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Obesity and STING1 genotype associate with 23-valent pneumococcal vaccination efficacy

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Introduction

Pneumococcal infections are a leading cause of adult and childhood hospitalizations and mortality, with an estimated 1.6 million annual deaths (1, 2). A high BMI is associated with an increased risk for contracting pneumococcal pneumonia, suggesting that it is desirable to provide effective vaccination to this susceptible, and growing, population (3). A number of studies have demonstrated that obesity may be a risk factor for vaccine nonresponsiveness, including hepatitis B and tetanus vaccines, which are both protein based (4, 5). However, the effects of obesity on immune response to the polysaccharide-based pneumonia vaccine, which elicits a T cell–independent response, have not been characterized.
Obesity constitutes one of the largest public health issues in the world and is associated with widespread comorbidities, including insulin resistance, hypertension, cardiovascular disease, stroke, sleep apnea, cancer, chronic inflammation, and mortality (6–9). Though inflammation in obesity is multifaceted, recent attention has turned to genetic contributions of underlying inflammatory mechanisms (10, 11). In particular, studies have shown that obesity induces cytosolic mitochondrial DNA release, which triggers activation of STING (stimulator of interferon genes; refs. 12, 13). This obesity-induced activation of STING may impact vaccination response (14). Indeed, STING has been implicated in vaccine efficacy, as reduced antibody responses to T cell–independent type II antigens have been demonstrated in Sting-deficient mice (15). Also, Sting–/– mice have impaired antibody responses to the 23-valent pneumococcal vaccine (PPSV23; Pneumovax 23), which also elicits a T cell–independent type II response (16). In humans, the STING1 gene is highly heterogeneous, and R71H-G230A-R293Q (HAQ), the second most common human STING1 allele, is carried by approximately 22.6% of Americans and approximately 63% of East Asians. A knockin mouse with the mouse equivalent of the human HAQ-STING1 allele has an attenuated PPSV23 response (16, 17). Whether the HAQ allele influences PPSV23 response in humans is an unknown.

The aim of the Result of Obesity on Vaccine Efficacy (ROVE) Study was to utilize a nonrandomized controlled trial to evaluate, in otherwise healthy adults, the effect of obesity (BMI ≥30) versus nonobesity (22 ≤ BMI ≤ 25) on humoral responses to PPSV23 in relation to STING1 genotypes. This is the first human study to our knowledge assessing the role of obesity and STING1 genotype in PPSV23 efficacy. Stated as null, we hypothesized that levels of antibodies to serotypes against PPSV23 before and after vaccination in nonobese and obese participants would be identical.

Results

Participants

Enrollment began in January 2017 and was completed in August 2018 after the prespecified number of 23 subjects per group was reached. A total of 136 potential participants were interested in the study and invited to take a brief online survey to assess eligibility. Eighty-eight participants were eligible and, after 32 lost interest or were unable to be contacted again, 56 consented. Six participants failed secondary screening during the first visit. Of the 50 remaining participants, 25 had BMI between 22 and 25, and 25 had BMI >30. In each group, 2 participants withdrew from the study. In total, 23 nonobese and 23 obese participants completed both visits (Figure 1). The baseline characteristics of the 2 groups are shown in Table 1. The nonobese individuals were younger than the obese individuals (mean ± SEM, 95% CI: 23.0 ± 0.643, 21.7–24.4 vs. 29.0 ± 1.11, 26.7–31.3; \( P < 0.001 \)). Participants in the obese group showed significantly elevated BMI, waist/hip ratio, WBC counts, hemoglobin A1C, erythrocyte sedimentation rate, and blood pressure compared with the nonobese group, which aligns with other studies involving obese participants (Table 1) (18–20). One adverse event occurred due to local swelling at the site of the vaccine injection, which resolved within 2 weeks.

Outcomes

No difference in baseline serotype titers or seroconversion.

Serum samples were collected both before and after vaccination (4–6 weeks later), and assessed for serotype-specific IgG responses. Since obesity is considered a risk factor for pneumonia, we measured prevaccination levels in nonobese and obese individuals, and found no significant difference (mean ± SEM, 95% CI: 8.06 ± 0.45, 7.18–8.94 vs. 9.3 ± 0.91, 7.51–11.1; \( P = 0.17 \); Figure 2A). Next, we evaluated whether there were clinically relevant differences in seroconversion between the groups. Participants in both groups showed significantly elevated BMI, waist/hip ratio, WBC counts, hemoglobin A1C, erythrocyte sedimentation rate, and blood pressure compared with the nonobese group, which aligns with other studies involving obese participants (Table 1) (18–20). One adverse event occurred due to local swelling at the site of the vaccine injection, which resolved within 2 weeks.

