Patients with autoimmune diseases are at higher risk for severe infection due to their underlying disease and immunosuppressive treatments. In this real-world observational study of 463 autoimmune subjects, we examined risk factors for poor B and T cell responses to SARS-CoV-2 vaccination. We show a high frequency of inadequate anti-spike IgG responses to vaccination and boosting in the autoimmune population but minimal suppression of T cell responses. Low IgG responses in B cell–depleted multiple sclerosis (MS) subjects were associated with higher CD8 T cell responses. By contrast, subjects taking mycophenolate mofetil exhibited concordant suppression of B and T cell responses. Treatments with highest risk for low IgG anti-spike response included B cell depletion within the last year, fingolimod, and combination treatment with mycophenolate mofetil (MMF) and belimumab. Our data show that the mRNA-1273 (Moderna) vaccine, is the most effective vaccine in the autoimmune population. There was minimal induction of either disease flares or autoantibodies by vaccination and no significant effect of pre-existing anti-type I interferon antibodies on either vaccine response or breakthrough infections. The low frequency of breakthrough infections and lack of SARS-CoV-2–related deaths suggest that T cell immunity contributes to protection in autoimmune disease.

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Factors associated with immune responses to SARS-CoV-2 vaccination in autoimmune disease individuals

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ABSTRACT

Patients with autoimmune diseases are at higher risk for severe infection due to their underlying disease and immunosuppressive treatments. In this real-world observational study of 463 autoimmune subjects, we examined risk factors for poor B and T cell responses to SARS-CoV-2 vaccination. We show a high frequency of inadequate anti-spike IgG responses to vaccination and boosting in the autoimmune population but minimal suppression of T cell responses. Low IgG responses in B cell–depleted multiple sclerosis (MS) subjects were associated with higher CD8 T cell responses. By contrast, subjects taking mycophenolate mofetil exhibited concordant suppression of B and T cell responses. Treatments with highest risk for low IgG anti-spike response included B cell depletion within the last year, fingolimod, and combination treatment with mycophenolate mofetil (MMF) and belimumab. Our data show that the mRNA-1273 (Moderna) vaccine, is the most effective vaccine in the autoimmune population. There was minimal induction of either disease flares or autoantibodies by vaccination and no significant effect of pre-existing anti-type I interferon antibodies on either vaccine response or breakthrough infections. The low frequency of breakthrough infections and lack of SARS-CoV-2–related deaths suggest that T cell immunity contributes to protection in autoimmune disease.

INTRODUCTION

Vaccinating individuals with autoimmune disease is a healthcare imperative due to their heightened susceptibility to severe infections, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). Understanding vaccine responses in autoimmune patients is crucial, as they may face increased risk of adverse reactions and mount less efficient immune responses (2). Many vaccines contain immune adjuvants that could potentially worsen existing autoimmunity (2). Of particular concern for systemic lupus erythematosus (SLE) patients, most SARS-CoV-2 vaccines contain mRNA that could act as a toll-like receptor (TLR) 7/8 agonist (3). Additionally, medications used in autoimmune populations have been linked to reduced antibody responses to SARS-CoV-2 vaccination (4-10). Strategies like withholding immunosuppressive medications prior to vaccination result in higher rates of seroprotection (11-13). Some studies have explored the impact of immunosuppression on T cell effector responses to SARS-CoV-2 vaccines during conventional or biologic immunotherapies (6, 14-17). While T cell immunity alone does not prevent SARS-CoV-2 infection, evidence suggests it limits disease severity (18, 19). Recent studies also suggest that antibodies to Type 1 interferon and other cytokines may negatively impact responses to infections and vaccinations, although the effects are still debated (20-22).

The COVID-19 pandemic provided an opportunity to examine how autoimmune diseases and immunosuppressive medications influence responses to new lipid nanoparticle encased mRNA vaccines. In this real-world observational study, we assessed factors affecting B and T cell vaccine responses in autoimmune disease subjects and evaluated the association of vaccination with disease activity, frequency of breakthrough infections, association of infections with anti-

type 1 IFN antibodies, and induction of autoantibodies following vaccination and boosting. The results of our studies help inform best practices for performing vaccination and boosting in subjects with a broad spectrum of autoimmune diseases and who are being treated with a range of immunotherapies.

RESULTS

Subject characteristics

Five hundred and thirty-four subjects consented to the study. Seventy-one subjects were subsequently deemed ineligible because they were not vaccinated or did not have a follow-up visit within the required period after vaccination. Demographic data are shown in **Table 1** and distribution of autoimmune diseases and number of subjects analyzed in each assay is shown in **Figure 1**.

Serologic response to SARS-CoV-2 vaccine in autoimmune subjects versus healthy controls

We assessed anti-nucleocapsid (anti-NC) and anti-spike IgG antibody values and trajectories in healthy control (HC) and autoimmune cohorts (**Supplementary Figures 1A–C**). Anti-spike IgG values showed no correlation with age or gender in autoimmune subjects (**Supplementary Figure 1D**). To assess the impact of previous SARS-CoV-2 infection on vaccine responses, subjects and controls were divided into 3 groups based on their prior exposure to SARS-CoV-2: (1) those who remained anti-NC- with no symptomatic infection (anti-NC-); (2) those who, at the Pre-vaccine (Pre V) visit, were positive for anti-NC or anti-spike antibodies, or for SARS-CoV-2 by polymerase chain reaction (PCR) or antigen test, or who at the first Post-vaccine (Post V1) visit 4 to 14 weeks post completion of initial vaccine, were anti-NC+ or reported a symptomatic

infection that predated vaccination verified by positive PCR or antigen test (anti-NC+); and (3)

those acquiring anti-NC positivity or testing positive for SARS-CoV-2 at or after the second

post-vaccine (Post V2) visit 24 +/- 8 weeks after completion of the initial vaccination series

