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Effect of atorvastatin on humoral immune response to 23-valent pneumococcal polysaccharide vaccination in healthy volunteers: The StatVax randomized clinical trial

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ABSTRACT

Background: The immunomodulatory effects of statins on vaccine response remain uncertain. Therefore, the objective of this study was to determine if atorvastatin enhances pneumococcal-specific antibody titer following 23-valent pneumococcal polysaccharide vaccination.
Methods: Double-blind, placebo-controlled, single-center randomized clinical trial entitled StatVax. Subjects were enrolled between June and July 2014 and followed up through September 2014. 33 healthy volunteers signed informed consent after volunteer sampling. 11 participants were excluded; 22 healthy volunteers without prior pneumococcal vaccination were enrolled and completed the study. Participants were randomized to receive a 28-day course of 40 mg atorvastatin (n = 12) or matching lactose placebo (n = 10). On day 7 of treatment, Pneumovax 23 was administered intramuscularly. The primary outcome was fold change in total pneumococcal-specific antibody titer determined by a ratio of post-vaccination titer over baseline titer. Secondary outcomes included serotype-specific pneumococcal antibody titer, seroconversion, complete blood counts (CBC), erythrocyte sedimentation rate (ESR), and serum cytokine analysis.
Results: Of the 22 randomized patients (mean age, 23.86; SD, 4.121; 11 women [50%]), 22 completed the trial. Total anti-pneumococcal antibody titer in the atorvastatin group went from a baseline mean of 32.58 (SD, 15.96) to 147.7 (SD, 71.52) μg/mL at 21 days post-vaccination while titer in the placebo group went from a mean of 30.81 (SD, 13.04) to 104.4 (SD, 45) μg/mL. When comparing fold change between treatment groups, there was a significant increase in fold change of total anti-pneumococcal antibody titer in the atorvastatin group compared to the placebo group (2-way ANOVA, p = .0177).
Conclusions: Atorvastatin enhances antigen-specific primary humoral immune response to a T cell-independent pneumonia vaccination. Pending confirmation by larger cohort studies of target populations, peri-vaccination conventional doses of statins can become a novel adjuvant for poorly-immunogenic polysaccharide-based vaccines.
Trial Registration: clinicaltrials.gov Identifier: NCT02097589

1. Introduction

Pneumococcal pneumonia is a major cause of morbidity and mortality in children and elderly patients [1–3]. Pneumonia vaccines which include Prevnar 13, a 13-valent conjugate vaccine, and Pneumovax 23, a T cell-independent, 23-valent polysaccharide vaccine, are scheduled for patients over the age of 65 to reduce the risk of infection [2,4,5]. However, vaccination responses are dampened in elderly patients by immune senescence rendering them vulnerable to infection [4,6]. Vaccination in these patients is further complicated by concurrent treatment with other medications. Over 80% of patients over the age of 65 take at least one prescription medication, and 39% take five or more prescriptions [7]. The

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effects of these medications on immunologic response to vaccination are largely unknown.

Statins are HMG-CoA reductase inhibitors used to block endogenous cholesterol generation in over 38.6 million Americans [8]. While statins are prescribed to reduce the risk of cardiovascular events, multiple recent reports have indicated a significant role for statins in modulating immunity. Statins have specifically been implicated in skewing Th1/Th2 cytokines [9–11], impairing T cell function [12], enhancing regulatory T cell function [13], impairing basophil activation and degranulation [14–16], and modulating acute phase reactants [17–23]. Retrospective cohort studies suggest that statins reduce the mortality associated with influenza infection and reduce the incidence and mortality of pneumonia by modulating humoral immune responses [24–31]. Although previous clinical evidence from a small cohort suggests that a short-term (10 day) conventional dose atorvastatin significantly enhanced production of antibody titers in a recall response to the T-cell dependent tetanus toxoid vaccine, no reports describe the effect of atorvastatin on primary humoral response to pneumonia vaccination [32]. What remains unclear is whether statin-mediated immune-modulation is only evident during T cell-dependent vaccines. Additionally, it remains unknown if prolonged conventional dose statin use during the immune response to vaccination differentially impacts immunity.

Therefore, the primary aim of the StatVax study was to evaluate the effect of a 28 day course of conventional dose atorvastatin on humoral responses to the T-cell independent pneumonia vaccine Pneumovax 23.

2. Methods

2.1. Study design and oversight

This single-center, randomized, double-blind, placebo-controlled study was approved by the University of Florida Internal Review Board, Research Advisory Committee, and Scientific Advisory Committee. All healthy volunteers provided written informed consent.

2.2. Participants

Posted flyers were used to recruit participants. After a telephone screening, 33 healthy volunteers between the ages of 18–30 gave written informed consent prior to a medical screening and 22 of those 33 were approved and completed the study. Subjects were excluded based on severe interactions with past vaccinations, past vaccination with Pneumovax 23 or Prevnar 13, drug allergies, BMI greater than 32, interactions with statins, pregnancy, creatinine greater than 2 times the reference interval, and creatine kinase (CK), aspartate aminotransferase (AST), and/or alanine transaminase (ALT) levels above reference interval. Of the 11 participants excluded after consent, 3 were excluded for BMI, 6 for high CK, AST, and/or ALT at baseline, and 2 dropped out due to scheduling conflicts.

2.3. Randomization and masking

Random assignment was performed by the Research Design and Analysis Program (RDAP) at the University of Florida Clinical Research Center (CRC) and kept from all providers including physicians, students, nurses, biostatisticians, technicians and study participants. Participants were blocked on sex and treatment. Participants were randomly assigned to 28 day daily treatment with lactose placebo or 40 mg atorvastatin, the conventional dose for hypercholesterolemia. The RDAP provided a randomized list of subject assignments to Investigative Pharmacy at the University of Florida CRC generated from a central automated randomization system. The sheet had a pin number, a place for a secondary ID, and the assignment. These PIN Numbers were 001, 002, . . .022. PIN numbers were assigned at the time of randomization as first come first serve. A back-up copy of the list was housed securely at RDAP. Initial intent was to obtain 20 completers and we attempted to accrue 24 participants to allow for a 20% attrition rate. A modification was made allowing for a larger accrual due to excess exclusions. 22 participants began and completed the trial. Data was managed in REDCap.

2.4. Interventions

Treatment of healthy volunteers with matching (for subject blinding) 40 mg atorvastatin or placebo (McKesson Pharmaceuticals) began on day −7 and ended on day 21. Pneumovax 23 (Merck & Co, lots K004930, J008799, and K005957) was administered intramuscularly on day 0 (Figs. 1 and 2).

2.5. Outcomes

Fasting blood samples were taken on the following days: −7, 0, 1, 7, 14, 21, and 24 in mid-morning. Heparinized whole blood was analyzed by the CRC for a complete blood count (CBC), erythrocyte sedimentation rate (ESR), and total immunoglobulins. Serum was analyzed by multiplex Luminex assay for 18 cytokines (ThermoFisher, EPX180-12172-901). Serotype-specific IgG antibody responses (14 of 23 serotypes) were analyzed by ARUP labs by quantitative multiplex bead assay in comparison to the standards for seroconversion set forth by the American Academy of Allergy, Asthma, & Immunology (AAAAI) [33,34]. Seroconversion was determined by a 2-fold increase in antibody titer for 50% of the serotypes [33].

2.6. Statistical analysis

The power analysis determined that a sample size of 20 patients (10 per group) would have 80% statistical power at $P < .05$ by two-sided *t*-test to detect a difference of 1.33 standard deviations (1.00 units in the log scale). We based the projection on data from a prior study based on tetanus toxoid immune response to conservatively power this study [32].

