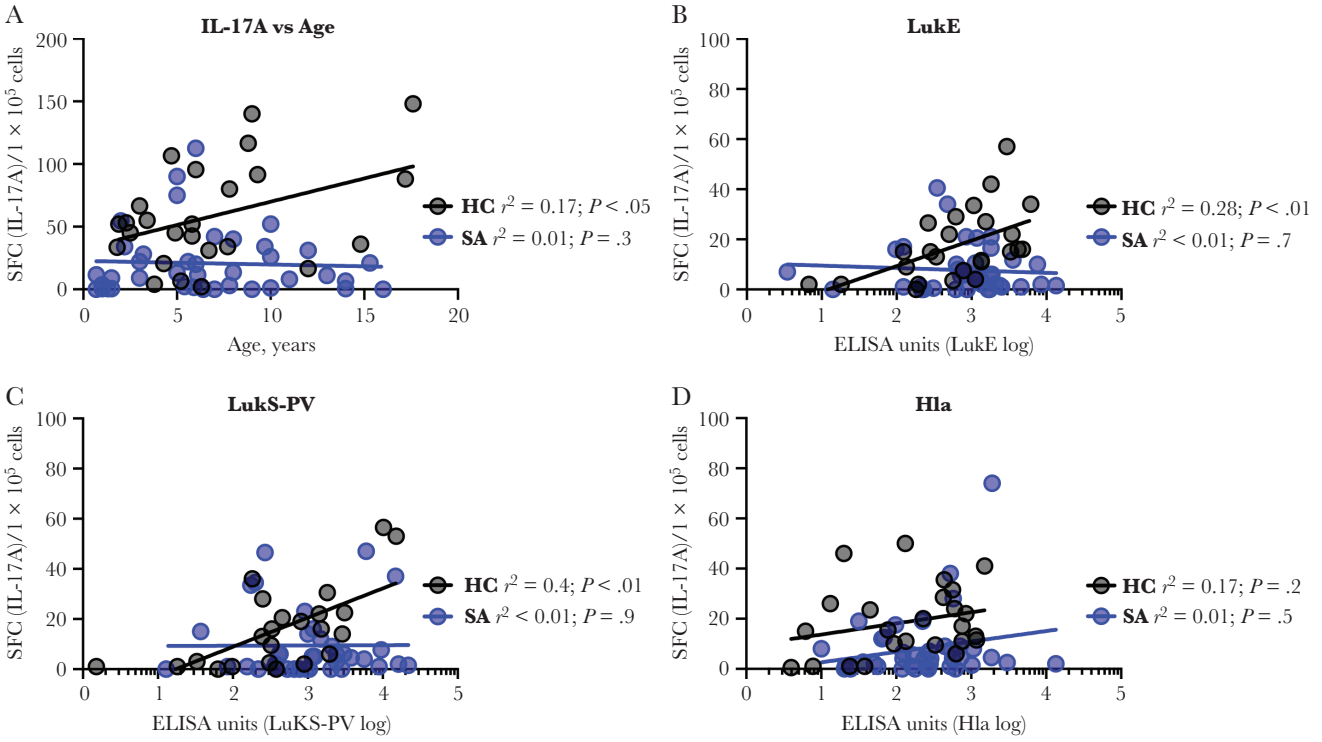

Figure 3. Correlation of T-cell responses with age and antibody levels. *A*, *Staphylococcus aureus*-specific (following incubation with heat-killed *S. aureus*) IL-17A responses were modestly correlated with age in healthy children but not children with *S. aureus* infection. *B*, LukE-specific IL-17A responses were positively associated with $\log_{10}$-transformed LukE-specific antibody levels in healthy children but not children with *S. aureus* infection. *C*, LukS-PV–specific IL-17A responses were positively associated with $\log_{10}$-transformed LukS-PV–specific antibody levels in healthy children but not children with *S. aureus* infection. *D*, There was no association between Hla-specific IL-17A responses and $\log_{10}$-transformed Hla-specific antibody levels in either group. Associations were determined using linear regression analysis. Abbreviations: ELISA, enzyme-linked immunosorbent assay; HC, healthy children; Hla, α-hemolysin; IL-17A, interleukin 17A; LukE, leukotoxin E; LukS-PV, Panton-Valentine leucocidin; NS, indicates not significant; SA, *S. aureus* infection.

active evasion of the adaptive immune response, providing a potential explanation for sustained susceptibility to recurrent infection.