Obesity is positively associated with PPSV23 responses.

After vaccination, the overall concentration of anti-PPSV23 titers in the obese group were significantly elevated compared with the nonobese group (mean ± SEM, 95% CI: 34.1 ± 3.51, 27.2–40.9 vs. 19.8 ± 1.52, 16.8–22.8, \( P < 0.001 \); Figure 3A). We next considered our primary end points. Controlling for age and sex, an ANCOVA showed a significant increase in fold change (mean ± SEM, 95% CI 9.56 ± 0.912, 7.77–11.4 vs. 4.75 ± 0.939, 3.98–5.23, \( P < 0.001 \); Figure 3B) and log fold change (\( P < 0.001 \)) in titers among obese participants. Post hoc comparisons showed that the obese group had a higher mean fold change in 21 of 23 serotypes (Supplemental Figure 1A; supplemental
material available online with this article; https://doi.org/10.1172/jci.insight.136141DS1). Due to differences in the proportion of ethnicities in the study, we also assessed fold change differences in White participants only. At baseline, nonobese White participants had higher serotype-specific titers (mean ± SEM, 95% CI: 7.95 ± 0.522, 6.93–8.98 vs. 5.44 ± 0.477, 4.50–6.38; \(P < 0.0001\); Supplemental Figure 1B). Though obese and nonobese White participants had similar antibody serotype-specific titers after vaccination (mean ± SEM, 95% CI: 21.7 ± 3.04, 15.7–27.6 vs. 19.4 ± 1.84, 15.8–23.0; \(P = 0.33\); Supplemental Figure 1C), obese White participants had a significantly greater fold change increase (mean ± SEM, 95% CI: 8.07 ± 1.07, 5.96–10.2 vs. 4.33 ± 0.393, 3.56–5.10; \(P < 0.0001\); Figure 3C). Together, these results suggest that obese participants mounted elevated PPSV23 IgG-specific responses compared with the nonobese group.

A timeline of 4–6 weeks after vaccination captures the latter portion of the primary humoral immune response. Since PPSV23 is a polysaccharide vaccine, we assessed IgM responses to serotypes that demonstrated the greatest group differences in IgG responses. The obese group had a significantly greater fold change in the selected IgM serotype-specific titers (mean ± SEM, 95% CI: 8.07 ± 1.07, 5.96–10.2 vs. 4.33 ± 0.393, 3.56–5.10; \(P < 0.0001\); Figure 3C). Together, these results suggest that obese participants also have an elevated IgM response to specific serotypes compared with the nonobese group.

**STING1 R232/R232 genotype is positively associated with the PPSV23 responses.** Next, we conducted genetic testing in our study population to assess the genotypes present for **STING1** in our secondary analysis. Ten genotypes were identified (Figure 4A). In the nonobese and obese groups, most participants had the WT genotype, **R232/R232** (13 of 23 and 14 of 23, respectively). Since the **HAQ** allele influences PPSV23 efficacy, specifically leading to an attenuated response in murine models, we assessed **HAQ** allelic frequency (16). Among the 11

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**Figure 1. Flow diagram of participating subjects.**
participants who had the \textit{HAQ} allele, 9 were in the nonobese group (Supplemental Figure 3). Next, we compared the 23 serotype-specific titers among the WT genotype, \textit{R232/R232}, and any genotype that had a single copy of the \textit{HAQ} allele. The WT genotype was associated with a significantly higher fold change in total anti-PPSV23 titers compared with any genotype that had a single copy of \textit{HAQ} (mean ± SEM, 95% CI: 7.97 ± 0.74, 6.52–9.43 vs 4.46 ± 0.514, 3.45–5.48; \textit{P} = 0.0025; Figure 4B). Thus, the \textit{HAQ} allele in humans is associated with decreased fold change in anti-PPSV23 titers.