The primary endpoint of this study was the fold change in total anti-pneumococcal antibody titers [24,29]. Anti-pneumococcal antibody titers were analyzed by ARUP laboratories with a quantitative multiplex bead assay. The analysis of variance (ANOVA) was used to test the difference between treatment groups for primary endpoint and secondary endpoints (serotype-specific titers, seroconversion, CBC, ESR, and Luminex). Significance was determined as $p < .05$ and displayed as $^{\dagger}p < .05$, $^{\dagger\dagger}p < .01$, $^{\dagger\dagger\dagger}p < .001$ for ANOVAs. For comparisons between only two populations, the paired or unpaired two-tailed *t*-test was performed. Significance was determined as $p < .05$ and displayed as $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. Data was analyzed in GraphPad Prism.

No major adverse events were reported. Data collection and analysis was completed prior to breaking the randomization assignment. Two patients' samples were not collected during the trial, one on day 14 (atorvastatin group) and day 21 (placebo group). Both missed collections were due to scheduling conflicts. Additionally, there were 13 insufficient samples; 1 insufficient sample on day −7 that only affected the serotype-specific antibody titer, 11 insufficient samples that only affected ESR on day 14, and 1 insufficient sample for CBC on day 7. All insufficient samples were due to insufficient volume of blood samples harvested by trial staff. We performed next observation carried backward (NOCB)

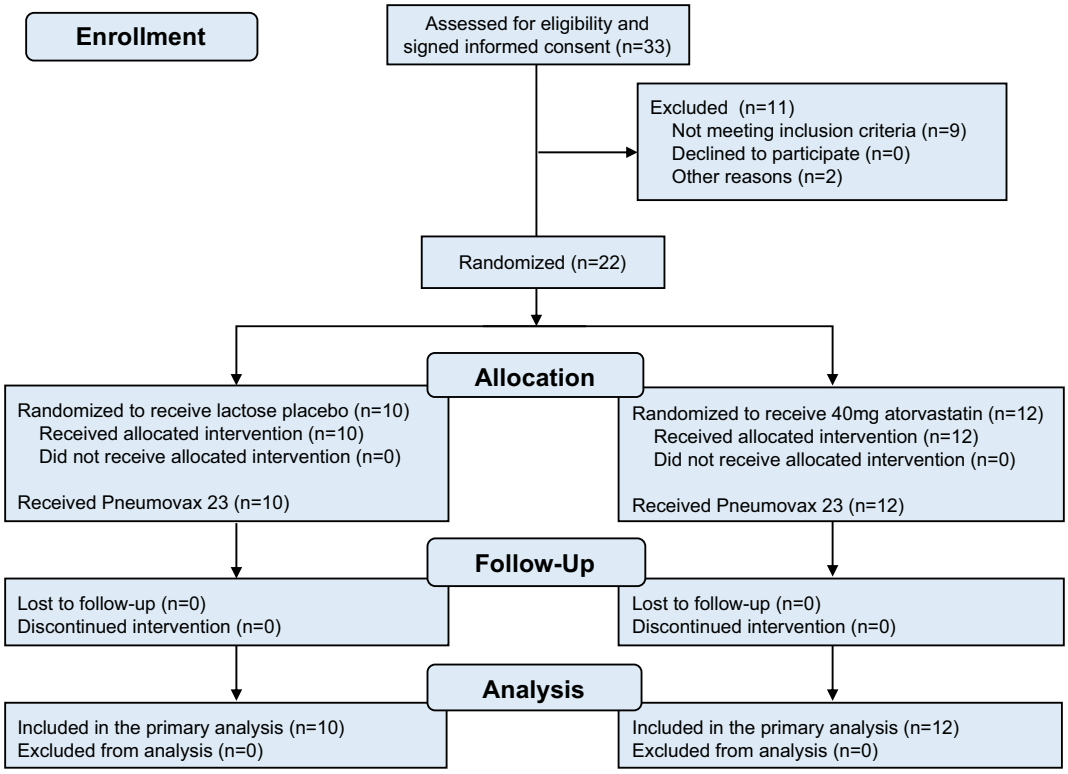

Fig. 1. StatVax CONSORT Diagram.

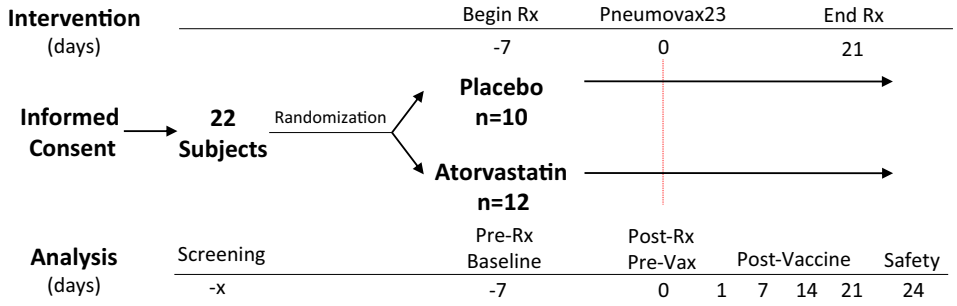

Fig. 2. StatVax Trial Design.

analysis for the insufficient sample for the anti-pneumococcal antibody titer on day −7. We utilized this structure because it was critical for calculating the fold change (dependent variable). We also utilized this structure considering the random nature of this insufficient sample, the small sample size of the trial, as well as the lack of a significant difference between the total pneumococcal-specific antibody titer between days −7 and day 0 of the trial (day −7, pre-tx and pre-vaccine; day 0, 7-day post-start of tx and pre-vaccine; placebo, r = 0.970, p = .95; atorvastatin, r = 0.824, p = .180; paired *t*-test). We additionally performed analysis of the primary endpoint without the NOCB data and identified significance without the imputed data (2-way ANOVA; without NOCB, p = .0194; with NOCB, p = .0177). This indicates a lack of dependence on NOCB for meeting significance of the primary endpoint.

3. Results

3.1. Participants

After screening, 33 patients signed informed consent and underwent a physical exam and preliminary blood draw. After meeting exclusion criteria, 22 patients were enrolled and 22 completed the study (Figs. 1 and 2). All participants displayed similar distribution of body mass index, heart rate, blood pressure, high-density lipoprotein (HDL), triglycerides, low-density lipoprotein (LDL), and Non-HDL cholesterol (Table 1). Of the 22 subjects, 12 were randomized to receive atorvastatin (6 females, 50%) and 10 were randomized to receive a lactose placebo (5 females, 50%). All subjects were compliant with their randomized treatment and when comparing the day 8 post-treatment mean of 44.08 (SD, 26.99) to the pre-treatment mean 86.33 (SD, 26.99) the atorvastatin cohort had a 49% reduction in LDL (LDL, difference, −42.25, [95% CI, −51.71 to −32.79]; paired two-tailed *t*-test, p < .0001; Figs. 3A; S1). Patients randomized to placebo displayed no change in cholesterol or triglycerides.

3.2. Outcomes

3.2.1. Antibody titers

The primary endpoint was fold change in total anti-pneumococcal antibody titer between treatment groups. This primary analysis revealed that the atorvastatin group had a signifi-

Table 1
Baseline Patient Characteristics. Baseline age, gender, blood pressure, weight, and heart rate were evaluated on the day of the screening visit. BMI, body mass index; HR, heart rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Mean +/− SD.