(anti-NC Acq). Twenty-four subjects with positive anti-NC antibodies at the Post V1 visit but no

history of symptomatic infection were considered to be anti-NC+. Since cutoffs for anti-spike

IgG positivity are not clearly defined, we based cutoffs on anti-spike responses at each visit in

HC (**Figures 2A, B**). We defined a low anti-spike IgG response to the initial vaccine series as <

250 U/mL for anti-NC- subjects and < 2000 U/mL for anti-NC+ subjects and a low anti-spike

IgG response to the booster vaccine as < 4000 U/mL in all subjects. These cutoffs are similar to

those recently reported in a similar study from the UK (23).

Anti-spike IgG values in autoimmune subjects at the Pre V visit are shown in **Supplementary**

Figure 1E. Anti-NC+ subjects had significantly higher anti-spike IgG values at Post V1 than

both anti-NC- and anti-NC Acq subjects (**Figure 2A, 2C, Supplementary Figure 1F, 1I,**

Supplementary Table 1). 45.1% of anti-NC- autoimmune subjects had a serologic response of <

250 U/mL at Post V1, while only 1/24 (1.5%) of HC (an 83-year-old male) had a low response.

Similarly, 25.5% of anti-NC+ autoimmune subjects had a serologic response of < 2000 U/mL at

Post V1, compared with 0/27 HC. At the post-booster (Post B1) visit, 42.1% of anti-NC- and

27.7% of anti-NC+ autoimmune subjects had a serologic response of < 4000 U/mL, compared

with none of the HC (**Figure 2B, 2C, Supplementary Figure 1H, 1I, Supplementary Table 1)**.

The percent increase in the anti-spike antibody response at the Post B1 compared with the Post

V1 visit was lower in anti-NC+ HC who already had high values of anti-spike IgG, compared

with anti-NC- HC (median fold increase 3.7 vs. 21.2, p < 0.0001; **Supplementary Figure 1J**)

but was not different in autoimmune subjects (**Supplementary Figure 1K, 1L** – median fold

increase 17.4 versus 11.0, p = NS).

We next examined the decline in anti-spike IgG values after the initial vaccine series. The

decline in anti-spike IgG between Post V1 and Post V2 in either anti-NC- or anti-NC+

autoimmune subjects was no different than in matched HC (**Figure 2D-G**). Few non-boosted

autoimmune subjects could be observed at Post V3, as most had either received booster

vaccinations or had acquired SARS-CoV-2 after Post V2. IgG anti-spike values continued to

decline in these subjects (**Supplementary Figure 1M**). In anti-NC- autoimmune subjects who

had a response < 250 U/mL to the initial vaccine series, anti-spike IgG remained stable from Post

V1 to Post V2 (**Figure 2H**).

To determine whether the anti-spike IgG response to booster vaccination was predicted by the

pre-booster anti-spike IgG level, we compared booster vaccination responses based on pre-

booster (Pre B) anti-spike IgG quartiles. Autoimmune anti-NC- subjects in the highest 2 quartiles

at the Pre B visit had significantly higher anti-spike IgG at Post B1 (equivalent to HC), unlike

those in the lower 2 quartiles (**Figure 2I**). By contrast, no difference was observed in anti-spike

antibody IgG between upper and lower 50th percentile of Pre B levels in anti-NC+ autoimmune

subjects (**Figure 2J**).

Immunosuppressant use and serologic response to vaccine
We assessed the effect of immunosuppressant drugs on the response to vaccination in 397

subjects with a Post V1 visit (**Supplementary Table 2**). Only 32% of 96 subjects on B cell–

depleting drugs were responders. Previous exposure to SARS-CoV-2 did not improve vaccine responses in this cohort. To evaluate the effect of mycophenolic acid (MPA) and MMF on the vaccine response, we excluded subjects also taking B cell–depleting drugs and those who were non-adherent to their medication based on serum drug levels. Sixty-one percent of 49 subjects taking MPA or MMF alone or together with hydroxychloroquine were responders. In this cohort, all 14 anti-NC+ subjects were responders, compared with only 46% of 35 anti-NC- subjects (p < 0.01). Only 26% of 19 subjects taking MPA or MMF together with a second immunosuppressive drug were responders (p < 0.01, compared with subjects taking MPA or MMF alone or together with hydroxychloroquine). Seventy-three percent of 49 subjects taking methotrexate and 68% of 16 subjects taking azathioprine were responders, and all non-responders were anti-NC-. Similarly, 73% of 26 subjects taking TNF inhibitors were responders and 4 of 5 non-responders were anti-NC-. Finally, only 9 of 25 (36%) subjects taking belimumab were responders; these 9 subjects were taking belimumab alone (1/9) or with MMF (1/9), methotrexate (2/9) or hydroxychloroquine (5/9). Of the belimumab NR, 11/16 were also taking other immunosuppressives including MMF (9/11), azathioprine (1/11), or methotrexate (1/11), and all were anti-NC-. Thus, the combination of MMF and belimumab conferred a 90% risk of NR.

