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BRIEF REPORT

Reduced Immunoglobulin (Ig) G Response to *Staphylococcus aureus* in STAT3 Hyper-IgE Syndrome

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STAT3 hyper-IgE syndrome (STAT3-HIES) patients presented with significantly lower *Staphylococcus aureus*-specific serum IgG compared to cystic fibrosis patients despite recurrent *S. aureus* infections. Immunoglobulin replacement therapy increased *S. aureus*-specific IgG in STAT3-HIES patients and attenuated the clinical course of disease suggesting a role of humoral immunity in *S. aureus* clearance.

Keywords. *S. aureus*; STAT3; hyper-IgE Syndrome; HIES; Th17; cystic fibrosis; adaptive immunity; specific IgG.

Staphylococcus aureus (*S. aureus)* is one of the leading human pathogens causing skin and soft-tissue as well as blood stream and respiratory infections. Increasing numbers of antibiotic-resistant *S. aureus* strains aggravate the situation and highlight the need of alternative preventive and therapeutic strategies, such as *S. aureus* vaccines [1, 2]. Clinical vaccine trials, however, have failed despite eliciting robust specific antibody responses [3]. Recurrent *S. aureus* infections of the skin and lung are frequent in patients with STAT3 hyper-IgE syndrome (STAT3-HIES) [4–7].

In these patients, heterozygous STAT3 mutations result in a dominant negative effect on the signal transducer and activator of transcription 3 (STAT3) signaling pathway, which impairs T-helper (Th) 17 differentiation and thus leads to markedly reduced numbers of Th17 cells [8–12]. Because the Th17/IL17-mediated immune responses are known to be crucial for neutrophil recruitment required for clearance of *S. aureus* infection, the low number of Th17 cells observed in STAT3-HIES is thought to be causative for the recurrent *S. aureus* infections seen in STAT3-HIES. Animal models corroborate the role of Th17 cells in combating *S. aureus* infections, raising the question whether specific antibodies are required to protect against *S. aureus* infections [3, 13, 14].

Although total serum IgG levels are in the normal range, patients with STAT3-HIES show reduced specific immunoglobulin G responses to neoantigen vaccination and present with a reduced memory B-cell compartment [15]. Because of the impaired humoral and cellular adaptive immunity. immunoglobulin replacement therapy (IgRT) has been suggested to be beneficial and is now used successfully in clinical practice [15–17]. With IgRT, patients receive regular infusions with purified IgG from healthy blood donors. The antibody response to *S. aureus* in patients with STAT3-HIES and the specific effects of IgRT, however, have not been comprehensively assessed.

METHODS

We assessed 16 patients with molecularly defined STAT3-HIES (8 male and 8 female; age range, 3–49 years; median, 17 years) from 15 unrelated families followed up at Children's Hospital, Ludwig Maximilian University, Munich. Results were compared with those in 20 patients with molecularly defined cystic fibrosis (CF) and chronic *S. aureus* airway infection (11 male and 9 female; age range, 8–38 years; median, 15 years; part of a recent multicenter study [18]), and 14 healthy *S. aureus* carriers (4 male and 10 female; age range, 19–26 years; median, 20 years). *S. aureus* carriage was defined by *S. aureus* positive cultures in 2 nose swab samples obtained ≥2 months apart. The study was approved by the local institutional ethics review boards. Written informed consent was obtained.

Two different immunoglobulin (lg) substitution products (Privigen [CSL Behring] and Intratect [Biotest]) were analyzed. *S. aureus*–specific IgG was quantified with extracellular *S. aureus* proteins and Ultraviolet-inactivated *S. aureus* cells as antigens. A protein A–deficient mutant of the *S. aureus* strain USA300 (USA300Δ*spa*) was used to avoid nonspecific antibody binding. *S. aureus* cells were cultured under iron limitation to the postexponential growth phase to induce the secretion of immunogenic proteins. Extracellular proteins from USA300Δ*spa* supernatant were prepared as described elsewhere [19]. Before enzyme-linked immunosorbent assay (ELISA) assessment, *S. aureus* cells were washed in phosphate-buffered saline (PBS), killed by UV light, and adjusted to an optical density of 1.0. IgG specific for *S. aureus* extracellular proteins was measured according to the Peggy Sue Simple Western Assay manual (ProteinSimple), using 1 μg/μL of extracellular USA300Δ*spa*

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proteins, 1:500 human serum dilution, and 0.16 μg/mL of goat anti-human IgG [Fc-specific] secondary HRP antibody (Jackson ImmunoResearch) (see also Supplementary Table 1 and reference [19]).