	Atorvastatin (n = 12)	Placebo (n = 10)
Age	24.5+/−4.1	23.2+/−4.4
Males	6	5
Females	6	5
Systolic Blood Pressure (mmHg)	119.7 +/−10.7	125.0+/−7.9
Diastolic Blood Pressure (mmHg)	71.2+/−7.2	71.4+/−6.2
BMI	23.4+/−2.5	25.1+/−2.7
HR (bpm)	63.0+/−7	65.8+/−7.2
HDL mg/dl	55.5+/−15.3	60.4+/−14.5
Triglycerides mg/dl	76.3+/−25.2	82.7+/−43.4
LDL mg/dl	83.7+/−26.7	87.0+/−30.6
Non-HDL Cholesterol	99.2+/−26.4	103.6+/−35.5

cantly higher fold change in total anti-pneumococcal antibody titer during the vaccine response (2-way ANOVA, p = .0177; Fig. 3B). Total anti-pneumococcal antibody titer in the atorvastatin group changed from a baseline mean of 32.58 (SD, 15.96) to 147.7 (SD, 71.52) μg/mL at 21 days post-vaccination while titers in the placebo group went from a mean of 30.81 (SD, 13.04) to 104.4 (SD, 45) μg/mL (two-way ANOVA, p = .0243; Fig. 3C). Comparing fold change in titer for each serotype between the atorvastatin and placebo group revealed that the atorvastatin group had significantly higher fold change at weeks 1, 2, and 3 post-vaccine (two-way ANOVA, p = .029, p = .0158, p = .0244, respectively; Fig. 4A and B).

For secondary analysis, there was no significant change in total IgG, IgM, and IgA between both groups (Fig. S2). We next analyzed the change in antibody titer over time for each individual serotype to determine if a fraction of serotypes were driving the total difference between groups. The atorvastatin group had a higher fold change in antibody titer when compared to placebo for 3 of 14 measured serotypes including serotype 1 (two-way ANOVA, p = .0056), serotype 7 (p = .0022), and serotype 9v (p = .0316; Table 2, Fig. 5A and B). Additionally, serotypes 5, 18c, and 19f approached significance (two-way ANOVA, p = .0762, 0.0585, and 0.0923, respectively; Table 2).

3.2.2. Seroconversion rates

We next evaluated differences in subject seroconversion between treatment groups. Seroconversion was determined by a 2-fold increase in antibody titer for 50% of the serotypes [33,34]. The analysis revealed that the atorvastatin group had a higher percentage of seroconversion when compared to placebo that approached significance (two-way ANOVA, p = .073; Fig. 5C). In addition, the atorvastatin group reached 100% seroconversion by two weeks post-vaccine while the placebo group never reached 100% seroconversion. We next evaluated the percent of individual serotypes that were converted for each patient. This analysis revealed that subjects in the atorvastatin group converted a non-significantly greater percent of serotypes compared to subjects in the placebo group (two-way ANOVA, p = .087; Fig. 5D).

3.2.3. Complete blood count

We next performed secondary analysis of complete blood counts over time. This determined that there are no significant differences in white blood cells, neutrophils, or eosinophils (Fig. S3). However, the fold change in basophils significantly increased in

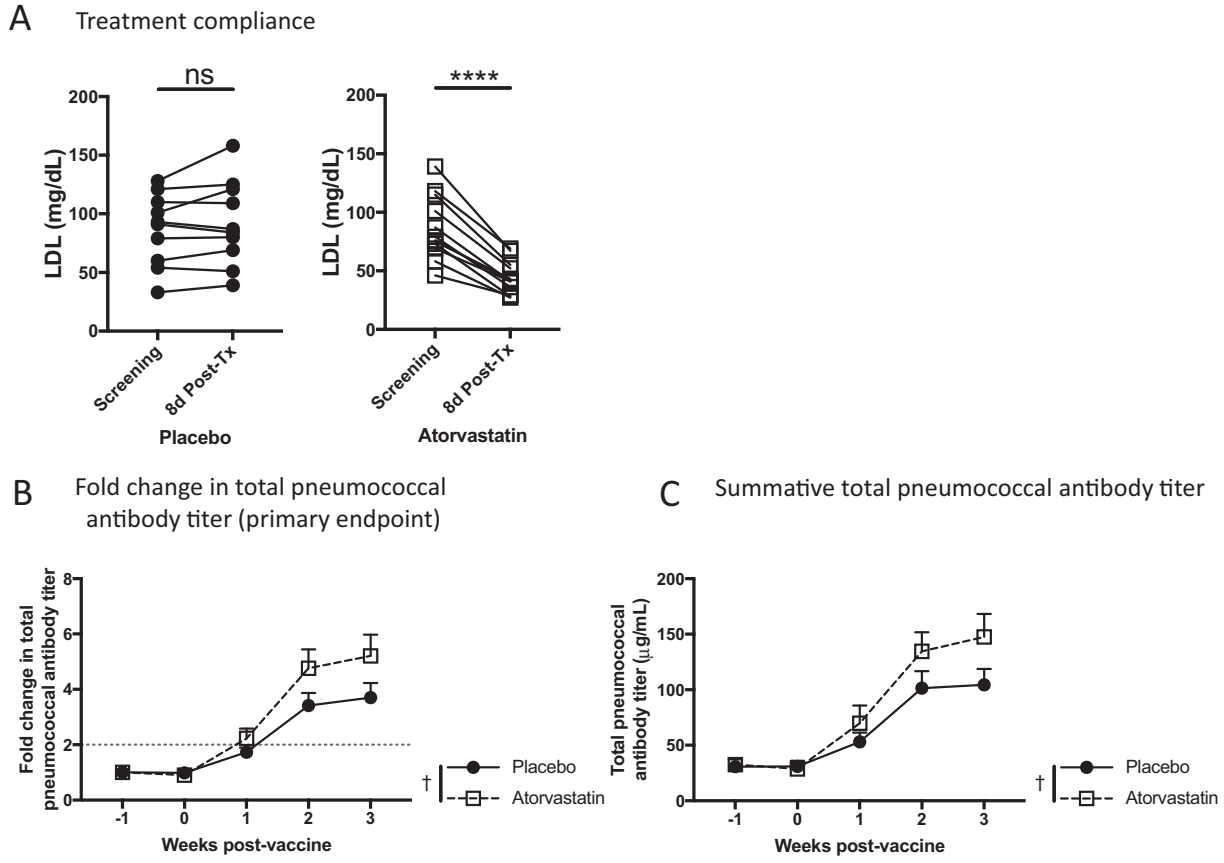

Fig. 3. LDL and Total Anti-pneumococcal Antibody Titers. LDL, low-density lipoprotein. (A), Paired analysis of the measurement of LDL before and after treatment with atorvastatin; ****, *p < 0.0001*. (B), Fold change in total anti-pneumococcal antibody titer over baseline and comparison between treatment groups. Fold change is the ratio of titer at each timepoint to the baseline titer; †, *p < .05*. (C), Total anti-pneumococcal antibody titer and comparison between treatment groups; †, *p < .05*.

