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Influenza vaccination responses in human systemic lupus erythematosus: impact of clinical and demographic features

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Abstract

Objective—Vaccination against common pathogens, such as influenza, is recommended for SLE patients to decrease infections and improve health. However, most vaccination response reports are limited to evaluation of SLE patients with quiescent disease. This study focuses on understanding the clinical, serological, therapeutic, and demographic factors which influence the response to influenza vaccination in patients with a range of disease activities.

Methods—Blood specimens and disease activity information were collected from seventy-two SLE patients at baseline and 2, 6 and 12 weeks after influenza vaccination. Influenza-specific antibody responses were assessed for antibody concentration (B_{max}), relative affinity (K_a), and hemagglutination inhibition (HAI). Using a cumulative score, the subjects were evenly divided into high and low responders. Autoantibody levels were evaluated at each time-point by immunofluorescence and standard ELISAs.

Results—Low responders to the vaccine were more likely to have hematologic criteria ($p=0.009$), exhibit more ACR criteria ($p=0.05$), and be on concurrent prednisone treatment ($p=0.04$). Interestingly, European American patients were more likely to be low responders than African Americans ($p = 0.03$). Following vaccination, low responders were more likely to experience disease flares ($p=0.01$) and to have increased ANA titers ($p = 0.04$). Baseline serum interferon alpha activity was significantly higher in patients that experienced a flare after vaccination compared to a matched group of patients that did not flare ($p= 0.04$).

Conclusions—Ancestral background, prednisone treatment, hematological criteria and evidence of increased disease flares were associated with low antibody responses to influenza vaccination in SLE patients.

Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease characterized by the presence of autoantibodies and multiple organ involvement. Infectious

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diseases are one of the leading causes of morbidity and mortality in SLE patients, accounting for 11–23% of all hospitalizations and 20–55% of all deaths (1, 2). This increased susceptibility to infection is likely due to immunosuppressive therapy and intrinsic immune defects. Indeed, corticosteroid use equivalent to ≥ 20 mg/daily of prednisone has been shown to increase susceptibility to infection (1). Additionally, SLE patients display immune abnormalities, such as decreased antigen presentation and disrupted T and B cell interactions, which could decrease immune responses to pathogens (3–5). This increased risk of infection in SLE patients has led to an emphasis on vaccination in this at-risk population.

Influenza infection is a major cause of morbidity and mortality in the United States with over 225,000 hospitalizations (6) and 36,000 deaths (7) annually. Immunocompromised individuals, such as SLE patients, are at high risk for all of the reasons discussed above. Therefore, vaccination of SLE patients with the influenza vaccine has become part of the standard of care. However, several reports have shown that SLE patients make lower responses to vaccinations than healthy controls (8–10). Four studies performed in the 1970s assessed the anti-influenza response in SLE patients vaccinated against the circulating H1N1. Two of the reports documented low seroconversion rates, determined by serum antibody titer and hemagglutination inhibition (HAI), in SLE patients (47–48%) as compared to healthy controls (62–94%) (11, 12). However, other studies reported no significant differences between the serum antibody or HAI titer of patients and controls (13, 14). This issue remains controversial in more recent studies as several groups have shown significantly lower HAI titers in SLE patients compared to controls (15, 16), while others have shown that patients have equivalent HAI titers compared to controls (17, 18).

Previous findings are also contradictory regarding the impact of vaccination upon autoantibody production and clinical disease (9, 10, 15, 16, 18–20). Several groups have shown that vaccination is associated with increased autoantibody levels in SLE patients (8, 13, 19) and healthy individuals (20). Application of these results to patients in general clinical practice has been limited due to the small number of unique individuals studied, the limited ethnic groups evaluated, and the selection of lupus patients with low disease activity or quiescent disease. Thus it remains unclear whether individuals with more active disease would be capable of mounting an effective immune response to influenza following vaccination.

Our objective was to evaluate the association between demographic, therapeutic, disease activity, and clinical features with influenza vaccine responsiveness in SLE patients from various ethnicities and a range of disease activities. A secondary objective was to monitor autoantibody production and disease activity following vaccination to determine if vaccination resulted in increased humoral autoimmunity or disease flares. We hypothesized that select disease activity criteria would correlate with reduced responsiveness to the vaccine and that in some patients vaccination would result in increased autoantibody production.

Methods

Study population

Seventy-two unique patients who met four or more ACR SLE classification criteria (21) were recruited from local rheumatology clinics and provided informed consent and demographic information (gender, age, and race). Seventy-two matched healthy controls were also recruited via patient friend referrals and local advertising, enrolled and followed. Exclusion criteria included severe anemia (hemoglobin < 8.5), egg allergies, and pregnancy. Clinical information was extracted using the Lupus Family Registry and Repository (22)

collection tool for ACR classification criteria, age at diagnosis, and medication usage. Peripheral blood was collected before vaccination, and at 2, 6, and 12 weeks post vaccination. Institutional Review Board approval was obtained from the Oklahoma Medical Research Foundation (OMRF) and Oklahoma University Health Sciences Center (OUHSC).