Antibody binding to *S. aureus* proteins was quantified using Compass 2.6.6 software (ProteinSimple). Areas under the curve of manually marked peaks (peak fit width, 7) were added up to obtain the total *S. aureus*–specific signal. Duplicates were assessed, and a third measurement performed when the 2 replicates differed by more than 30% [19].

ELISA plates (Maxisorpt; Thermo Fisher Scientific) were coated with 100 μL of UV-killed USA300Δ*spa* cells overnight at 4°C, washed with 0.05% Tween 20 in PBS, and blocked with 150 μL of 10% fetal calf serum in PBS for 1 hour. Then, 50 μL of human serum dilutions (1:2500, 1:12 500, 1:62 500, and 1:312 500) were incubated in duplicates for 1 hour. After washing, 50 μL of secondary antibody was added for 1 hour and tetramethylbenzidinesubstrate (BD) for 10 minutes. After addition of 20 μL of NH_2SO_4, samples were measured in an ELISA reader at 450 nm. Arbitrary units were calculated with the duplicate means, using GraphPad Prism 6.01 software for nonlinear standard curves, and analyzed with the Kruskal-Wallis test with Dunn posttest; differences were considered statistically significant at $P < .05$. All results were standardized to the serum of 1 healthy donor.

RESULTS

Recurrent skin and respiratory tract infections with *S. aureus* as well as the classic findings of STAT3-HIES were present in all patients with STAT3-HIES with findings reported herein. *S. aureus*–specific IgG in serum samples from 16 patients with STAT3-HIES before and during IgRT were assessed and compared with findings in 20 patients with CF and chronic *S. aureus* airway infections and 14 healthy *S. aureus* carriers. There was significantly less IgG specific for extracellular *S. aureus* proteins as well as for *S. aureus* cells in serum samples from patients with STAT3-HIES than from those with CF ($P < .001$; Figure 1A and 1B). Compared with healthy *S. aureus* carriers, the amount of specific IgG was slightly but nonsignificantly reduced. At clinical follow-up, patients reported improvement of eczema and chronic infections, resulting in a subjectively better state of health. Objectively, the number of severe infections, such as pneumonia, decreased in all patients except patient 4 with STAT3-HIES, who had several pulmonary exacerbations despite the start of IgRT and needed several intravenous antibiotic treatment cycles.

Because all except 1 patient with STAT3-HIES benefitted clinically from IgRT, we assessed 2 Ig substitution products (Privigen [CSL Behring] and Intratect [Biotest]) for their content of *S. aureus*–specific IgG. Both Ig substitution products contained *S. aureus*–specific IgG (Figure 1C and 1D). Furthermore, we showed that *S. aureus*–specific IgG levels rose in 3 of 4 patients with STAT3-HIES during IgRT (Figure 1E and 1F). Patient 4 with STAT3-HIES had chronic destructive *S. aureus* lung infection and unusually high baseline levels of *S. aureus*–specific IgG, within the range of levels in patients with CF (615 000 AUC, Figure 1A). This might explain why IgRT did not further increase *S. aureus*–specific IgG levels in this patient.

DISCUSSION

Although patients with STAT3-HIES consistently experience skin and respiratory tract infections with *S. aureus*, they had significantly lower levels of serum IgG specific for extracellular *S. aureus* proteins and *S. aureus* cells than patients with CF who had chronic and persistent *S. aureus* airway infections, and nonsignificantly lower levels compared with healthy *S. aureus* carriers. Patients with CF were selected as a disease control group because they are extensively exposed to *S. aureus,* similarly to patients with STAT3-HIES [20, 21].