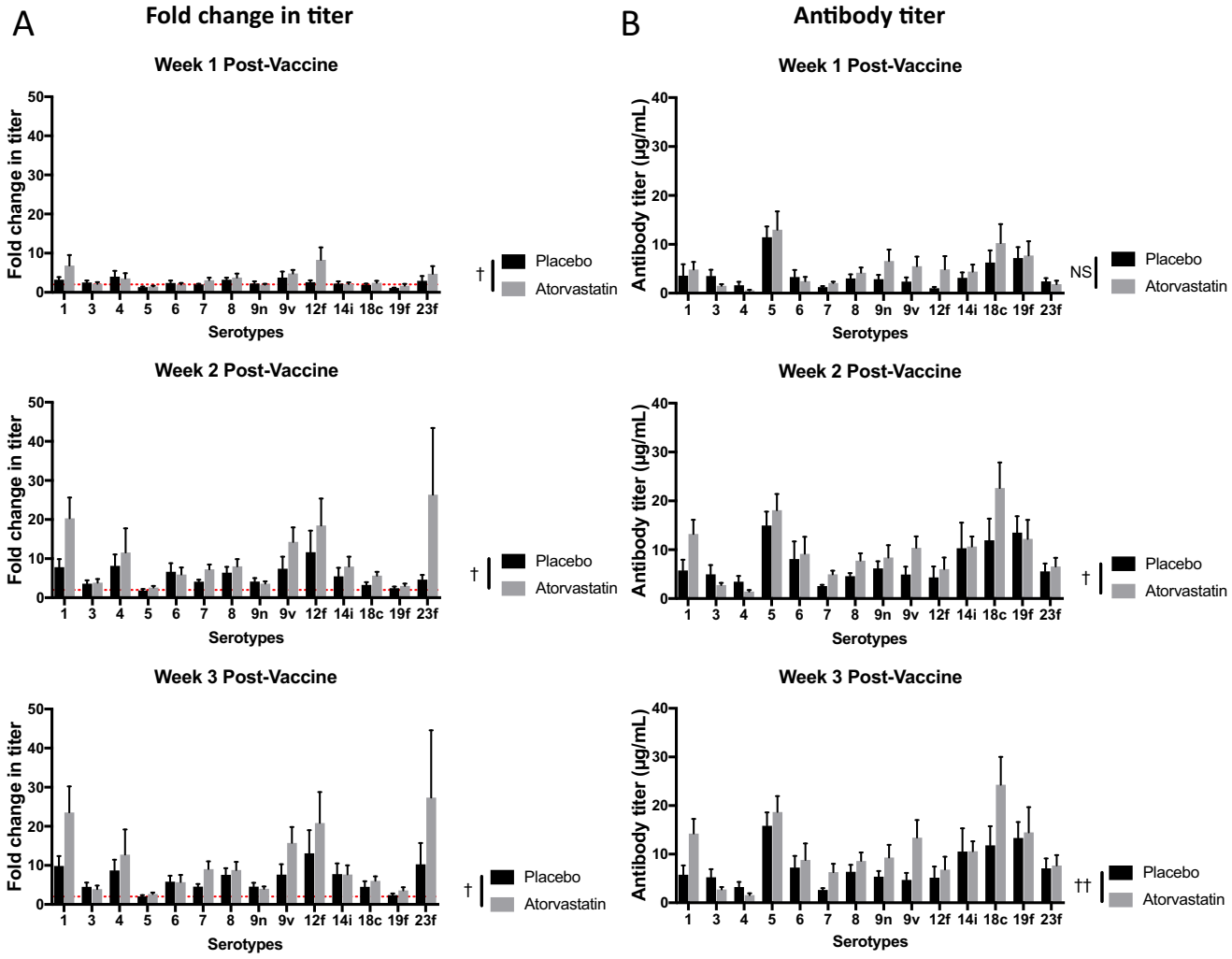

Fig. 4. Weekly Serotype-specific Anti-pneumococcal Antibody Titer. (A), Comparison of fold change in anti-pneumococcal antibody titers between treatment groups at weeks 1–3 post-vaccine; †, $p < .05$. *(B),* Comparison of anti-pneumococcal antibody titers between treatment groups at weeks 1–3 post-vaccine; †, $p < .05$.

Table 2
Serotype-specific Pneumococcal Antibody Titers. Mean and SD of serotype-specific anti-pneumococcal antibody titers at each timepoint. ANOVAs performed for titer value or fold change in titer value. Significant values are bolded.

		Placebo	Statin	Placebo	Statin		
Serotype	Time (week)	Mean (µ)		SD(σ)		ANOVA (p-value)	Fold change ANOVA (p-value)
1	Baseline	1.50	0.91	3.42	0.66	**0.0162**	**0.0056**
	Vaccine	1.99	0.70	4.96	0.41		
	Week 1	3.58	4.87	7.44	5.05		
	Week 2	5.78	13.26	6.53	10.05		
	Week 3	5.77	14.24	6.08	10.38		
3	Baseline	1.81	1.10	1.56	1.35	**0.0048**	0.67
	Vaccine	1.78	1.14	1.53	1.35		
	Week 1	3.53	1.51	3.95	1.12		
	Week 2	4.99	2.77	5.67	1.55		
	Week 3	5.24	2.75	5.34	1.72		
4	Baseline	0.46	0.29	0.41	0.31	**0.0026**	0.524
	Vaccine	0.41	0.22	0.36	0.20		
	Week 1	1.62	0.55	2.18	0.45		
	Week 2	3.47	1.44	3.54	1.13		
	Week 3	3.24	1.59	3.35	1.20		
5	Baseline	8.78	8.65	5.18	8.25	0.554	0.0762
	Vaccine	8.79	6.51	6.37	4.59		
	Week 1	11.44	12.99	6.96	12.39		
	Week 2	14.97	18.12	8.57	11.47		
	Week 3	15.84	18.68	8.69	11.37		

(*continued on next page*)

Table 2 (*continued*)

Serotype	Time (week)	Placebo Mean (μ)	Statin Mean (μ)	Placebo SD(σ)	Statin SD(σ)	ANOVA (p-value)	Fold change ANOVA (p-value)
6	Baseline	1.29	1.08	1.09	0.68	0.82	0.718
	Vaccine	1.29	1.31	1.11	1.42		
	Week 1	3.32	2.45	4.47	2.83		
	Week 2	8.13	9.18	10.74	11.99		
	Week 3	7.24	8.83	7.68	11.78		
7	Baseline	0.67	1.13	0.35	1.01	**0.0007**	**0.0022**
	Vaccine	0.66	0.94	0.44	0.83		
	Week 1	1.24	2.08	0.62	0.94		
	Week 2	2.57	4.99	0.79	2.58		
	Week 3	2.66	6.32	1.11	5.84		
8	Baseline	1.00	1.84	0.62	2.24	**0.0182**	0.389
	Vaccine	0.98	1.80	0.69	2.38		
	Week 1	3.03	4.20	2.60	3.52		
	Week 2	4.62	7.78	1.74	5.15		
	Week 3	6.39	8.63	4.53	6.01		
9n	Baseline	1.38	4.08	1.11	5.11	**0.009**	0.377
	Vaccine	1.37	3.65	1.24	4.70		
	Week 1	2.90	6.58	2.65	7.69		
	Week 2	6.18	8.43	4.39	8.73		
	Week 3	5.37	9.32	3.78	9.05		
9v	Baseline	0.77	1.26	0.41	1.42	**0.0019**	**0.0316**
	Vaccine	0.76	1.16	0.41	1.39		
	Week 1	2.40	5.52	2.59	6.52		
	Week 2	4.96	10.43	4.78	8.08		
	Week 3	4.72	13.40	4.51	12.50		
12f	Baseline	0.38	0.50	0.16	0.41	0.193	0.158
	Vaccine	0.37	0.45	0.17	0.38		
	Week 1	1.01	4.88	0.82	8.99		
	Week 2	4.37	6.04	6.69	8.21		
	Week 3	5.19	6.86	7.17	9.09		
14i	Baseline	1.48	2.86	0.97	2.97	0.5359	0.659
	Vaccine	1.46	2.99	1.11	3.14		
	Week 1	3.15	4.41	3.45	4.77		
	Week 2	10.32	10.67	15.64	7.11		
	Week 3	10.55	10.63	15.11	6.96		
18c	Baseline	3.05	3.32	3.14	1.83	**0.0155**	0.0585
	Vaccine	3.01	3.57	2.83	2.23		
	Week 1	6.30	10.27	7.75	12.82		
	Week 2	11.96	22.64	13.27	18.19		
	Week 3	11.80	24.27	12.47	19.92		
19f	Baseline	6.99	4.42	7.22	3.57	0.5829	0.0923
	Vaccine	6.84	3.66	6.64	2.56		
	Week 1	7.20	7.73	7.00	9.59		
	Week 2	13.51	12.24	10.12	13.43		
	Week 3	13.36	14.48	10.26	18.04		
23f	Baseline	1.27	0.59	1.33	0.53	0.9632	0.148
	Vaccine	1.21	0.67	1.21	0.65		
	Week 1	2.42	1.86	2.11	2.29		
	Week 2	5.57	6.58	4.84	6.19		
	Week 3	7.09	7.68	6.36	7.40		