Disease Activity Evaluation

Disease activity was measured by SLE Disease Activity Index (SLEDAI) and Physician's Global Assessment (PGA) (23) before vaccination and at 6 and 12 weeks post vaccination. Data on medications and hospitalizations were also gathered. Using these data, the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-SLEDAI flare composite score was computed (24, 25) and used to classify flares as mild/moderate or severe.

Trivalent Influenza Vaccine

All patients received the subunit influenza vaccine approved for use in the United States. The 2005–2006 vaccine consisted of an A/New Caledonia/20/99 (H1N1)-like, an A/California/7/2004 (H3N2)-like, and a B/Shanghai/361/2002-like virus (Aventis Pasteur, Swiftwater, PA). The 2006–2007 vaccine consisted of an A/New Caledonia/20/99 (H1N1)-like, an A/Wisconsin/67/2005 (H3N2)-like, and a B/Malaysia/2506/2004-like virus (Chiron Vaccines Limited, Liverpool, UK). The 2007–2008 vaccine consisted of an A/Solomon Islands/3/2006 (H1N1)-like, and A/Wisconsin/67/2005 (H3N2)-like, and a B/Malaysia/2506/2004-like virus (Novartis, Emeryville, CA).

Characterization of humoral influenza responses

To quantify antibodies to native glycoproteins a sandwich ELISA was used (26) and data were subjected to a nonlinear regression model to calculate B_{\max} , the relative measure of total antibody concentration, and $K_{a(\text{app})}$, the apparent overall association constant of the serum antibodies (26). Hemagglutinin assays were performed using human red blood cells (28). Each of the three vaccine response measurements were ranked (including all patients and controls for a given year) and then scaled to fall between 0 and 100 for each study year. The sum of these three ranked and re-scaled measurements will fall between 0 and 300 with an average of approximately 150 (what we call sum of ranks). Therefore, high and low responders were classified by identifying a noticeable gap in the sum of ranks for each study year, resulting in equal groups of 36 high and 36 low patient responders.

Detection of ANA and dsDNA

Anti-nuclear antibodies (ANA) were detected using the NOVALite Hep-2 assay (INOVA Diagnostics, San Diego, CA, positive $\geq 1:120$) (29, 30). The NOVALite *Crithidia luciliae* assay was used to measure anti-dsDNA (positive $\geq 1:30$) (29, 31).

Autoantibody Detection

Ro, La, Sm, and nRNP (Immunovision, Springdale, AR) antibodies were tested by standard ELISA (29, 31). A sample was considered positive if the optical density (OD) was greater than the average OD plus two times the standard deviation (SD) determined for 36 healthy individuals. Antibodies against the ribosomal P antigen, the 22-amino acid carboxyl terminal peptide (R5HUP0) were detected (32) using a standard ELISA. To quantify IgG antibodies to cardiolipin antigen, Linbro/Titertek E.I.A Microtitration (ICN BioMedicals, Irvine, CA) plates were used in a modified ELISA (33, 34).

Serum IFN α activity

Cells (Wistar Institute, Susan Hayflick (WISH) cells, ATCC, Manassas, VA) were cultured with 50% patient sera and lysed (35, 36). mRNA was purified and cDNA was made from total cellular mRNA and quantified using real-time PCR (37). Forward and reverse primers for three genes known to be highly and specifically induced by IFN α (MX1, PKR and IFIT) were used and gene expression levels were normalized for GAPDH. Experimental samples were compared to the mean and standard deviation of healthy controls (n=141), and IFN α activity values reported represent the number of standard deviations above the mean of healthy donors.

Statistical analysis

Exact (permutation) Chi-square tests determined the association between vaccine response and race, age, and type of ACR criteria. Comparisons of number of ACR criteria, baseline disease activity scores and pre-vaccination to post-vaccination changes in antibody responses between SLE patients and controls were evaluated using Wilcoxon two-sample tests. Differences in the number of responders taking each medication were evaluated using a Fisher's exact test. Differences between responders in regards to changes in autoantibodies were assessed using conditional logistic regression. A one-tailed paired Student's t test was used to evaluate the IFN α serum activity.

Results

SLE patients have reduced humoral immune responses following influenza vaccination

African American patients comprised 44% of this cohort and over 90% of participants were female (Table 1). As shown in Figure 1, we looked at \log_{10} transformed ratios of post-vaccination to pre-vaccination measurements. Using the INA approach we classified patients as either high or low responders based upon their overall anti-influenza response. Although controls showed a greater increase in the total amount of native antibody after vaccination compared to all SLE patients ($p = 0.035$), neither the high responding patients (B_{max} , Figure 1A, $p = 0.254$) nor the low responding patients (B_{max} , Figure 1A, $p = 0.081$) alone showed a significantly smaller increase in total native antibody after vaccination compared to controls. Both the high ($p = 0.008$) and low ($p < 0.001$) responding patients had a significantly smaller increase in apparent affinity after vaccination compared to controls (K_a , Figure 1B). While a non-significant difference in hemagglutination inhibition was noted between SLE patients and controls ($p=0.17$), it should be noted that few of these individuals (patients or controls) had substantial increases in HAI titers after vaccination. Furthermore, low responding patients showed a significantly smaller increase in HAI after vaccination than either high responding patients (HAI, Figure 1C, $p < 0.001$) or healthy controls (HAI, Figure 1C, $p < 0.001$). Using the INA approach to look at differences of pre- and post-vaccination, controls showed significantly improved humoral influenza responses after vaccination than patients ($p < 0.001$), and this effect is driven by the poor overall antibody responses of low responding patients (Figure 1D).