The observation that *S. aureus* exposure results in high levels of *S. aureus*–specific serum IgG is not unique to patients with CF [18, 22]. In atopic dermatitis, the skin lesions are chronically colonized with *S. aureus* in about 90% of patients [23]. We recently showed that in most patients with atopic dermatitis, *S. aureus*–specific serum IgG is higher than in healthy controls [24]. Hence, we conclude patients with STAT3-HIES produce much lower amounts of *S. aureus*–specific IgG than expected considering their extensive exposure during recurrent and chronic *S. aureus* infections. Similarly, low production of phage-specific IgG has been observed in patients with STAT3-HIES after experimental vaccination [15]. Thus, even though total serum IgG titers in these patients are comparable to those in healthy controls, there is impairment in the production of specific IgG [15, 17].

IgRT has been shown to benefit the clinical course of *S. aureus* infection in patients with STAT3-HIES [9, 15, 16]. *S. aureus* infection control was achieved with a combination of IgRT and antibiotic treatment despite the persistent Th17 defect. In the current study, we show that IgRT can increase the threshold *S. aureus*–specific IgG levels in patients with STAT3-HIES. We conclude that impaired generation of Th17 cells is not the only reason for the pronounced susceptibility to *S. aureus* infections observed in these patients. This is corroborated by observations in other monogenetic defects impairing IL17 signaling, which have been linked to chronic mucocutaneous candidiasis without recurrent *S. aureus* infections [25]. Hence, while the reduced Th17 cell counts can account for the chronic mucocutaneous candidiasis observed in STAT3-HIES patients, they may not fully explain why the patients also suffer from recurrent *S. aureus* infections. The data further suggest that the defective Th17 response not only affects neutrophil recruitment but may also impair the specific humoral immune response to infectious agents, which may be due to a lack of T-cell help for B cells and a broader impact of the STAT3 signaling defect. As