the atorvastatin group compared to the placebo group over time (two-way ANOVA, p = .0068; Fig. 6A). To analyze the kinetics of the basophil expansion we performed paired t-tests. Participants in the atorvastatin and placebo groups experienced no change in absolute basophils one week after treatment or during the first day post-vaccine (Fig. 6B and C). However, from day 1 post-vaccine through the study end at day 21 post-vaccine the atorvastatin group displayed a marked increase in absolute basophils and basophil fold change (mean fold change, 1.11 vs. 1.32, difference, 0.206, [95% CI, 0.027 to 0.384], p = .028, paired *t*-test; Fig. 6D). On the contrary, the placebo group displayed no significant increase in absolute basophils and basophil fold change.

Investigation of absolute lymphocytes revealed no significant difference between groups by ANOVA (two-way ANOVA, p = .1009, Fig. 7A). However, participants in the atorvastatin group experienced a significant increase in absolute lymphocytes one week after treatment with atorvastatin, but before vaccination (mean lymphocyte count, 1882 vs. 1667, difference, 214.4 cells/μL, [95% CI, 67.05 to 361.7], p = .0084, paired *t*-test; Fig. 7B). On the contrary, the placebo group displayed no change during this period. Subjects in the placebo group displayed a marked decrease in absolute lymphocytes and fold change in lymphocytes during the 1-day post-vaccination period (mean fold change, 0.937 vs. 1.063, difference, −0.126, [95% CI, −0.227 to −0.025], p = .02, paired *t*-test; Fig. 7C). On the contrary, the atorvastatin group displayed no change immediately after vaccination. Lastly, the placebo group displayed a marked increase in absolute lymphocytes and lymphocyte fold change during the remaining three weeks post-vaccine (mean fold change, 1.162 vs. 0.937, difference, 0.226, [95% CI, 0.0833 to 0.369], p = .0059, paired *t*-test; Fig. 7D).

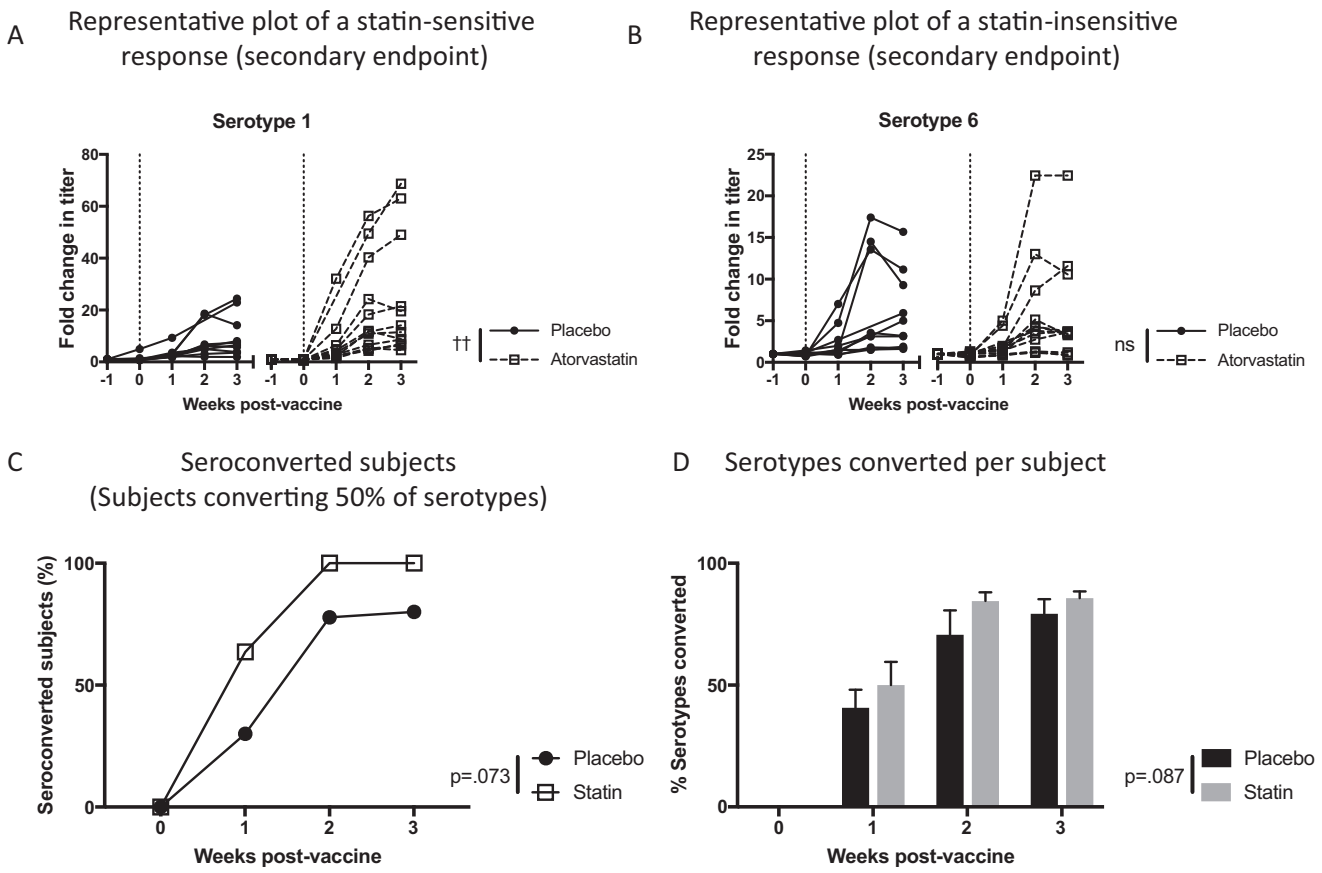

Fig. 5. Serotype-specific Pneumococcal Antibody Seroconversion. A and B, Representative plots of the comparison of the impact of treatment on serotype-specific antibody responses during a statin-sensitive response (A) or statin-insensitive response (B). †, $p < .05$, ††, $p < .01$. C, Seroconverted subjects. Graph represents subjects that converted 50% of their serotypes (converting is a 2-fold change in titer); p = .073. D, Serotypes converted by subject; p = .087.

On the contrary, the atorvastatin group displayed no increase in absolute lymphocytes and lymphocyte fold change. Additionally, the atorvastatin group had a quicker lymphocyte spike that peaked at 1.5 weeks post-vaccine whereas the placebo group peaked at 2.4 weeks post-vaccine (mean week number, 1.5 vs. 2.4, difference, −0.9, [95% CI, −1.57 to −0.226], p = .0115; Fig. 7E).

3.2.4. Erythrocyte sedimentation rate

Fold change in ESR was compared between the atorvastatin group (mean, 0.95 [95% CI, 0.75 to 1.16]) and placebo group (mean, 1.28 [95% CI, 1.05 to 1.51]). The atorvastatin group had a significantly lower ESR (two-way ANOVA, p = .0025; Fig. 8A).