\textbf{Obesity and the HAQ allele both affect PPSV23 response.} Next, we assessed the role of the \textit{STING1} genotype in nonobese and obese participants. This analysis included only the genotypes \textit{R232/R232} and \textit{R232/HAQ}, as each was represented by at least 2 participants per nonobese or obese group. Among participants with the WT genotype, \textit{R232/R232}, we found the obese group to have significantly higher anti-PPSV23 titers (mean ± SEM, 95% CI: 7.97 ± 0.74, 6.52–9.43 vs 4.46 ± 0.514, 3.45–5.48; \textit{P} = 0.0025; Figure 4B). Thus, the \textit{HAQ} allele in humans is associated with decreased fold change in anti-PPSV23 titers.

\textbf{Discussion}n

As a growing population of obese individuals inevitably ages and becomes susceptible to pneumococcal infections, it is important to consider how obesity and genetics impact the efficacy of PPSV23. The ROVE study investigated the impact of obesity on immune response to the polysaccharide-based
pneumococcal vaccination. Despite the fact that nonobese and obese participants had similar baseline serotype-specific titers and achieved similar clinically relevant responses, the obese group consistently had greater fold changes in serotype-specific titers compared with the nonobese group — the primary endpoint of ROVE. These data align with our observation that obese individuals had elevated inflammatory markers, such as erythrocyte sedimentation rate and WBC counts. Because studies regarding hepatitis B and tetanus vaccinations in obese participants have demonstrated reduced antibody response, our results were unexpected. This may be due to the nature of polysaccharide antigens involving the STING pathway. Indeed, Sting−/− mice have decreased antibody production to PPSV23 (16).

Recently, the role of genetics in individual variability in weight has become better elucidated (21–23). Approximately 22.6% of Americans carry the HAQ-STING1 allele (24). Intriguingly, we observed that 9 of the 11 individuals with a copy of HAQ were in the nonobese group. The WT genotype, R232/R232, was associated with significantly greater fold changes in total anti-PPSV23 titers compared with any genotype with a single copy of HAQ, regardless of BMI. When assessing only the WT genotype of STING1, R232/R232, we found that obese individuals had higher serotype-specific titers to the polysaccharide vaccine. However, this increase in titers was lost in obese individuals with a single copy of HAQ. Together, these exploratory data from our secondary analysis are the first to our knowledge to demonstrate that the HAQ allele is associated with a lower PPSV23 response in humans and are in line with published findings that the vaccine was ineffective in an HAQ-knockin mouse (16). Indeed, we found that the HAQ allele was enriched in the nonobese individuals. Future studies are needed to determine whether the HAQ allele is inversely associated with obesity.

Though our data suggest that the association of obesity and increased serotype-specific titers is related to a lack of HAQ, there may be other explanations. As PPSV23 is a polysaccharide vaccine, its
efficacy depends on B cells. Immune cells play a critical role in the inflammatory state that accompanies obesity (25–27). Human adipose tissue in the obese state is known to secrete leptin and adiponectin (28, 29). Though macrophages and T cells have been implicated in the process of inflammation in obesity, emerging evidence suggests that B cells also modulate obesity-induced adipose tissue inflammation (30). Adipokines may alter B cell function and, in the context of a nonconjugated vaccine, may change the secretion of serotype-specific antibodies, as observed in this study.

Although overweight and obese individuals are at a significantly increased risk of pneumonia, a meta-analysis of 10 cohort studies on pneumonia-related mortality showed an inverse relationship between overweight and obese subjects and pneumonia-associated mortality (31). This “obesity survival paradox” could reflect our finding that obese individuals are more responsive to vaccination. It is thought that PPSV23 protects hosts from pneumococcal infection exclusively via production of opsonizing antibodies to capsular polysaccharides, with increases in serotype-specific antibody levels above reference thresholds leading to better clinical responses (32).

Among this study’s strengths is the finding that PPSV23 induces equal seroconversion in nonobese and obese individuals, suggesting that it is an effective vaccine in the obese population. The use of healthy volunteers in this study minimized some confounding variables and isolated obesity-mediated functions. This study provides findings that warrant future preclinical studies to assess whether enhanced vaccine-specific titers in obese individuals who have the WT *STING1* genotype can be extended to the 13-valent protein conjugate pneumococcal vaccine or other T cell–independent polysaccharide vaccinations, such as those against *Neisseria meningitidis* and *Salmonella enterica* serovar Typhi.