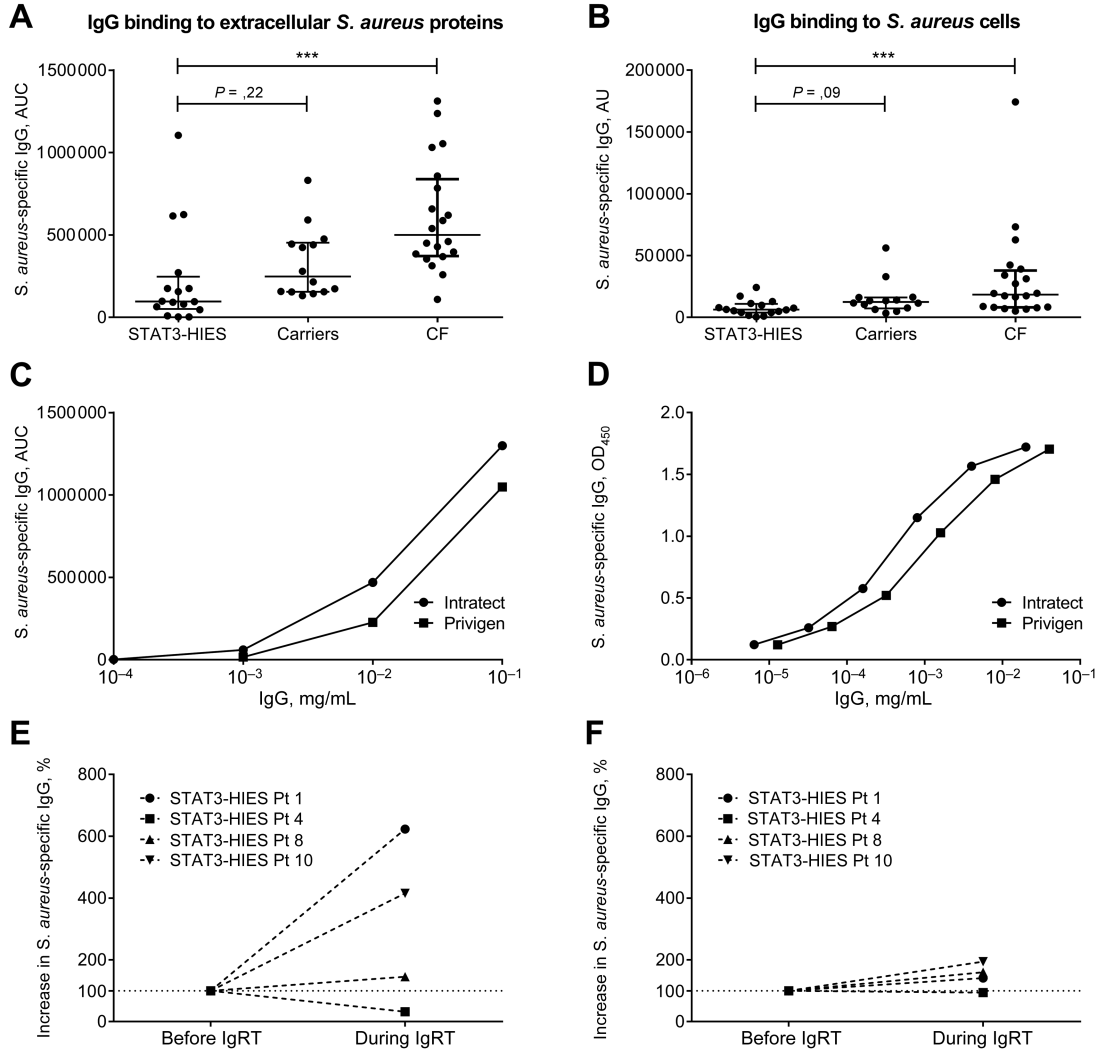

Figure 1. *Staphylococcus aureus*–specific serum immunoglobulin (Ig) G in patients with STAT3-hyper IgE syndrome (STAT3-HIES), patients with cystic fibrosis (CF), healthy *S. aureus* carriers, and 2 different Ig substitution products. IgG binding to extracellular *S. aureus* (USA300Δ*spa*) proteins (*A, C, E*) and to *S. aureus* cells (*B, D, F*) was quantified using the Peggy Sue Simple Western Assay and enzyme-linked immunosorbent assay (ELISA), respectively. *A, B,* Serum samples from patients with STAT3-HIES contained the lowest amount of *S. aureus*–specific IgG, with significant differences compared to patients with CF (***$P < .001$ for both *A* and *B*: Dunn posttest). Single dots represent the result for 1 serum sample; medians and interquartile ranges are indicated. AU, arbitrary units; AUC, area under the curve. *C, D, S. aureus*–specific IgG was detectable in both IG substitution products (Privigen [CSL Behring; 100 mg/mL] and Intratect [Biotest; 50 mg/mL]). OD_{450}, optical density at 450 nm. *E, F,* Relative increase in *S. aureus*–specific IgG during immunoglobulin replacement therapy (IgRT), calculated as the percentage of *S. aureus*–specific IgG before IgRT (100%); Pt, patient.

suggested in the literature and according to our clinical experience, neither IgRT nor antibiotic treatment alone can fully prevent *S. aureus* infections in STAT3-HIES; rather, a combination therapy is required [15, 16].

Our findings in STAT3-HIES patients are in agreement with earlier findings in immunocompetent individuals documenting an association of high-titer anti–*S. aureus* antibodies with protection from severe *S. aureus* disease [26–28]. In those studies, however, it was not possible to exclude an important effect of antigen-specific T cells, which in immunocompetent individuals consistently accompany an antigen-driven antibody response. Our present study separates the antibody-mediated effects from those of T cells, because T-cell immunity remained defective in IgRT treated patients with STAT3-HIES. Therefore, our observations strongly support a fundamental role of *S. aureus*–specific IgG in controlling *S. aureus* infections.

In contrast to experimental monovalent *S. aureus* vaccines, which induce an IgG response selectively directed at the vaccine antigen, IgRT is purified from serum of many healthy blood donors and contains antibodies that bind to a broad range of *S. aureus* antigens, including numerous virulence factors. In view of the plethora of *S. aureus* virulence factors, this may explain the difference in efficacy between IgRT and the experimental *S. aureus* vaccines tested in clinical trials. It might be interesting to explore whether higher amounts of *S. aureus*–specific antibodies, beyond the average serum levels

of healthy adults in currently used therapeutic immunoglobulin preparations, would further improve IgRT-mediated control of *S. aureus* infections.

Current knowledge holds that particularly T-cell immunity is essential for clearing *S. aureus*. Our results now make a strong case for a Th17-independent protective role of specific antibodies in the immune defense against *S. aureus*. This is encouraging for the development of passive as well as active *S. aureus* vaccines.

Supplementary Data

Supplementary materials are available at *Clinical 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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