Among 153 subjects with both a Post V1 and Post B1 visit, we assessed medication use among those who did not respond to both the initial vaccine series and the booster ("Double Non-Responders" [Double NR], n = 36), those who did not respond to the initial vaccine series but responded to the booster ("Single Non-Responders" [Single NR], n = 41), and those who responded to both vaccinations ("Responders," n = 76; **Supplementary Table 3**). A majority of Double NR received B cell depletion alone (20/36, 56%) or with another drug (6/36, 9%). The

other Double NR subjects received belimumab with a second immunosuppressive drug (4/36), MMF alone (1/36), and leflunomide together with a TNF inhibitor (1/36). In addition, 4 of 5 subjects receiving fingolimod were Double NR, while the other was a Single NR. Most subjects who were on MMF alone or with hydroxychloroquine were Responders (8/13, 63%). However, all subjects who were on MMF with an additional immunosuppressant (n = 8) were either Single (5/8) or Double NR (3/8). Ten out of 13 (77%) subjects on belimumab were either Single (6/10) or Double NR (4/10). Of 16 subjects on methotrexate, 50% were Single NR and 50% were Responders, while none were Double NR. Only 5 subjects were taking a prednisone equivalent of 30 mg daily at the time of the initial vaccine series, and only 1 subject was taking this dose of corticosteroid at the time of the booster.

Vaccine type, immunosuppressant adjustment, and vaccine response

Of 234 autoimmune subjects who received the BNT162b2 (Pfizer), 107 (45%) were NR at Post V1, compared with 15/24 subjects (62%) who received Ad26.COV2.S (Johnson & Johnson) and 27/116 subjects (23%) who received mRNA-1273 (Moderna). In anti-NC- subjects, those who received the Moderna vaccine had significantly higher anti-spike IgG values, compared to subjects who received either the Pfizer ($p < 0.001$) or Johnson & Johnson ($p < 0.001$) vaccines (**Figure 3A**). This was not due to a difference in age or gender of the subjects receiving the different vaccines (**Figure 3B**). There was no difference in anti-spike IgG values in anti-NC+ subjects according to vaccine type received (**Figure 3C**). Of the 64 subjects taking B cell depletion who received the Pfizer vaccine, 48 (75%) were NR, whereas 59/170 (34%) not receiving B cell depletion were NR. Anti-NC- subjects who received the Moderna vaccine had higher anti-spike IgG values at the Post V1 visit compared to those receiving the Pfizer vaccine,

regardless of whether they were unexposed (**Figure 3D**) or exposed (**Figure 3E**) to B cell depletion. There were insufficient anti-NC+ subjects taking B cell depletion to evaluate the differences between vaccine types (**Figure 3E**).

We evaluated the effect of stopping methotrexate and MMF on anti-spike IgG values. Methotrexate was held or stopped at initial vaccination in 11 subjects of whom 8 (73%) were Responders and 3 (27%) were NR. In 41 subjects who continued methotrexate at Post V1, there were 28/41 (68%) Responders and 13/41 (32%) NR (p = 0.08). No differences were observed between subjects who held or continued MMF or MPA during the initial vaccination series or MMF, MPA or methotrexate at the time of boosting (**Supplementary Table 4**).

T cell assays

T cell assays were performed on matched samples from autoimmune subjects at the Pre V, Post V1, and Post B1 visits and on HC at the Pre V and Post V1 visits (**Figure 1**). Samples with $\leq$ 1000 CD4 T cells and/or $\leq$ 750 CD8 T cells were considered to have insufficient cells for accurate evaluation and were removed prior to analysis (**Supplementary Figure 2A, 2B**). Vaccination of SARS-CoV-2–unexposed HC elicited robust T cell responses to SARS-CoV-2 peptides as expected (**Supplementary Figure 2C, 2D**) with no effect on responses to CMV peptides (**Supplementary Figure 2E, 2F**). Two healthy donors were used as controls for each batch and showed similar activation-induced marker (AIM) results over multiple blood draws (**Supplementary Figure 2G, 2H**).

We first analyzed T cell responses in all autoimmune subjects using a linear regression model. At the Pre V visit, anti-NC- subjects had significantly lower CD4 and CD8 T cell reactivity to SARS-CoV-2 peptides than those subjects with known SARS-CoV-2 exposure as expected (**Figure 4A**). Unlike what we observed with antibody responses, however, CD4 and CD8 responses to initial vaccination did not differ between autoimmune subjects and HC or between SARS-CoV-2 exposed and non-exposed individuals (**Figure 4B**). The treatment analyses were therefore carried out regardless of exposure status. We analyzed T cell responses in subjects taking B cell–depleting agents, MMF/MPA, methotrexate, or other drugs (**Supplementary Table 5**). The final linear regression model showed no difference in CD4 responses at the Post V1 visit among treatment groups (**Figure 4C, 4E**; p = 0.86). By contrast, there was a significant difference in CD8 responses among treatment groups, (**Figure 4D, 4F**; p = 0.013) reflecting a higher CD8 response in the B cell depletion group compared to the MMF and other groups. No differences were observed in T cell responses to CMV peptides (**Supplementary Figure 5I, 5J**). Unlike what we observed for anti-spike IgG, there was no relationship between the time of the last dose of B cell depletion and either the CD4 or CD8 response to SARS-CoV-2 peptides (**Figure 4G**).