Based on the INA approach used to classify patients as either high or low responders, we can see that the high responder group of patients generated an anti-influenza response which was much closer to the behavior of the controls than that of the low responding patients. For each measure of responsiveness (B_{max} , K_a , HAI and overall response), the low responder group had impaired responses compared to both the high responder and control groups, particularly when evaluating HAI differences and overall differences. In the following experiments we use this classification of high and low responders to further examine correlates of poor SLE vaccine response.

African-American SLE patients are more likely to have strong influenza vaccination responses

African-American patients were more likely to have strong influenza vaccination responses than European American patients ($p=0.03$, Table 1). Specifically, African-American patients were 3 times more likely to be high responders than European Americans (95% CI: 1.07, 9.94). This racial association with vaccination response was not due to age since the ages between the two groups did not significantly differ (42 ± 12.4 vs. 46 ± 15.8 , respectively). This discrepancy may be due to the impact of HLA haplotypes on vaccine responsiveness, although this requires further investigation.

Patients ranged in age from 20 to 89 years, with 42% between 29 and 45. The relationship between age and the response to vaccination was examined since older individuals may demonstrate decreased influenza antibody levels (38). Although we had only a small number of elderly individuals, we found no relationship between age and the vaccine response. Due to the predilection of SLE for females, the small number of male patients did not allow evaluation of gender differences.

Poor influenza vaccination responses are associated with hematologic manifestations and more SLE ACR classification criteria

We next analyzed age at diagnosis, number of cumulative ACR criteria, and select ACR classification criteria to determine whether these factors correlated with the subsequent response (Table 1). Low responders had more ACR criteria (median=6) than high responders (median=5, $p=0.05$). Indeed, 23 of the 36 (64%) low responders met six or more of the ACR SLE criteria compared to 15 of 36 (42%) high responders.

We then analyzed the types of ACR criteria found in both groups. Twenty-five out of 36 low responders (69.4%, Figure 2A) exhibited at least one hematological criterion, compared to 12 out of 36 high responders (33.3%, $p=0.009$). All of the individuals with hemolytic anemia were low responders (Figure 2b, $p=0.01$). The low responders also had a higher prevalence of thrombocytopenia (22.2% vs. 13.9%), lymphopenia (38.9% vs. 27.8%), and leucopenia (36.1% vs. 22.2%), although these were not statistically significant (Figure 2B). While not statistically significant, nine of the thirty-six (25%) high responders exhibited discoid rash compared to three of thirty-six (12.5%) low responders ($p=0.1$). No differences were seen in the prevalence of renal criteria or arthritis.

Poor influenza humoral immune responses are more common in lupus patients using steroids

While some report that medication has no effect on anti-influenza antibodies (11), others have shown decreases in antigen-specific responses in patients taking azathioprine or corticosteroids equivalent to ≥ 10 mg prednisone daily (19, 39). Twenty-four of 36 (67%) patients who were low responders took prednisone at a level equivalent to ≥ 10 mg/day compared to 17 of 36 patients (47%) who were high responders ($p=0.04$, Figure 2C). Although the sample sizes are small, higher doses of corticosteroids (≥ 20 mg daily) did not appear to further lessen the magnitude of the influenza immune response. No other immunomodulatory medication was associated with a low response.

Of course, the number of individuals taking these medications is relatively small. Therefore, we divided patients into those on “low immunosuppressive therapies” (no medications, hydroxychloroquine only, or hydroxychloroquine in combination with the equivalent of 7.5 mg of prednisone daily) versus those on “high immunosuppressive therapies” (methotrexate, mycophenolate mofetil, azathioprine, cyclophosphamide, greater than 7.5mg of prednisone daily, or a combination of these). Of the thirty-nine individuals on “high immunosuppressive

therapies”, twenty-one were high responders (54%). Of the thirty-three individuals on “low immunosuppressive therapies”, eighteen were high responders (55%). Thus, while steroid use is associated with a lower antibody response to influenza vaccination, other immunosuppressive medications do not account for the decreased responses in some patients.

No differences were detected in baseline autoantibody specificities of patients with poor vaccination responses

To help determine what predicts a low response to vaccination in SLE patients, autoantibody levels were measured at the time of enrollment. Nearly all patients were ANA positive (Figure 2D) and high and low responders did not significantly differ in regards to ANA titer (median 1:1080 and 1:953, respectively; data not shown). No significant differences in the dsDNA antibody titer were observed between the responders (median 1:90 for each; data not shown).