Emerging clinical evidence and data from mouse models demonstrates roles for both the Th17/IL-17 and Th1/IFN-γ pathways in defense against *S. aureus*. Th17/IL-17-mediated defense is critical for protection in models of *S. aureus* bacteremia, SSTI, and pneumonia [14, 15, 23–26], with γδ T cells a major source of IL-17 [15, 27]. Th1/IFN-γ responses protect in some mouse models of *S. aureus* infection [16, 28], but inhibit protection in others [14, 29]. Human studies are inconclusive; for example, Th17-polarized responses in adults with *S. aureus* sepsis were associated with higher mortality [30], while adults with *S. aureus* bacteremia have strongly induced Th1 responses [16] that may be associated with better survival [30]. Together, these studies demonstrate the need to translate findings on the role of T cells in controlling *S. aureus* infection from preclinical studies to the human population.

The most striking finding of this study is the severely impaired effector T-cell IL-17A and IFN-γ responses in children with active *S. aureus* infection, independent of the site or extent of infection. Global impairment of T-cell responses was observed, but *S. aureus*-specific responses were even more profoundly impaired. The impaired T-cell IL-17A and IFN-γ production may reflect a preinfection T-cell phenotype that explains predisposition of individuals to infection. However, nearly 80% of the children in our cohort presented with first-time infection, and infected children had higher levels of antibodies against LukE and LukS-PV and increased percentages of B cells compared with healthy children. Because T cells promote B-cell–mediated antibody responses, these observations argue against baseline immune deficits in these patients. Because we did not quantify lymphocyte subsets prior to cryopreservation of PBMCs, we also cannot exclude the possibility that infected children presented with lymphopenia. An alternative hypothesis is that infection actively suppresses T-cell responses. These findings are consistent with those of Ardura et al, who used a transcriptomic approach to identify decreased T-cell responses in children with active *S. aureus* infection [31]. Future studies that quantify these responses in children during and following recovery from infection should resolve this question. The suppression of adaptive immune responses may be explained by direct toxicity of staphylococcal toxins to T lymphocytes and DCs, a critical APC population that drives T-cell responses. For example, Hla impairs DC and T-cell responses during murine *S. aureus* infection [32], and Hla induces apoptosis of T lymphocytes [33]. Similarly, LukED triggers cellular toxicity on T cells and DCs that express its cellular receptor, CCR5 [34]. LukAB also directly kills DCs, impairing their ability to activate $CD4^+$ T cells [35]. Although it remains to be proven, we speculate that expression of one or more of these toxins during infection, together with impaired toxin neutralizing antibody responses, results in suppression of T-cell responses via direct toxicity toward lymphocytes and APCs [32, 36]. Mechanistic studies to test this hypothesis are underway.

Our observation that antibodies against *S. aureus* toxins increase with age in children are consistent with previous reports [10, 11, 22, 37] and support a conceptual model that exposure to colonizing *S. aureus* occurs throughout childhood, and the sum of these exposures results in a quantifiable level of immunity. Our findings are also consistent with the notion that the plasticity in *S. aureus*-specific antibody responses during childhood is lost by adulthood, resulting in the anti-staphylococcal antibody repertoire being fixed by adulthood [38–40]. Nevertheless, the role of antibodies in protection against *S. aureus* infection in humans remains unclear, despite evidence from mouse models that antibodies, primarily those directed against Hla, are protective [12–14, 41]. Although we found no differences in Hla-specific antibody levels between healthy and infected children during acute infection, an infection-elicited increase in anti-Hla antibodies in children from acute infection to convalescence correlates with lower rates of recurrent infection [4]. Therefore, Hla-specific antibodies may play a more important role in defense against recurrent infection than primary infection. We did not assess the functionality of toxin-specific antibodies. This will be important to assess in the future because healthy children have lower levels of Hla-specific neutralizing antibodies despite similar levels of anti-Hla IgG [37].

A limitation to this study is that immune responses were quantified during acute infection and compared to healthy controls, but we lack baseline responses prior to infection and follow-up studies after the infection has cleared. Nevertheless, this work has several potentially important implications. First, the development of anti-staphylococcal immunity in healthy children follows a predictable trajectory, while children who present with active *S. aureus* infection appear to have dysregulated responses. Second, impaired T-cell responses may impair clearance of bacteria and prolong resolution of infection. This may also explain why recurrent infections are common in children. Third, a seemingly nonprotective antibody response is elicited at the expense of a protective response. Finally, these findings may have implications for vaccine studies. For example, vaccine efficacy may be optimal during early childhood, prior to the onset of natural nonprotective responses. Additionally, surrogate endpoints for vaccine efficacy might need to include elicited T-cell responses.

In conclusion, we found that *S. aureus*-specific antibody and T-cell responses develop throughout childhood, but these responses are abnormal in children who develop *S. aureus* infections. T-cell responses are markedly impaired in children with *S. aureus* infections, suggesting a mechanism by which the bacterium modulates host immune responses to both impair host defense and prevent the development of protective immunity against subsequent infection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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