**Limitations.** Our study has several important limitations. First, like many human studies, our study cannot prove causality, in this case between obesity or *STING1* genotype and PPSV23 efficacy. Second, though the age range was set between 18 and 35 to avoid comorbidities due to age, a significant difference in age was observed between the 2 groups. Also, as adults between the ages of 18 and 35 are not generally indicated to receive PPSV23, our study may have limited application. The small cohort size and the even smaller pools of participants constituting subgroups (e.g., White individuals, *STING1* genotypes) limit the ability to rule out residual confounding effects and thus limit the external validity of the study. Last, a study that assessed the effect of obesity on influenza vaccine response found that though obesity correlated positively with the ability to mount a protective immune response at 1 month after vaccination, this increase was lost at 12 months after vaccination (33). However, this study used an inactivated vaccine that relies on a mechanism of action different from that of polysaccharide vaccines.

Overall, in this nonrandomized controlled trial, obese participants had greater serotype-specific antibody responses to PPSV23 than nonobese participants. When assessing the *STING1* genotype, we found that WT *STING1* obese participants had greater PPSV23 responses than nonobese WT *STING1* participants. The increases were lost when we assessed nonobese versus obese participants with the HAQ-*STING1* allele. In conclusion, our study indicated a stronger PPSV23 vaccine response in obese participants, which may lead to better antibody-mediated protection against pneumococcal infection.
Methods

Further information can be found in the Supplemental Appendix, available online.

Participants. Recruitment for the study included the use of posted flyers, emails, and phone calls, and of recruitment databases such as University of Florida (UF) HealthStreet and ResearchMatch, a national health volunteer registry that was created by several academic institutions and supported by the NIH as part of the Clinical Translational Science Award program. Eligible participants were between 18 and 35 years of age, with either a BMI between 22 and 25 kg/m²; or ≥30 kg/m² and a waist-to-hip ratio of at least 0.9 in males or 0.85 in females. Participants were excluded if they were pregnant, breastfeeding, diagnosed with diabetes, unable to fast for 8 hours prior to the initial blood draw, or previously immunized with PPSV23 or the 13-valent conjugate pneumococcal vaccine. Exclusion criteria also included use of immunosuppressive drugs, chemotherapeutic agents, or steroids; or a history of any of the following: pneumonia, splenectomy or damage to spleen, lung disease, history of chronic inflammatory disease, or autoimmune diseases. Subjects were also excluded if they planned to lose weight within the time frame of the study, experienced excessive weight loss or gain within 2 months prior to the study, planned to undergo any weight loss procedures during the study period, or partook in excessive weight training. The trial protocol, statistical analysis, and complete inclusion and exclusion criteria are presented in the Supplemental Appendix.

Study procedures. After passing an initial screening questionnaire, eligible participants were invited for 2 visits at the UF Clinical Research Center. During the first visit, the informed consent form was reviewed and signed. A physical exam, history, urine pregnancy test, and hemoglobin A1C test (<6.5%) were conducted to reassess eligibility with study criteria via a second screening. If these criteria were met, study nurses acquired baseline measurements, collected blood, and administered a single dose of
PPSV23 intramuscularly (Merck, M043987 and N032092). The participants returned 4–6 weeks after vaccination for 1 additional blood draw. In compensation for their time and participation, participants were given US$50 for each visit completed.

Outcomes. Fasting blood samples were taken during both visits and analyzed by the UF Clinical Research Center for a complete blood count, complete metabolic panel, erythrocyte sedimentation rate, and total lgs. Serotype-specific IgG antibody responses against the 23 pneumococcal serotypes (i.e., 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F) were analyzed by Mayo Clinic Laboratories (test ID: PN23) using microsphere photometry. If the test was unable to quantitate specific serotypes due to a nonlinear dilution response, the interpretation of pneumococcal antibody serology was based on the remaining serotypes, as per the recommendations for the test (34). Clinically relevant responses to Streptococcus pneumoniae vaccination were assessed using the following 2 definitions: (i) antibody concentrations ≥the reference value for at least 50% of serotypes present in either a pre- or postvaccination sample; (ii) antibody concentrations increased by 2-fold or greater for at least 50% of serotypes present when comparing post- and prevaccination results. Since PPSV23 is a polysaccharide vaccine, IgM responses to serotypes that demonstrated the greatest group differences in IgG responses were assessed using ELISAs. The following reagents were used: pneumococcal polysaccharide type 2 (PPS2) (ATCC 500-X), PPS4 (ATCC 18-X), PPS8 (ATCC 503-X), PPS18C (ATCC 285-X), PPS33F (ATCC 67-X), ELISA Diluent (eBioscience 00-4202-56), TMB Solution 1× (eBioscience 00-4201-56), Stop Solution (Invitrogen SS04), ELISA Coating Buffer Powder (eBioscience 00-0044-59), Tween 20 (Fisher Scientific BP337-100), glycine hydrochloride (Acros Organics 411011000), and 10× PBS (Fisher Scientific BP3991).