The linear mixed regression model further showed no difference in the overall CD4 response between the Post V1 and Post B1 visits (**Figure 4E, 4H**) but a statistically significant increase in the overall CD8 response after adjusting for treatment group (**Figure 4F, 4H**; p = 0.002). When we examined the effect of the 4 different treatment regimens on booster responses, we found a significant increase in the CD4 response between the Post V1 and Post B1 visits only in the

MMF group (p = 0.002), with a trend for CD8 (p = 0.01), but no significant differences in any of the other treatment groups (**Figure 4H–J**).

We next determined whether there was a relationship between the anti-spike IgG antibody response and the T cell response to spike peptides at the Post V1 visit. We found no relationship between CD4 or CD8 responses and anti-spike IgG values in the B cell depletion group (**Figure 4K**). However, in the final linear regression model, we found an inverse association between both CD4 and CD8 and anti-spike IgG among the small number of B cell depletion subjects who had previously been exposed to SARS-CoV-2 (p = 0.013 and p = 0.035, respectively). A similar inverse effect was observed for CD4 responses among SARS-CoV-2–exposed healthy donors (p = 0.017). By contrast, CD8 responses positively correlated with anti-spike IgG in subjects on MMF regardless of SARS-CoV-2 exposure ($R^2 = 0.58$, p = 0.014; **Figure 4L**).

Finally, we found no differences in the CD4 T cell response according to the vaccine received and only minimal differences in the anti-CD8 response (**Figure 3F, 3G**).

Disease diagnosis and vaccine response

Anti-NC- subjects with multiple sclerosis (MS) had lower anti-spike IgG values at the Post V1 visit than anti-NC- HC or subjects with pemphigus or SLE (**Figure 5A**), most likely due to the high frequency of B cell depletion use in MS. In subjects taking B cell depleting drugs, the most important determinant of the anti-spike IgG response was the time since the last dose of B cell–depleting drug. There was a significantly lower percentage of responders among those who received B cell depletion within the previous 6 months (9%), compared to those who received it

≥ 7–11 (45%) or ≥ 12 months (81%) prior to vaccination (p < 0.05 and p < 0.001, respectively;

Figure 5B). MS subjects had taken their last dose of B cell depletion more recently than subjects

with pemphigus (median 5.5 versus 24 months since last dose, p < 0.0001). There were no

differences in CD4 and CD8 responses at the Post V1 visit between subjects with the most

represented autoimmune diseases in our cohort, except for subjects with SLE who had lower

CD4 and CD8 responses than non-SLE subjects after correcting for medication use (p < 0.05 and

p < 0.001, respectively; **Figures 5C, 5D**). The difference in the CD8 response was still

significant when SLE and non-SLE subjects taking B cell–depleting drugs (n = 15 versus 85) or

any other therapy except MMF/MPA (n = 56 versus 50) were compared (p < 0.001 and p < 0.05,

respectively; **Figure 5E**) but was not significant when SLE and non-SLE subjects taking MMF

(n = 40 versus 13) were compared (**Figure 5E**).

Disease flares and vaccine type

There were 16 disease flares (**Supplementary Table 6**) in 251 subjects in whom flares were

assessed at the Post V1 visit for an overall flare rate of 6.4%. The Post V1 flare rate in SLE

subjects was 4/204 (2%), lower than reported in the literature (24, 25), and the flare rate in

rheumatoid arthritis (RA) subjects was 5/56 (9%)—similar to that reported in the literature (26,

27). There were 19 flares in 208 subjects in whom flares were assessed after booster vaccination

(**Supplementary Table 6**), for an overall flare rate of 9.1%. The Post B1 flare rate in SLE

subjects was 8/71 (11%), similar to that reported in the literature, and the flare rate of RA

subjects was 5/22 (23%). The vaccine type was not associated with the rate of flares at either the

Post V1 or Post B1 visit (**Supplementary Table 7**).

Breakthrough infections

SARS-CoV-2 infection frequency and severity at each visit are shown in **Supplementary Table 8** and **Supplementary Figure 3**. At the time of the Pre V visit, 29.4% of the cohort had already been infected with SARS-CoV-2, with 2.1% requiring hospitalization. At the first follow-up visit (n = 253), 4.4% of subjects had acquired a new infection, of whom only one was symptomatic. Subjects seen for the first time 4 to 14 weeks after completion of the first vaccination series also had a high rate of previous exposure (14.6%): all but 2 of these infections were acquired before vaccination based on the timing of the sample collection in relation to completion of vaccination. The frequency of breakthrough infections was 4.8% at Post V2 but had increased to 25% by Post V3. By contrast, only 7.5% of subjects receiving booster injections had breakthrough infections at the time of the Post B1 visit; the 1 subject in this group with a severe infection was hospitalized in the 2-week window immediately after receiving the booster. Medical charts of all subjects who enrolled at the Pre V or Post V1 visit were examined regardless of whether they completed all visits, and there were no deaths due to SARS-CoV-2 in our cohort after 12 months of follow up, a period encompassing the Delta and Omicron variant waves.