3.2.5. Serum luminex assay

Using a multiplex Luminex assay we compared serum cytokines between treatment groups that were altered after vaccination. On the whole, cytokines decreased over the post-vaccination immune response (Figs. 8B and C, S4). However, there were some notable differences between treatment groups. In the atorvastatin group, fold change in cytokines IFN-α, LT-α, and IL-31 was significantly elevated (two-way ANOVA, p = .0023, 0.0005, and 0.0157) while IFN-γ approached significant elevation (p = .0689) in comparison to the placebo group (Figs. 8B, S4). On the contrary, fold change in cytokines IL-5, IL-12p70, IL-1RA, and GM-CSF was significantly diminished (two-way ANOVA, p = .0063, 0.0376, 0.0234, and 0.0295) while IL-1β approached significance (p = .0778) in comparison to the placebo group (Figs. 8C, S4).

4. Discussion

Statins have considerable immunomodulatory potential and are accessible and scalable [9–23,32,35–39]. Previous studies have demonstrated considerable anti-inflammatory properties of statins as demonstrated by promoting decreases in c-reactive protein (CRP) and ESR, impairment of major histocompatibility complex class II (MHC II) upregulation [17], and decreases in TNF and other pro-inflammatory cytokines [9–11,18,19,23,37,38]. Additional studies have demonstrated considerable statin-mediated immune suppression of adaptive immune cells including decreasing T cell quantity [20], impairing antigen-presenting cell activation of T cells [12], and promoting a shift towards type 2 helper T cell cytokines (Th2) that can resolve autoimmunity [9–11]. On the contrary, recent studies have demonstrated considerable immune-stimulating functions of statins including induction of diabetes and increasing the number of IFN-γ-secreting T cells [21,22]. Clinically, statin use elicits reduced mortality in influenza patients [25], can be associated with a decrease in ventilator-associated pneumonia (VAP) in hospitalized patients [29], and can reduce the risk of pneumonia-related death [26]. While some studies have determined that statins administered to acute cases of critically ill patients with VAP are ineffective [40], other studies have demonstrated the considerable impact on humoral immunity of short-term (10-day) statin doses during vaccination in healthy volunteers [32]. Based upon the observations of statin-mediated T cell modulation and previous impact of short-term statin treatment on T cell-dependent vaccination response, we were interested in the impact of statins in T cell independent vaccination.

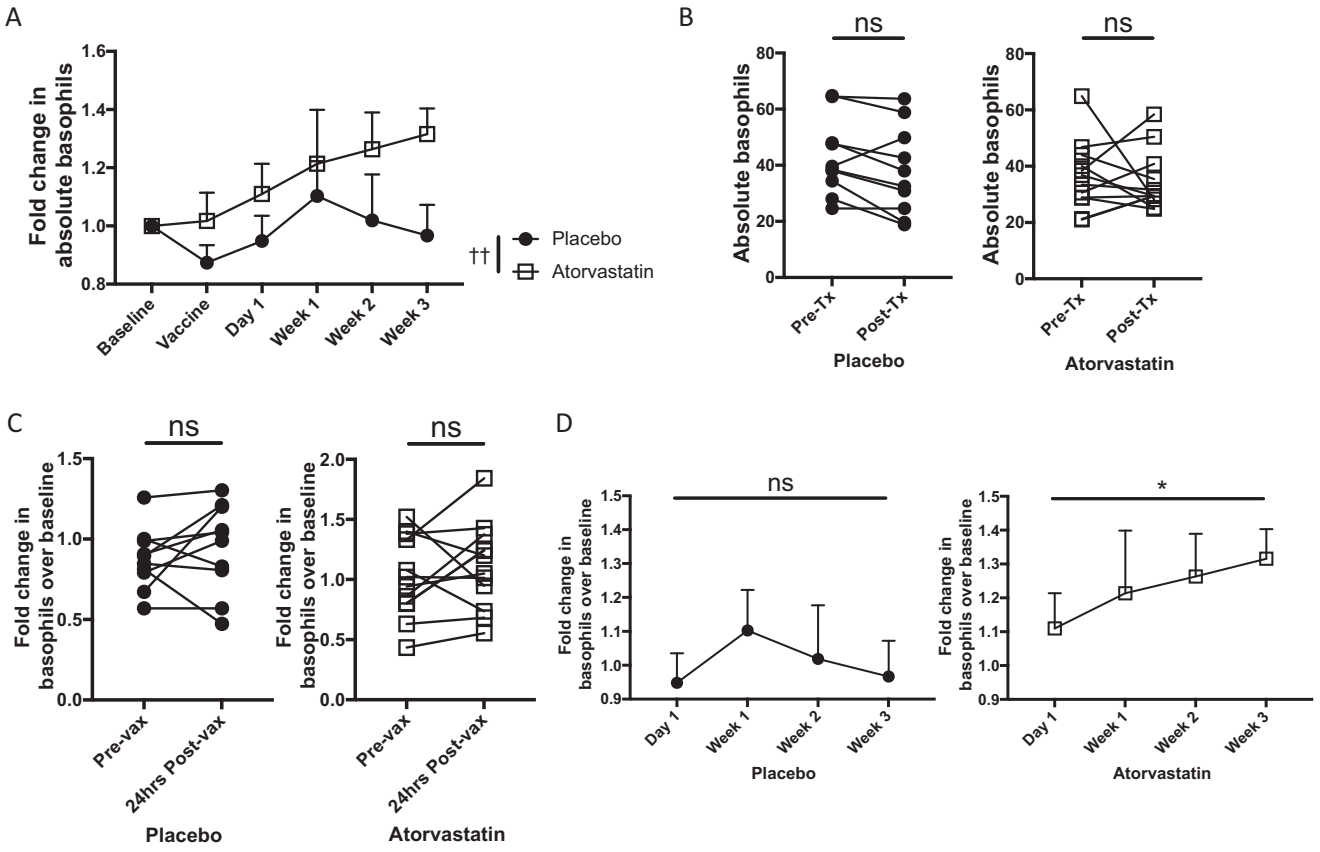

Fig. 6. Basophil Analysis. A, Comparison of the fold change in absolute basophil count. Calculation is the ratio of the absolute basophil count at each timepoint to the baseline value; p = .101. B–D, Paired analysis of absolute basophil count and fold change in basophil count. Comparisons include pre-treatment to 7 days post-treatment (B); pre-vax (same as 7 days post-treatment) to 24 h post-vax (C); and 1 day post-vax (same as 24 h post-vax) to 3 weeks post-vax (D); *, $p < 0.05$, **, $p < 0.01$.

StatVax investigated the impact of statins on the primary immune response to polysaccharide-based pneumococcal pneumonia vaccination. The primary endpoint of the StatVax trial demonstrated that atorvastatin significantly enhances anti-pneumococcal antibody titer after vaccination with Pneumovax 23. Secondary analysis revealed that statins increase the number of serotypes that patients respond to and increase the number of seroconverters while decreasing ESR and modulating immune cell and cytokine kinetics in the peripheral blood. While the short-term dose of statins used in the Lee et al. study could have indicated that rebound from statin treatment was the causative mechanism for enhanced humoral immunity, our study indicates that longer course statins induce a similar improvement in humoral immunity. This indicates three major findings: 1. statins can enhance humoral immunity to a T cell-independent polysaccharide vaccine, 2. Statins can enhance the primary humoral immune response to pneumococcal serotypes for which the immune system has no pre-existing memory, and 3. Statin-mediated enhancement of humoral immunity is not a response to washout or rebound to short-term statin therapy. The first finding signals a fundamental shift in the clinical utility of statins as an adjuvant strategy for vaccination. This opens up the use of statins as an adjuvant for poorly-immunogenic vaccines including polysaccharide-based vaccines that induce T cell-independent immune responses. The second finding signals a novel discovery understanding the role of statins in modulating immunity. In combination with Lee et al. [32], our data suggests that statins do not require immune memory to enhance humoral immunity. Instead, in pneumococcal pneumonia-naïve subjects, the primary immune response to pneumococcus exposure was sensitive to statin treatment. The third finding signals that statin-mediated enhancement of humoral immunity is a function that persists during longer-term statin regimens [32]. This is critical to understanding the dynamics of immunomodulatory effects of statins and for translation to the 38.6 million Americans that are on long-term statin regimens [8].