While a trend toward higher cardiolipin and lower nRNP antibody frequencies was seen in the low responders, these differences were not significant, which may relate to the low number of individuals positive for these antibodies (Figure 2D). No differences were seen between the high and low responders in the proportion of individuals positive for any of the other autoantibody specificities at baseline. There was also no difference in the average number of autoantibodies in high and low responders (average 2.6 and 2.4, respectively).

Low responders have increases in autoantibody levels and new specificities after vaccination

We next examined the impact of vaccination upon autoantibody specificities and levels. Figure 3 shows the number of individuals who had new onset, a two-fold or greater increase, or a two-fold or greater decrease in the specified antibody. These data show patterns of changes in certain autoantibodies, including ANA, anti-La, and anti-cardiolipin among the high responders, and ANA, anti-Ro, and anti-RiboP among the low responders. Nineteen (26%) of the patients had a change in their ANA titer at two weeks post vaccination: two with new onset, eight with increased titer, and nine with decreased titer. Low responders were more likely to have increased ANA titer than high responders (14% versus 8%, respectively), with 5 of the thirty-six low responders having an increase in ANA titer post vaccination. Eight of the high responders (22%) had decreased ANA titers compared to only 1 (3%) of the low responders. Indeed, among high responders, post-vaccination decreases in ANA were twice as common as increased or new ANA expression. Among low responders, decreases in ANA titers were one-sixth as common as increased or new ANA expression ($p=0.05$, Figure 3).

The small number of patients exhibiting changes in antibodies to La, Ro, cardiolipin, and RiboP do not allow a definitive statistical evaluation of differences between high and low responders. However, both groups had new onset of autoantibodies directed against La and cardiolipin (Figure 3). Three low responders and one high responder had new onset/increased antibodies to the La antigen. Both the high responder group and the low responder group had two individuals with new onset/increased antibodies to cardiolipin.

Low responders to influenza vaccination are more likely to have a disease flare

We next examined disease activity following vaccination. Five individuals (6.9%), four of which were low responders, had an increase in SLEDAI of three or more points at 6 weeks post vaccination. Eight individuals (11%), four high and four low responders, had a three point SLEDAI change at 12 weeks post vaccination. Overall, no increased frequency nor severity of flares were seen in the time period immediately after vaccination (6 weeks)

compared to a time period further from vaccination (12 weeks). However, this method does not capture some indications of disease flare such as medication changes or hospitalizations.

To further refine our method of determining flares, the SELENA-SLEDAI flare index was used (24, 25). Using this index, fourteen patients (19.4%) flared at six weeks post vaccination including 10 (13.8%) with mild/moderate and 4 (5.6%) with severe flares (Figure 4a). At twelve weeks there were nineteen (26.4%) flares including 16 (22.2%) mild/moderate and 3 (4.2%) severe flares (Figure 4a). Of the patients flaring at 6 weeks, 10 (71.4%) were low responders and 4 (28.6%) were high responders (Figure 4b, $p = 0.01$). By twelve weeks after vaccination, no significant difference was observed in the flare rate between high and low responders, 53% and 47% respectively (Figure 4c). Using data collected from previous studies where patients did not receive influenza vaccination, we observed that 1 of 8 patients (12.5% vs 19.4% in our study) had a mild/moderate flare when observed over a six to eight week time frame. Of 41 patients observed over a nine to eighteen week timeframe without vaccination, 8 (19.5% vs 26.4% in our study) had mild/moderate flares. The differences between flare rates over similar time periods in vaccinated versus non-vaccinated SLE patients were not statistically significant.

Serum Interferon alpha activity associates with disease flare following vaccination

We next asked if there were characteristics that would predict which patients would flare following vaccination. To this end we compared groups of patients that flared at six weeks post vaccination ($n = 14$) to a group of matched patients that did not experience a flare. We found that age at diagnosis, number of SLE ACR criteria, and baseline disease activity did not correlate with a flare. Select SLE ACR criteria were more prevalent in the individuals experiencing a flare and these included renal disease (43% vs. 29%), central nervous system involvement (21% vs. 0), and hematological disorder (50% vs. 29%). In contrast, fewer individuals with serositis (29% vs. 50%) or oral ulcers (57% vs. 71%) were in the flare group. Unfortunately, the small numbers of patients in these groups do not allow a definitive statistical evaluation of the impact of these criteria upon flare.

Since increased IFN α induced gene expression correlates with higher disease activity in SLE patients (37), we examined serum IFN α activity. We found that patients experiencing a flare six weeks post vaccination had higher baseline serum IFN α activity than patients that did not have a flare (mean activity 19.3 vs. 2.7, $p = 0.04$, Figure 5a). While patients that experienced a flare had a decrease in IFN α activity at two weeks post vaccination (mean activity of 10.95), this activity was still higher than seen in individuals that did not experience a flare (Figure 5a). This difference in serum IFN α activity was not observed in the individuals that had a flare at twelve weeks post vaccination (mean activity 2.9 vs. 5.3, $p = 0.2$, Figure 5b). Of note, we found no significant correlation between IFN α activity level and the overall magnitude of the influenza-specific response.