The medications used by subjects with breakthrough infections are listed in **Supplementary Table 8**. Sixty-five percent of breakthrough infections were symptomatic. The 2 hospitalized subjects were both taking rituximab and had a negative anti-spike IgG titer. Subjects with symptomatic infections were more often taking B cell–depleting agents (27% versus 17%), MMF (27% versus 17%), or azathioprine (15% versus 6%) compared to subjects with asymptomatic infections, whereas those with asymptomatic infections were more often taking methotrexate (28% versus 6%) or hydroxychloroquine alone (17% versus 9%). There was

insufficient power to determine the statistical significance of these findings. Thirty percent of subjects with breakthrough infections had anti-spike IgG values of < 250U/ml at the visit prior to the breakthrough infection, which was no different than the overall cohort. Sixty percent of subjects with symptomatic infections prior to boosting had a pre-infection anti-spike IgG titer of < 250U/ml compared with 20% of subjects with asymptomatic infections (p = 0.07). Similarly, 50% of subjects with symptomatic infections after boosting had a pre-infection anti-spike IgG value of < 250U/ml compared with 22% of subjects with asymptomatic infections. Notably, 9 subjects failed to mount an anti-NC response to their breakthrough infections. Eight out of 9 of these subjects had anti-spike IgG values of < 250U/ml, indicating that we may have underestimated the frequency of asymptomatic breakthrough infections in subjects who did not mount IgG responses to vaccination.

Using a cutoff of 0.2 for a low CD4 response and 0.05 for a low CD8 response at the Post V1 visit, we found that 9 of 12 (75%) autoimmune subjects with a breakthrough infection prior to boosting manifested low T cell responses at the Post V1 visit, compared with 90/228 (39%) of subjects without breakthrough infections (p < 0.02). The other 3 subjects, 1 of whom requiring hospitalization, had low anti-spike IgG values. Only 2 of 8 tested subjects with breakthrough infections after boosting had low T cell responses; both had negative anti-spike IgG and symptomatic infections, and 1 required hospitalization.

Autoantibodies

To determine whether vaccination with spike protein induced new or increased titers of autoantibodies in individuals with autoimmune disease, autoantibody profiles were examined

using a previously described autoantigen array (28) (**Figure 6**). As expected, subjects with SLE displayed autoantibodies to nuclear antigens including SSA (Ro), SSB (La), Smith, and RNP, whereas subjects with pemphigus and MS had few such autoantibodies. Antibodies against thyroid peroxidase (TPO) were common and found across autoimmune diseases. Antibodies against Type 1 IFNs, particularly IFNα7 and α8, were found most frequently in subjects with SLE, whereas antibodies against IL6 were most commonly found in subjects with MS. Antibodies against TNF were detected in RA subjects who were taking TNF inhibiting drugs, reflecting the circulating drugs in their serum.

We next examined autoantibody induction using matched subject sera before and after vaccination and boosting. We found no statistical difference in autoantibody median fluorescence intensity (MFI) for any analyte after vaccination or boosting, although a few subjects had either an increase or decrease in the MFI of some autoantibodies.

Anti-SSA (Ro) antibodies have been reported in subjects with severe SARS-CoV-2 infection (28, 29). We examined autoantibodies against Ro52 and Ro60 and La (**Supplementary Figure 4A–C**). Autoantibodies against Ro60 and La remained stable over time. Although there was more variability in antibodies to Ro52, differences in MFI at the 3 time points were not significant. Autoantibodies against RNA associated antigens Sm and RNP also remained stable (**Supplementary Figure 4D**). Two subjects developed new autoantibodies to TPO after vaccination (**Supplementary Figure 4E**), confirmed by TPO ELISA, but neither developed thyroid dysfunction and 1 had a subsequent negative test after 24 months of follow up.

Autoantibodies to cardiolipin are induced by SARS-CoV-2 infection, and there was a significant incidence of thrombosis in subjects infected during the initial waves of the pandemic (30-33). We therefore determined whether vaccination could induce or boost a preexisting anti-cardiolipin response. We found no difference in either IgM or IgG anti-CL titers between anti-NC- and anti-NC+ subjects at the pre-vaccination visit and no difference in titers between time points in the 2 groups (**Supplementary Figure 4F–I**). Three subjects had a modest increase in pre-existing anti-CL antibodies and 3 subjects developed a de novo IgG or IgM anti-CL antibody of > 20U. Of these, 1 with SLE had been previously positive, 1 with RA had a previous clot without anti-phospholipid antibodies, and 1 had eosinophilic granulomatosis with polyangiitis (EGPA). None of these subjects developed thrombotic sequelae over the course of the study or at routine follow up visits 24 months after initial vaccination.

Autoantibodies to desmogleins 1 and 3 were measured in paired serum samples from 23 pemphigus subjects who received SARS-CoV-2 vaccination, including 14 subjects with Pre V and Post V1 paired samples (10 PV, 4 PF) and 9 subjects with Pre B and Post B1 paired samples (8 PV, 1 PF). Except for 1 subject who had a rising titer of anti-desmoglein antibodies prior to vaccination that continued after vaccination, there was no change in titers of autoantibodies to either antigen or development of a new autoantibody in this subject group after vaccination or boosting (**Supplementary Figure 4J, 4K**).

Recent large studies using electronic medical data have suggested that there is an increase in the incidence of autoimmune diseases including rheumatoid arthritis, SLE, vasculitis, inflammatory bowel disease (IBD), and type I diabetes in SARS-CoV-2–exposed individuals (34). We

therefore determined whether there was a difference in autoantibody profiles of autoimmune subjects who had been previously exposed to SARS-CoV-2 compared to those who had not. We found no differences between these 2 groups (**Supplementary Figure 5**).

Antibodies to Type 1 interferons have been associated with worse outcomes of SARS-CoV-2 infection (22). We therefore examined the correlation of antibodies to Type 1 IFNs with the values of anti-spike IgG antibodies and with the severity of reported SARS-CoV-2 infections. There was no correlation between anti-IFN MFI and anti-spike IgG values (**Figure 7A**). Furthermore, there was no difference in anti-IFN MFI between anti-NC- and anti-NC+ subjects (**Figure 7B**) or between subjects who had pre-vaccination symptomatic infections versus asymptomatic infections (**Figure 7C**). Furthermore, we found no difference in anti-IFN MFI between subjects with or without breakthrough infections (**Figure 7D**).