The kinetics of peripheral blood cell populations during StatVax highlight some important areas for future discovery. Atorvastatin and Cerivastatin have been reported to induce anti-inflammatory and LDL-lowering effects specifically on human basophils [14,16]. This included statin diminished release of histamine after IgE-stimulation as well as diminished basophil activation marker CD203c expression [16]. In a separate study, statins mediated an increase in LDL-receptors on basophils and mast cells while also increasing the uptake and degradation of LDL within basophils [15]. In our trial, basophils were surprisingly increased during the course of statin treatment. While we have no direct evidence of the long-term effect of statins on basophils independent of vaccination, in the immediate post-treatment pre-vaccine week atorvastatin appeared to exert minimal effects on basophil levels. However, once vaccination began, basophil levels increased in the atorvastatin group. While future studies are required to clarify this system, the increase in basophils may be a compensatory mechanism to induce requisite inflammation towards vaccination in the presence of the inflammation-limiting atorvastatin. The downstream effects of this expansion of basophils may have an influence on antibody titers and pneumococcus infection prevention that should be explored further. Perhaps statins are mediating enhanced allergic sensitization that promotes increased antibody generation.

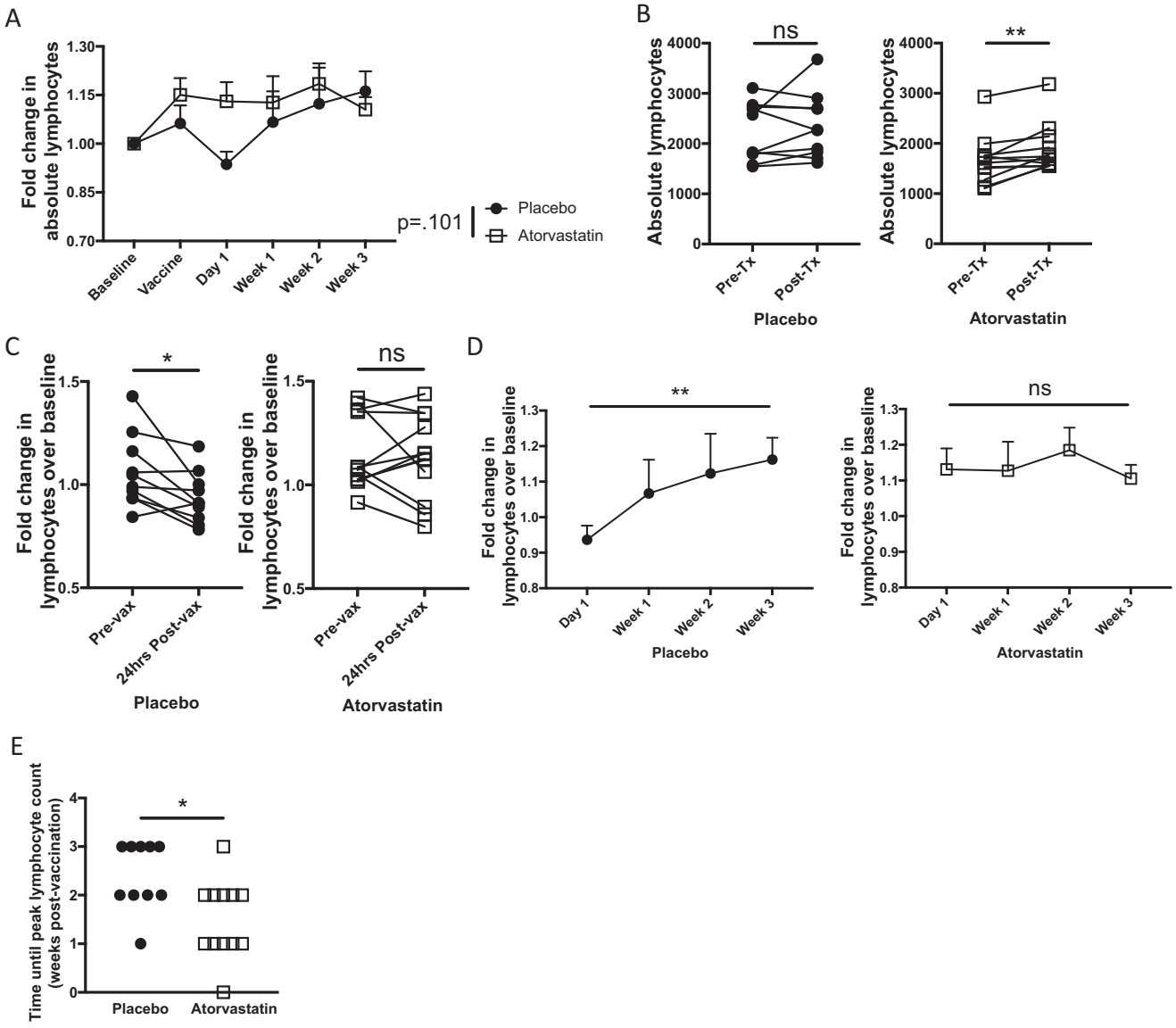

Fig. 7. Lymphocyte Analysis. A, Comparison of the fold change in absolute lymphocyte count. Calculation is the ratio of the absolute lymphocyte count at each timepoint to the baseline value; p = .101. B-D, Paired analysis of absolute lymphocyte count and fold change in lymphocyte count. Comparisons include pre-treatment to 7 days post-treatment (B); pre-vax (same as 7 days post-treatment) to 24 h post-vax (C); and 1 day post-vax (same as 24 h post-vax) to 3 weeks post-vax (D); *, $p < 0.05$, **, $p < 0.01$. E, Comparison between groups of the time point during post-vaccination response at which each participant reached their peak lymphocyte count; *, $p < 0.05$.

Future studies should focus on the mechanisms by which statins modulate innate and adaptive immunity to lead to the enhanced generation of antibodies. In this study, we demonstrated a significant expansion of lymphocytes after induction of statin treatment that persisted throughout the following weeks. While these are indeterminate lymphocytes, further investigation should determine how this expansion occurs. This is especially interesting in light of observations that atorvastatin can decrease T cell activation or modulate T helper cytokines to enhance Th2 or even T regulatory-polarized responses [9–13,20]. Of additional interest is studying the impact of statin-mediated LFA-1 inhibition and subsequent reduced lymphocyte diapedesis from the blood on antibody titer [10,36]. That mechanism could have an impact on migration to lymphoid organs during the vaccine response.