Discussion

The goal of this study was to identify factors which correlate with the immune response to influenza vaccination in SLE patients and examine the impact of vaccination on autoimmune disease activity. We found no differences in the immune response based on age or gender; however, high responders were enriched for African American patients. Previous reports about influenza vaccination in SLE patients were performed in other geographic regions, using Israeli (8, 17, 19, 40, 41), Italian (18), Mexican (16), or Dutch subjects (15, 39, 42, 43) or were not able to evaluate race (11–14). Surprisingly, little is known about racial differences in immune responses to vaccinations. Healthy young African Americans had higher levels of neutralizing antibodies against HIV gp-120 after vaccination compared to European Americans (44), yet older African Americans had impaired T cell responses to

influenza vaccination (45). Additional work is necessary to fully understand the influence of race on vaccination responses.

SLE patients with a history of hemolytic anemia were more likely to be low responders. Our initial assumption was that this association would be driven by patients with significant lymphopenia and/or leucopenia. However, no significant differences were found in the percentage of lymphocytes at baseline between the low and high responders (28.1% vs. 34.8%, respectively). In addition, severe lymphopenia (those individuals with $\leq 500/\mu\text{l}$) was found in 14% of low responders compared to 11% of high responders, indicating that lymphopenia was not the main factor in this association. Rather, hemolytic anemia was the dominant hematologic manifestation driving the association with low vaccination response. No other reports could be found examining the response to vaccination in patients with hemolytic anemia or thrombotic thrombocytopenic purpura. Examinations of vaccination responses of larger collections of patients with hemolytic anemia are warranted to uncover mechanisms for this epidemiologic association.

Reports regarding changes in autoantibody concentration and/or specificities in SLE patients after vaccination have been conflicting (17–20). These studies have been limited by small sample sizes ($n = 14$ to 18) (16, 18), differences in methodologies, and limited number of autoantibodies selected for analysis (16). Abu-Shakra *et al.* found that influenza vaccination had no effect on anti-dsDNA, a transient effect on Sm, nRNP, Ro, and La, and a prolonged effect on anti-cardiolipin antibodies (19). These results are supported in part by our findings.

We observed two striking findings in regards to autoantibodies following vaccination. First, patients who mounted a strong response were significantly more likely to decrease their ANA titer. This suggests that some SLE patients have an immune system poised to generate antigen-specific immune responses while minimizing the stimulation, expansion, and maturation of autoreactive B cells. Second, over half of the low responders developed new autoantibodies or increased the concentration of existing autoantibodies, suggesting that these patients are more likely to have immune responses focused, at least in part, against self antigens. Further investigation into the longevity of these responses and associated long-term clinical consequences are underway. Additional evaluation of the influence of genetic, environmental and other disease-specific factors on determining whether SLE patients will focus their immune responses on antigen-specific or autoantigen-specific pathways is warranted.

Several groups have reported no differences in disease activity measures between influenza vaccinated and unvaccinated SLE patients (41, 46). However, the majority of these studies were small ($n = 23$ –24) (41, 46). Holvast *et al.* (15) found that influenza vaccination did not result in significant changes to disease activity scores in 56 SLE patients. We showed no increased flare rates within six weeks post-vaccination compared to the six to twelve week post-vaccination time interval. However, patients who flared within six weeks post vaccination were predominantly low responders. Being able to identify these individuals before vaccination and modify approaches for influenza vaccination in ways that do not negatively impact their underlying disease would be clinically useful.

Several differences between the published studies and our work may influence the flare rates and disease activity changes detected. First, in previous studies, a flare was defined as an increase of three or more points as measured by SLEDAI. Our study calculated flares using the SELENA-SLEDAI index which also takes into account hospitalization and medication changes (24, 25). Second, the studies cited above enrolled patients with quiescent disease (SLEDAI ≤ 4) whereas our patient population was composed of individuals with both quiescent and active disease. Indeed, 41 of our 72 patients (57%) had a SLEDAI of 6 or

more at baseline. Lastly, as mentioned earlier, our study was composed of roughly equal numbers of European Americans and African American SLE patients whereas the majority of the previously reported studies were composed primarily of patients of European descent.

When we examined patients that flared following vaccination, we found that select ACR criteria were either over or under-represented. Renal disease, central nervous system involvement, and hematological disorder were more prevalent at baseline in the patients that flared. Additionally, patients that flared at six weeks post vaccination had higher baseline serum IFN α activity. Others have shown that IFN α activity correlates with SLE disease criteria and that an elevated IFN α score is associated with severe manifestations of SLE (47, 48). The relationships between interferon activity, vaccination responses and subsequent disease flares need to be confirmed in a larger cohort and the biological significance examined. This information is especially important to investigate since several groups have advocated using IFN α as an adjuvant for influenza vaccination (48, 49).

Our data indicate that some SLE patients, especially those with a history of hematologic disorder or individuals taking prednisone, mount weak responses to the influenza vaccine. Furthermore, low responders are more likely than high responders to increase autoantibody production and experience disease flares following vaccination. Current studies are underway to explore biomarkers which might better predict which patients are likely to flare following vaccination. Coupled with select serologic biomarkers in patients who are anticipated to have low responses, this information would help identify the subset of patients who may need to have their underlying disease more aggressively treated before receiving influenza or other vaccination.