Unbiased predictive model for B and T cell responses to vaccination
To identify additional variables associated with compromised B and T cell responses to vaccination, we developed predictive models of anti-spike IgG and T cell responses to the initial COVID-19 vaccination in an unbiased approach that included all the variables we recorded. The modeling was performed both with and without autoimmune diagnosis since the association of an autoimmune diagnosis with immune responses may be confounded by immunosuppressant use. The model that included autoimmune diagnosis had modest predictive power ($R^2 = 0.28$) and identified the diagnosis of MS as a predictor of low anti-spike IgG response (**Supplementary Figure 6A**) except in subjects treated with interferon-beta or glatiramer acetate who mounted significantly higher anti-spike IgG values than the rest of the autoimmune cohort

(p < 0.01), comparable to the HC (**Supplementary Figure 6B**). The model that excluded the autoimmune diagnosis also had modest predictive power ($R^2 = 0.25$) and also found that interferon-beta predicted a higher anti-spike IgG response at Post V1. In addition, B cell depletion or belimumab predicted a lower anti-spike IgG response (**Supplementary Figure 6C**). The models for CD4 and CD8 T cell response did not reveal additional meaningful associations beyond those already described.

DISCUSSION

Our real-world study yields a comprehensive overview of SARS-CoV-2 vaccine responses in individuals with multiple autoimmune diseases compared with HC. We show significant differences in the B cell but not the T cell response to vaccination between SARS-CoV-2 naive and pre-exposed subjects, discordance between anti-spike antibody and T cell responses in subjects with different autoimmune diseases, and minimal effects of vaccination on autoantibody and anti-cytokine reactivities after vaccination.

Using the Roche Elecsys assay to evaluate the IgG anti-spike protein response post vaccination, we established that thresholds for a normal response depend on prior SARS-CoV-2 exposure. Response rates below the cutoff values after the first vaccination occurred in 25% and 44% of exposed and unexposed cohorts, respectively. Dissipation of the humoral immune response over time and response to boosting were similar in autoimmune subjects and controls.

Medication use was a major determinant of low antibody response in autoimmune subjects. Consistent with prior literature, most subjects with an inadequate humoral response to both the

initial and booster vaccinations were taking B cell–depleting drugs (5, 7, 8, 35, 36), with the time

from the last dose of B cell depletion being a critical factor (37). There were low humoral

responses in > 50% of subjects even in the 6- to 12-month window after the last dose of B cell–

depleting drug. These findings have general implications for the immunization of patients taking

B cell–depleting drugs for whom current guidelines recommend a window of 6 months post-

treatment or 4 weeks prior to the next treatment cycle for delivery of vaccines (38). Other

immunosuppressive agents that conferred a high frequency of suppression of IgG anti-spike

responses were the combination of MMF and belimumab and treatment with fingolimod.

Because we assessed MMF levels and found that approximately 20% of subjects were not taking

their medication, we were able to evaluate a true rate of low IgG response in subjects taking

MMF without an additional immunosuppressant at 39%, higher than previously reported (4, 7,

8). Most subjects did not contact their physicians or hold their medications prior to vaccination.

Methotrexate was held most frequently with a trend for an improved vaccine response, consistent

with recent clinical trials (12, 13, 39). We were unable to show that holding MMF improved

vaccine responses. Few of our subjects were taking high doses of steroids, but doses of < 20

mg/day were not associated with low IgG responses. These findings identify those subjects at

highest risk of non-response to vaccination.

The vaccine type also influenced immune responses in our subjects. In a previous study of

autoimmune subjects in the UK, the viral vector vaccine ChAdOx1 nCoV-19 was less effective

than the Pfizer vaccine at eliciting IgG anti-spike responses but it induced a stronger T cell

response (23). In our study, differences between vaccines were observed only in SARS-CoV-2–

naive subjects. Subjects immunized with the Johnson & Johnson vaccine mounted lower IgG

responses than subjects immunized with mRNA vaccines. Notably, the IgG anti-spike response was higher in subjects immunized with the Moderna vaccine than in those immunized with the Pfizer vaccine even after adjusting for usage of B cell–depleting drugs. A recent meta-analysis of observational studies of immunocompromising conditions, including autoimmune disease, found that the Moderna vaccine was associated with reduced risk of SARS-CoV-2 infection hospitalization and mortality compared with the Pfizer vaccine (40). Additionally, the Moderna vaccine was associated with increased seroconversion rates compared to the Pfizer vaccine in immunosuppressed transplant recipients (41). Increased TLR stimulation with the higher dose Moderna vaccine, compared to the Pfizer vaccine, has been hypothesized to lead to improved seroconversion in immunocompromised subjects, which may account for the differences in conversion rates found in this study and others (42, 43). Alternatively, differences in formulation or in the prime-boost timing of the 2 vaccines could contribute to differences in efficacy. The vaccine type had much less effect on the quality of the T cell response, and we could not confirm a previously reported higher T cell response to the Moderna compared with the Pfizer vaccine (44).