STING1 genotyping. For assessment of STING1 alleles, peripheral blood samples were drawn in EDTA-coated vacutainers, and DNA was extracted from buffy coats using a DNeasy Blood & Tissue Kit (QIAGEN 69504). The following primers were used for amplification: exon 3-4-5 Fwd 5'-GGAAATGGTCATGGATTTCTTGG-3', exon 3-4-5 Rev 5'-AGAATGGTCATGGAATTCTTGG-3'; exon 4-5-6 Fwd 5'-GTCTGTTTTGTAGATC-3', exon 4-5-6 Rev 5'-CTGGCCTCCTGTAATGAGAT-3'; exon 7a Fwd 5'-CTCCCATAGCCCTTCTGACTTT-3', exon 7a Rev 5'-GGCTTAGCTGGTCTTCCATTAC-3'. PCR products were purified using QIAquick PCR purification kit (QIAGEN 28104) and run on agarose gel for product size confirmation. Products were then sent to GENEWIZ for sequencing.

Statistics. The study was powered based on previous work assessing the effect of atorvastatin on immune response to PPSV23 (35). The sample size was calculated by comparing the antibody titer of each serotype to its specific reference value to determine whether a subject mounted a positive immune response per titer. Subjects received a score from 0 to 14 corresponding to the number of positive titers. The cutoff value used for sample size calculation was a BMI of 25. For the BMI <25 group, the mean number of positive titers was 9.2, with an SD of 2.3; and for the BMI >25 group, the mean was 7.4, with an SD of 1.5. At a significance level of α = 0.05 for a 2-sided t test at 80% power, 23 subjects per group was necessary.

Our primary outcome variable was fold change in titer levels from before to after vaccination. In our primary analysis, we used an ANCOVA to test for differences in fold change titer levels across groups (nonobese and obese) while controlling for sex and age. Our secondary outcome was analyzed using 2-way ANOVA to assess fold change in titer levels between the STING1 WT genotype, R232/R232, and genotypes that include the HAQ allele. Secondary analyses were interpreted as exploratory. In post hoc analyses, we examined titers for each of the specific antipneumococcal antibodies without multiple P value correction. For data comparisons between 2 populations, as seen in the baseline characteristics table, normality was assessed by Shapiro-Wilk test, followed by either an unpaired 2-sided t test for parametric data or Wilcoxon's signed-rank test for nonparametric data to assess differences between groups for individual serotype-specific titers, complete blood count, complete metabolic panel, erythrocyte sedimentation rate, and vaccine efficacy due to STING1 genotype between groups. All hypothesis tests were 2 sided, and a P value less than 0.05 was considered significant. A χ² test was used to determine differences in sex and ethnicity. Data were analyzed in Prism (GraphPad) and R 3.5 (http://r-project.org).

Study approval. This study was approved by the UF Institutional Review Board (IRB 201401069). Written informed consent was obtained from all participants.

Author contributions

MS, RSE, LD, CJH, HSF, AMC, LJ, and MLB participated in the study design. MS, RSE, LD, CJH, HSF, AC, SP, MG, DSK, and JC participated in acquisition, analysis, and interpretation of data. MS wrote the initial draft of the manuscript, which was reviewed, edited, and approved by all authors. MS, RSE, LD,
CJH, and HSF conducted primary data analysis. MS, RSE, LD, CJH, HSF, LJ, and MLB participated in administrative, technical, and material support. Study supervision was provided by LS, CDH, LJ, and MLB. Authorship was assigned among co–first authors in reverse alphabetical order.

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