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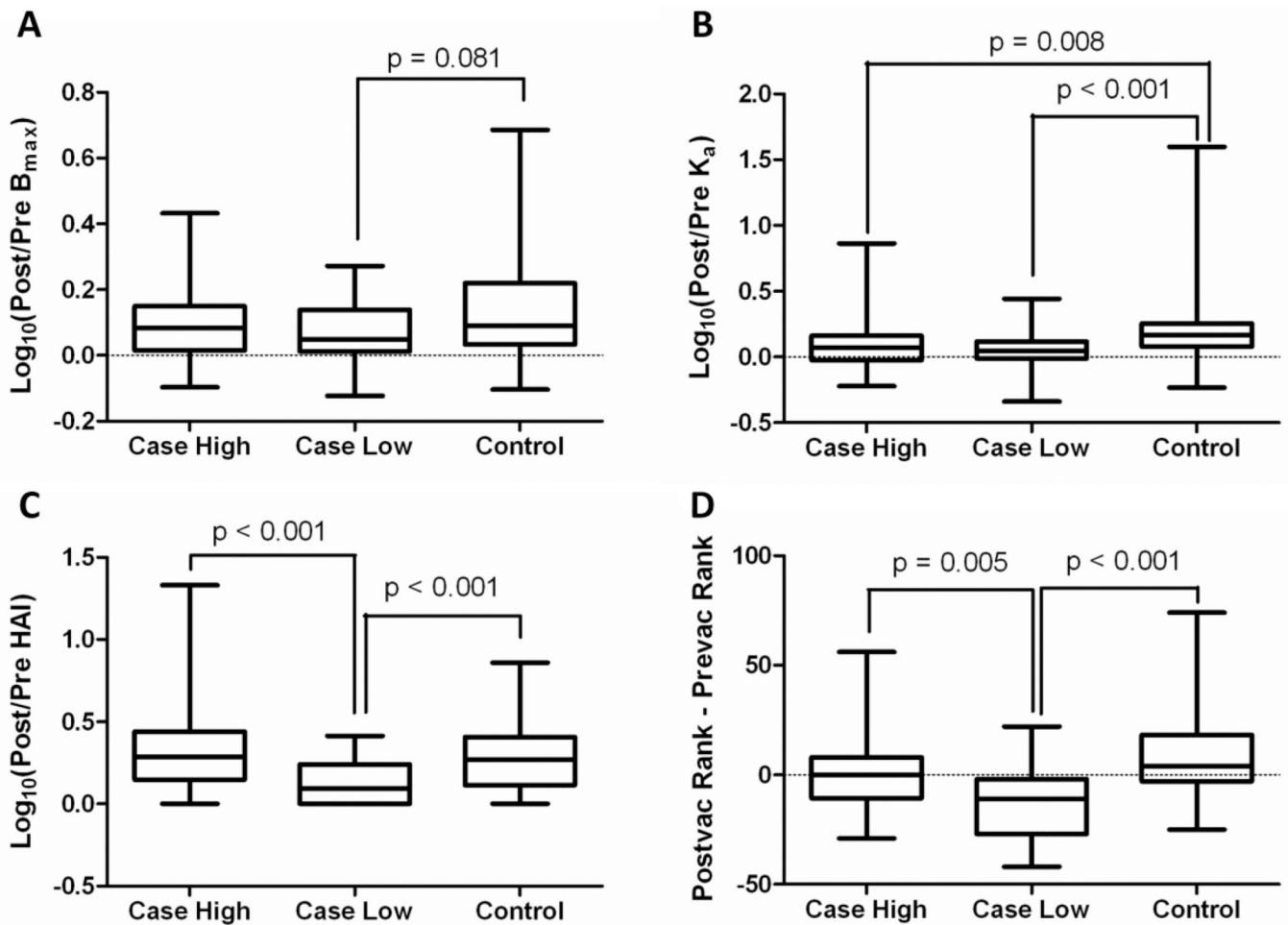


Figure 1. SLE patients with poor antibody responses have reduced humoral immunity compared to healthy controls following influenza vaccination

Plasma from SLE patients (case) and normal healthy controls (control) were tested for humoral immune responses to the influenza vaccine. Cases were divided into high and low responders based upon cumulative rank as outlined in the methods section of the manuscript. Shown are the log₁₀ transformed ratios of post-vaccination to pre-vaccination measurements for (A) antibody concentration (B_{max}), (B) antibody affinity (K_a), and (C) hemagglutination inhibition (HAI). Panel D shows the overall vaccine response, expressed as the difference of the percentile ranks (postvacc minus prevacc). Shown are box and whisker plots where the middle line is the median, the box represents the middle 50% of the data, and the whiskers extend to the minimum and maximum values. P-values are derived from nonparametric Mann-Whitney tests using a Bonferroni multiple comparison correction.