We observed differential effects of immunosuppression on B and T cell responses across various classes of immunosuppressive drugs. Consistent with prior findings, MS subjects taking B cell–depleting drugs exhibited lower IgG anti-spike responses and higher CD8 responses to spike protein peptides (14, 16, 17, 36, 45). This heightened CD8 response could result from reduced IgG anti-spike levels in B cell–depleted subjects, prolonging spike protein clearance post vaccination. Conversely, MMF usage coordinately suppressed both B and T cell responses, reflecting the drug's anti-proliferative activity.

While differences in the humoral response to vaccine between subjects with different autoimmune diseases could be attributed to medications, intriguingly, we found that the CD8 response to vaccine was lower in SLE subjects than in HC and in subjects with other autoimmune diseases, including those receiving B cell–depleting drugs. Comparable results comparing SLE subjects with HC have been recently reported (46). Mitochondrial and metabolic defects in T cell function occur in SLE; these defects are more pronounced in CD8 than in CD4 T cells and are associated with an increased risk of recurrent infections (47, 48). Furthermore, a subset of SLE patients has a profile of CD8 T cell exhaustion, a phenotype that has been associated with worse response to vaccinations and viral infections (49).

Despite the relatively high occurrence of subnormal vaccination responses, severe breakthrough infections were rare in our cohort. Importantly, unlike a recent report from a similar UK cohort (23), we had no deaths among our immunosuppressed subjects. This discrepancy likely arises from the UK cohort's inclusion of more vulnerable individuals. Moreover, the overall frequency of breakthrough infections in autoimmune subjects was lower than in HC and was not higher in SLE subjects than in subjects with other autoimmune diseases despite their CD8 T cell defect. The reasons for this difference are multifactorial and could include the earlier immunization timeframe of our autoimmune cohort relative to the pandemic's evolution as well as variations in the prevalent variant during immunization and boosting. Additionally, cautious behavior among subjects with autoimmune diseases might have contributed as well as the utilization of tixagevimab with cilgavimab (Evusheld), although this drug received emergency use authorization in December 2021 and was prescribed to only 3 subjects in our cohort during the

study period. Protection of our subjects was sustained until the second post-vaccination visit but waned by the third (12-month) visit in subjects who did not receive a timely dose of booster.

Data on the association between anti-type I IFN antibodies and SARS-CoV-2 infection to date is mixed (22, 50), likely as a consequence of differing characteristics of individual cohorts. Our data shows that anti-cytokine antibodies do not correlate either with the magnitude of the immune response to vaccination or the frequency or severity of breakthrough infections in autoimmune subjects.

Pre-SARS-CoV-2–era studies in autoimmune subjects have demonstrated minimal induction of new autoantibodies by vaccines or adjuvants in subjects with autoimmune diseases (51). Large cohort studies have linked SARS-CoV-2 infection to autoantibody induction and increased risk of new-onset autoimmune disease (34, 52, 53). The SARS-CoV-2 mRNA vaccines contain pseudouridine nucleoside–modified mRNA designed for reduced inflammation compared to unmodified RNA. Nevertheless, these vaccines still activate the MDA5/Type 1 IFN innate immune pathway, leading to CD8 T cell response and production of inflammatory chemokines and cytokines like IL1 and IFNγ along with potent activation of T follicular helper cell and germinal center responses (54, 55). Furthermore, observational studies have reported a variable rate of disease flares ranging from 0.4 to 20% following vaccination (56). We were therefore particularly interested in whether vaccination in the context of an innate immune stimulus would induce an increase in existing autoantibody MFI or new autoantibodies or disease flares. Reassuringly, although a few subjects developed new autoantibodies to TPO and cardiolipin without clinical autoimmune disease, we did not observe emergence of new specificities or

significant increases in autoantibodies in our array panel or in either anti-cardiolipin or anti-desmoglein antibodies by ELISA. In accordance with these data, disease flares of either RA or SLE did not occur at a higher frequency than reported in the literature, and there was no association of vaccine type with disease flares.

This real-world study has several strengths. The study encompassed multiple autoimmune diseases spanning rheumatologic, neurologic, and dermatologic domains, and it included subjects of diverse race, gender, and age. By using matched pre- and post-vaccine samples, we were able to analyze the impact of SARS-CoV-2 vaccination on existing autoimmunity, including the potential elicitation of new autoantibodies and the effects of anti-cytokine antibodies on vaccine responses. The study involved subjects on various immune-suppressive medications, including conventional immunosuppressives and biologics, confirming previously described medication effects on vaccine responses while evaluating both B and T cell responses concurrently. In a reflection of the real-world scenario, the study included subjects who interrupted and those who did not interrupt their immunosuppressive medications.

There were also some limitations to the study, notably the challenge of small sample sizes for some of the subanalyses. Additionally, the interruption of medications lacked standardization, reflecting how subjects obtained their vaccines (often without first contacting their physicians). The non-randomized interruption of medications introduced potential confounding factors, such as disease activity and subject beliefs. The evolution of SARS-CoV-2 variants and the compromise of anti-viral responses by some immunosuppressive regimens may also have impacted our analysis of the frequency of asymptomatic breakthrough infections. Moreover,

dropouts and assessments occurring outside of the timing window added complexities to the study's interpretation. Finally, we used the anti-spike IgG and anti-IFN values as measures of the antibody response without concomitant neutralization assays. This may have resulted in overestimation of the quality of the response in autoimmune subjects in which the neutralization capacity of anti-spike IgG may be compromised (46) and underestimation of the impact of cytokine neutralization on anti-spike IgG responses.