On the innate side of immunity, future studies should investigate the impact of statin-mediated modulation of acute phase reactants on antibody titers post-vaccination [10,17–19,39]. In our study, some key proinflammatory cytokines including IL-12 and GM-CSF were decreased in the statin group. Additionally, we replicated the result that ESR is reduced in statin-treated individuals. While those findings may be in line with some previous hypotheses of statins anti-inflammatory action, other cytokine analyses raise more questions. First, the statin group has a demonstrably higher level of pro-inflammatory cytokines IFN-α, IFN-γ, and LT-α (formerly TNF-β). That largely contradicts previous reports of the statin-mediated decrease in Th1 cytokines; yet is potentially supportive of the finding that statins can enhance IFN-γ production [22]. Perhaps the most interesting discoveries in this trial revolve around Th2 cytokines and basophil expansion. Basophils are known to drive Th2 cytokine shifts and promote Th2 T cell immune responses [41], which can be critical for T cell-dependent antibody generation. While Pneumovax 23 is a T cell-independent polysaccharide-based vaccine, the statin group has significantly lower IL-5, one of the Th2 cytokines, while there is a concomitant expansion of basophils. This suggests that there may be more to the mechanism behind Th1/Th2 cytokines, lym-

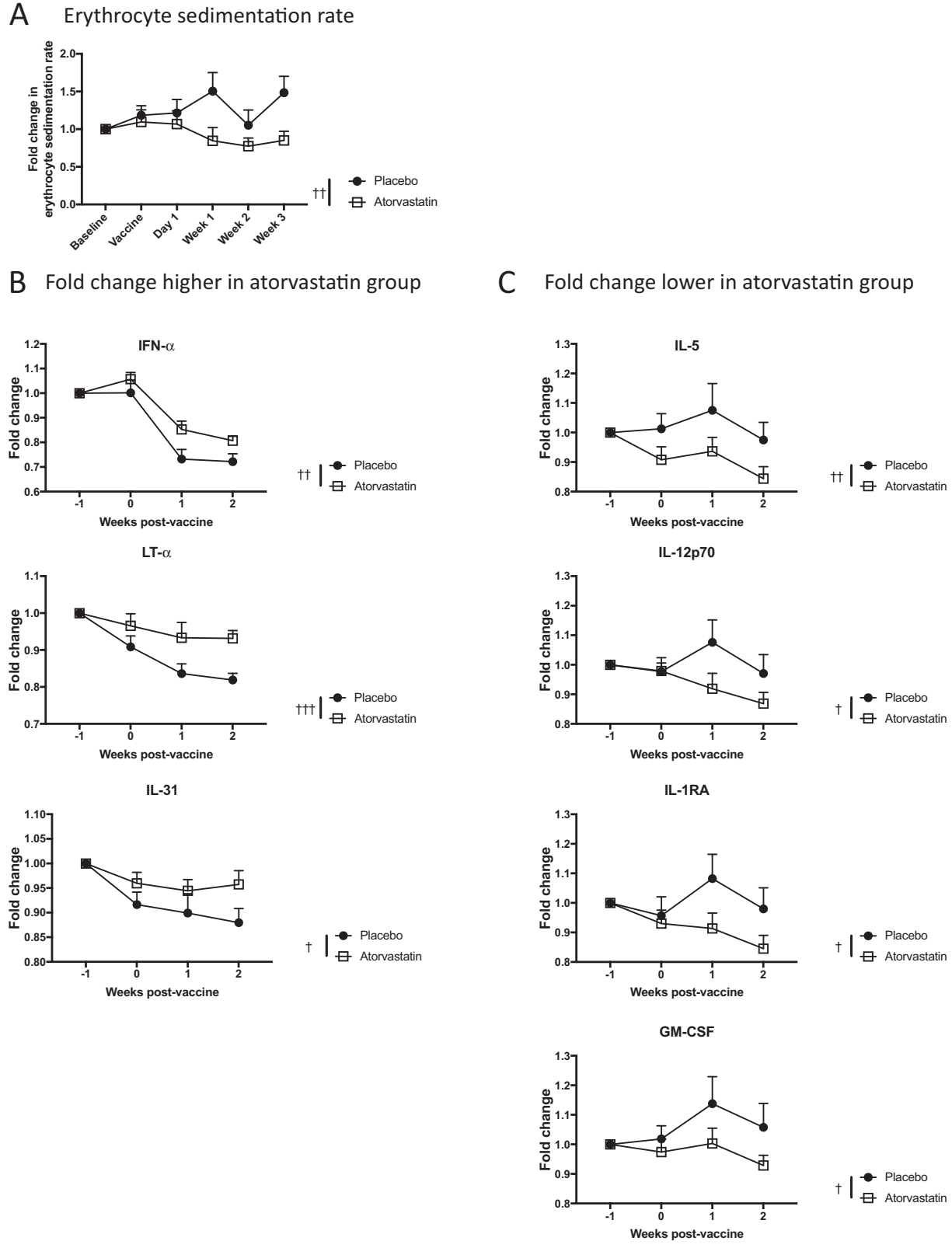

Fig. 8. Inflammatory Response. ESR, erythrocyte sedimentation rate. A, Comparison of ESR between treatment groups; †, $p < .05$. B, Comparison of the fold change in absolute lymphocyte count. Calculation is the ratio of the absolute lymphocyte count at each timepoint to the baseline value; NS, not significant. C, Paired analysis of absolute lymphocyte count and fold change in lymphocyte count. Comparisons include pre-treatment to 7 days post-treatment; pre-vax (same as 7 days post-treatment) to 24 h post-vax; and 1 day post-vax (same as 24 h post-vax) to 3 weeks post-vax; *, $p < 0.05$, **, $p < 0.01$. D, Comparison between groups of the time point during post-vaccination response at which each participant reached their peak lymphocyte count; *, $p < 0.05$.

phocyte expansion/activation, and the role of basophils in statin-mediated enhancement of vaccination response. Future studies should investigate a complete panel of cytokines and immune cells in model systems where it is possible to study multiple immune locations including lymph nodes. Through those studies we can gain an understanding of the causative mechanisms behind statin-mediated enhancement of humoral immunity.

This study has many strengths that include identifying significance in the primary endpoint of the atorvastatin group, even within a small cohort design. Also, the use of healthy volunteers in this study minimizes confounding variables and isolates statin-mediated functions. This study provides multiple fascinating secondary findings that warrant future preclinical and clinical investigations. In addition to the previously described future directions, the most obvious next clinical steps are to test this statin-mediated effect in elderly patients scheduled to receive pneumonia vaccination and in other vaccination strategies, including T cell-independent vaccinations for meningococcus and *Salmonella* Typhi.

5. Limitations

The small cohort size limits the external validity of the study. Additionally, the study could be enhanced by a more diverse participant pool that more accurately represents heterogenous patient populations. The age of the participants is 18–30; therefore, future studies will need to enroll target populations that encompass the elderly and participants with comorbidities and indications for statins. Future study populations should also include immunocompromised patients, a population in which we do not understand the role of statins on vaccination response. Given the fact that statins are indicated for patients that may sometimes have elevated BMI, additional studies should address the impact of statins on subjects with high BMI. A current study is underway at our institution investigating the effect of obesity on pneumovax 23 vaccine efficacy (ROVE, NCT02471014). Additionally, opsonophagocytic activity should be measured in future studies to fully appreciate the functional activity of the enhanced antibody response. While a previous study identified the role of statins in protein conjugated vaccines [32], this study did not investigate the role of stains on Prevnar 13, the conjugated Pneumococcus vaccine. Future investigation of the impact of statins on this vaccine may be warranted given its recent indication for adults in addition to Pneumovax 23.

6. Conclusions

In healthy volunteers, atorvastatin significantly enhances anti-pneumococcal antibody titer response to the T cell-independent Pneumovax 23 vaccine. Peri-vaccination conventional doses of statins can become a novel adjuvant for poorly-immunogenic polysaccharide-based vaccines. Future studies are needed to understand the complete mechanism of statin-mediated immunomodulation in the clinical setting.

Author contributions

Study concept and design: Wildes, Grippin, Fasanya, Dyson, Brantly.

Acquisition, analysis and interpretation of data: Wildes, Grippin, Fasanya, Dyson, Brantly.

Drafting of the manuscript: Wildes, Fasanya, Grippin.

Critical revision of the manuscript for important intellectual content: Wildes, Fasanya, Grippin.

Statistical analysis: Wildes, Grippin, Fasanya, Dyson, Brantly.

Administrative, technical, or material support: Wildes, Grippin, Fasanya, Dyson, Brantly.

Study supervision: Brantly.

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Conflict of interest

TW, AG, HF, KD, and MB declare no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.01.023.

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