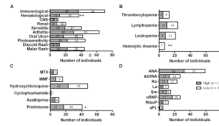


Figure 2. Patients with low influenza vaccination responses have more hematologic SLE classification criteria and more steroid use
 Subjects were divided into high (gray bar) and low (white bar) responders to influenza vaccination. Shown are the number of individuals that (A) met the indicated ACR SLE classification criteria, $**p = 0.009$ by exact permutation Chi-square test, (B) exhibited specific aspects of hematological disorders, $*p = 0.01$ by exact permutation Chi-square test, (C) taking specific medications, $*p = 0.037$ by Fisher's exact test and (D) exhibiting specific autoantibodies prior to vaccination.

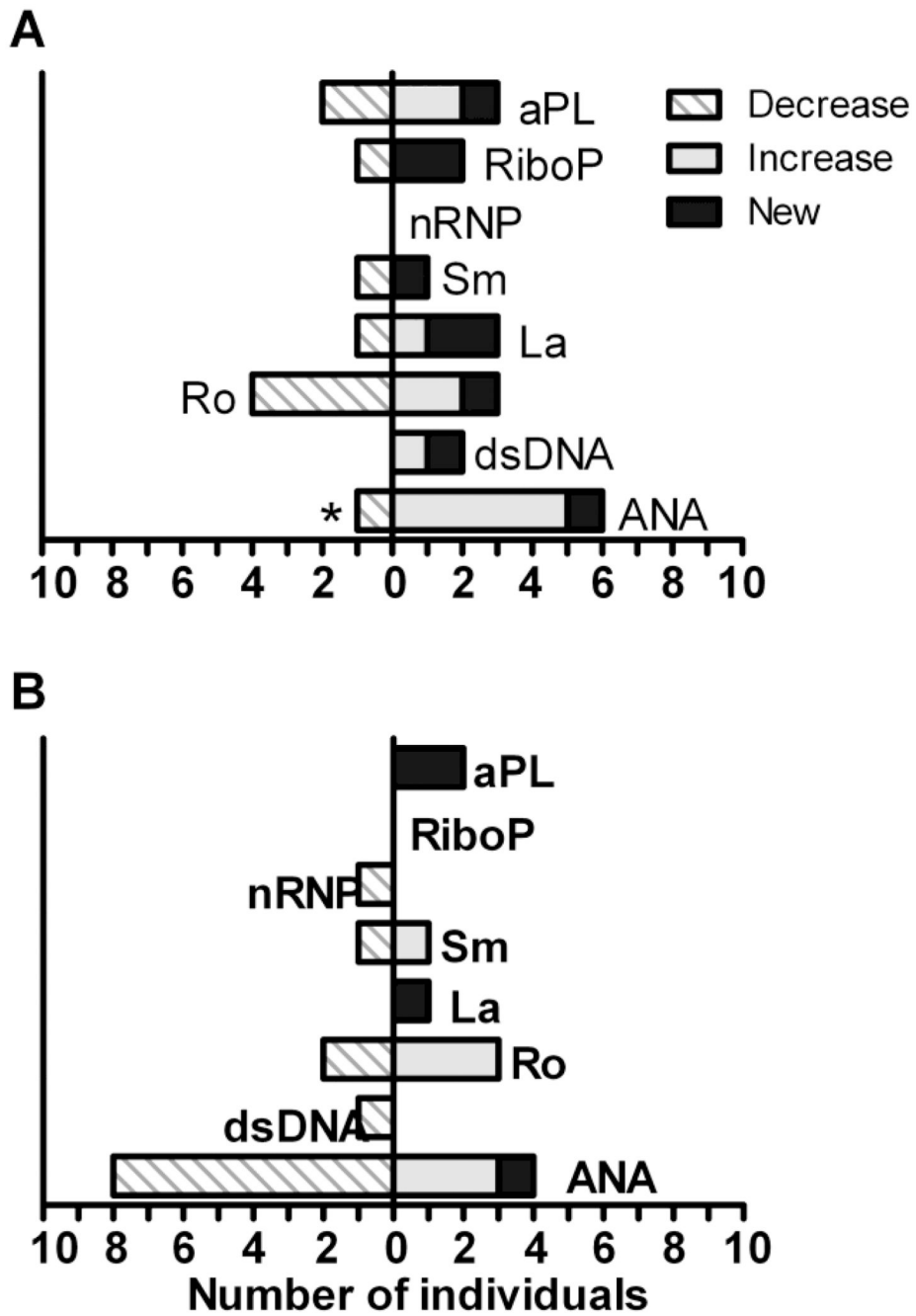


Figure 3. Patients with low responses to influenza vaccination have increases in autoantibody levels and new specificities after vaccination
 Autoantibody titers were measured in sera from the initial (pre-vaccination) and post-vaccination time-points. Shown is the number of (A) low responders or (B) high responders with a two-fold increase (solid gray bar), a two-fold decrease (hatched bar), or new onset (solid black bar) of the specified autoantibody, *p = 0.045 by conditional logistic regression.

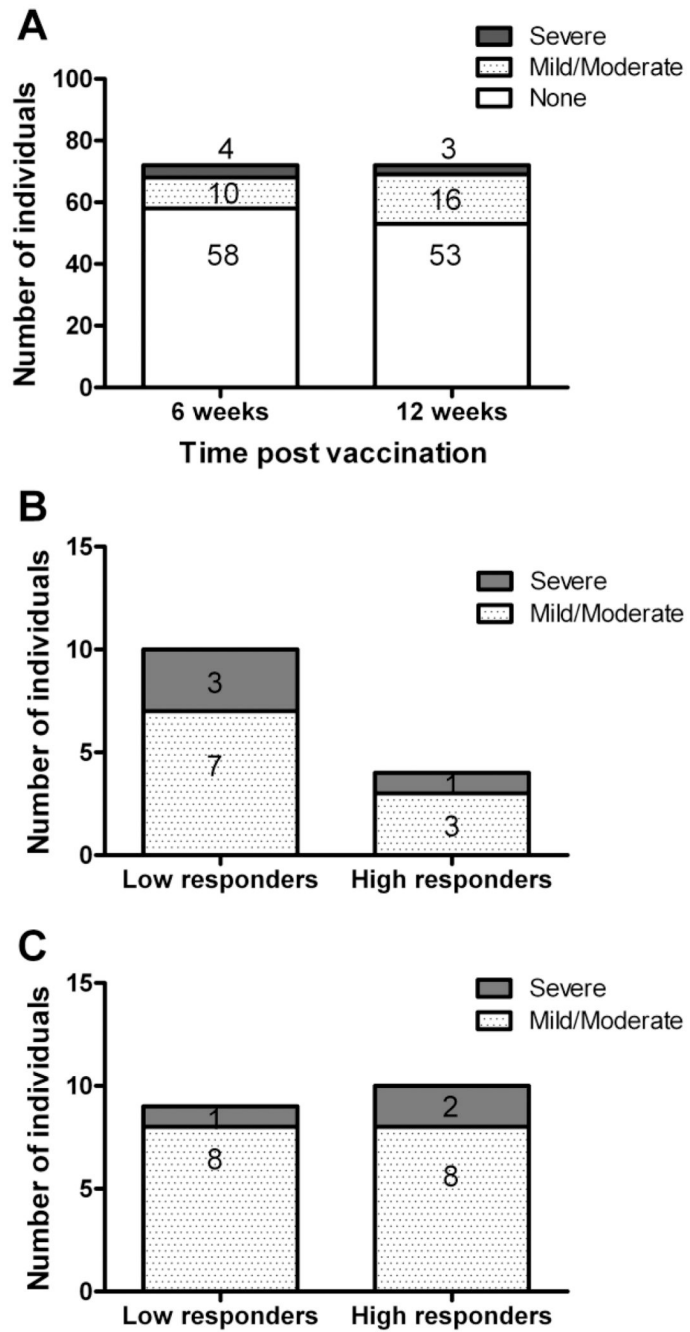


Figure 4. SLE disease flares are more frequent in low responders at 6 weeks, but not 12 weeks, after influenza vaccination

High and low antibody responders to influenza vaccination were evaluated for the presence of flares using the SELENA-SLEDAI method (24, 25) for all individuals at six and twelve weeks post vaccination. Shown is the total number of individuals experiencing no flare (white bars), mild/moderate flare (dotted bars), or severe flares (gray bar) (A), the vaccine responsiveness (high or low) of individuals with a flare at 6 weeks post vaccination (B), and the vaccine responsiveness (high or low) of individuals with a flare at 12 weeks post vaccination (C).

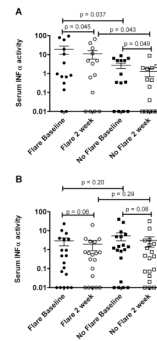


Figure 5. Patients that flare at six weeks post vaccination had higher baseline IFN alpha serum activity

Patients were divided into individuals that had a flare (flare) and age and race matched SLE patients that did not have a flare during the course of the study (no flare). Shown is the serum IFN alpha activity at baseline and two weeks in the patients that had a flare at 6 weeks (panel A, p value determined by unpaired t test) and those with a flare at 12 weeks (panel B, no significance by unpaired t test). Each symbol represents one individual and the mean and standard error of the mean are shown.

Table 1

Demographics of this influenza vaccination SLE patient cohort.

	All Individuals (n = 72)	Low Responders (n = 36)	High Responders (n= 36)
Average Age (SD) ^a	43 (14)	43 (13)	43 (15)
African American ^b	44%	31%	58% ^e
European American	49%	61%	36%
Hispanic and Other ^c	7%	8%	6%
Female Sex	92%	97%	86%
Median Age at Diagnosis (SD)	31 (14)	29 (15)	33 (14)
Average number of ACR criteria (SD)	5.8 (1.6)	6.2 (1.4)	5.5 (1.7)
Median Baseline SLEDAI (SD) ^d	6.0 (3.8)	6.0 (4.1)	5.0(3.4) ^f
Steroid Treatment	58%	53%	64%
Anti-Malarial Treatment	69%	69%	69%
Combination Steroid and Anti-Malarial	51%	50%	53%

^aSD = standard deviation^bRace was self defined^cOther designation includes individuals that self identified with more than one race (n = 1)^dBaseline refers to the day of vaccination^ep = 0.028, by exact Chi-square test^fp = 0.053, by Wilcoxon two-sample test