Overall, our study provides data on factors associated with poor B and T cell responses to SARS-CoV-2 vaccination in autoimmune subjects. The significantly enhanced responsiveness to vaccination among subjects previously exposed to SARS-CoV-2 virus infection indicates that most autoimmune subjects can mount anti-viral responses. This suggests that safe immunization strategies for autoimmune populations could include increased mRNA vaccine dose, extra boosting, or boosting with a wider range of viral antigens. Using both clinical and laboratory-based measures, we offer reassuring data regarding the minimal induction of autoimmunity by SARS-CoV-2 vaccines. We also demonstrate little effect of anti-cytokine responses on vaccine response or breakthrough infections. Importantly, we show that B and T cell responses to vaccination are not always correlated and that T cell responses, except in SLE subjects, resemble those in HC with enhanced CD8 but not CD4 responses after boosting. Despite the high rate of compromised anti-spike IgG responses, the low rate of breakthrough infections in our cohort provides additional support for the protective function of vaccine-induced T cell responses across a broad swath of autoimmune subjects.

METHODS

Subject enrollment and sex as a biological variable

Clinical data and biospecimens were obtained from a prospective observational study involving 75 male and 388 female subjects aged ≥ 18 with autoimmune diseases at 4 National Institutes of Health (NIH) Autoimmunity Centers of Excellence (Feinstein Institutes for Medical Research, University of Pennsylvania, Oklahoma Medical Research Foundation, and Emory University) from January 2021 to September 2022. Males and females behaved similarly (**Figure 3B, Supplementary Figure 1D**) and are reported together. The autoimmune diseases studied are shown in **Figure 1**. Clinical assessments (see **Supplementary Methods**) occurred before receiving an mRNA or vector-based SARS-CoV-2 vaccination (Pre V) and at subsequent post-vaccine visits. Post V1, V2, and V3 occurred at 4 to 14 weeks, 24 +/- 8 weeks, and 52 +/- 8 weeks, respectively, after completion of full vaccination (1 dose for protein vaccine or 2 doses for mRNA vaccines). Additional assessments occurred before the first booster for newly enrolled subjects (Pre B) and 2 to 8 weeks after receiving the first SARS-CoV-2 booster (Post B1). One hundred and twelve sera from healthy donors, matched by age, gender, and ethnicity to the autoimmune cohort, were obtained from the Serological Sciences Network for COVID-19 (Seronet) database (57) (**Figure 1**) during the same time windows relative to vaccination. Samples were collected from 2 separate cohorts of HC: 1 with matched Pre V and Post V1 visits and the other with matched Pre B and Post B1 visits. Control peripheral blood mononuclear cells (PBMCs) were processed from whole blood collected from healthy donors at the Feinstein Institutes for Medical Research and Emory University, and pre–COVID-era serum samples were obtained from Stanford University.

Chart reviews to evaluate SARS-CoV-2–related deaths were conducted on all subjects a minimum of 1 year after initial vaccination regardless of inclusion in the analyses.

Serologic testing

IgG antibodies to SARS-CoV-2 spike and NC proteins were measured at Northwell Core Laboratories using the Roche Elecsys® assay (Roche Diagnostic Corporation, Indianapolis, IN), with serial 10 fold dilutions in Roche assay buffer if the initial anti-spike IgG value was > 250 U/mL. Anti-NC antibodies were considered positive when the assay result was > 0.5 U/mL for autoimmune subjects and > 0.8 U/mL for healthy donors, as these sera were diluted 1:2 for the assay with 0.4 U/mL being the lower limit.

Mycophenolate mofetil (MMF) or mycophenolic acid (MPA) levels

For those subjects being prescribed MMF or MPA, sera from the Post V1 and/or Post B1 visits were tested for drug levels using a commercial assay (Labcorp). Subjects with values of < 5ug/mL were considered non-adherent.

T cell activation induced marker (AIM) assays and flow cytometry

CD4 and CD8 responses to SARS-CoV-2 peptides were assessed using activation-induced marker (AIM) flow cytometric assays employing the following 3 activation markers: CD137, Ox40 and CD69. Cells were stimulated for 20-24 hours at 37°C in 1 of 4 conditions: (1) vehicle control, (2) anti-CD3/CD28 beads, (3) PepTivator CMV pp65, or (4) PepTivator SARS-CoV-2 Prot_S Complete (**Supplementary Methods**). The gating strategy for identifying B and T cells

and the distribution of immune cell counts is shown in **Supplementary Figure 7**. After gating on

CD4 or CD8, the percent AIM+ values were calculated using Boolean 'OR' gating as follows:

$CD137^+OX40^+$ 'OR' $CD137^+CD69^+$ 'OR' $OX40^+CD69^+$. The vehicle control percent AIM+

values were subtracted from each stimulation percent AIM+ value to control for non-specific

background activation. Representative FACS plots and AIM assay gating are shown in

Supplementary Figure 8.

Autoantibodies

An 83-plex custom, bead-based antigen array consisted of 3 broad categories of antigens

(**Supplementary Table 9**). Each array was constructed as previously described (28) by

conjugating antigens to uniquely barcoded, carboxylated magnetic beads (MagPlex-C, Luminex

Corp – **Supplementary Methods**). The "Cytokine" content included 49 cytokines, chemokines,

growth factors, acute phase proteins, and cell surface proteins. The "Traditional Autoimmune

Associated" content included 21 commercial protein antigens associated with connective tissue

diseases. The "Viral" content included 8 antigens derived from viruses such as SARS-CoV-2,

respiratory syncytial virus (RSV), and cytomegalovirus (CMV).

Samples were initially run in singlet and selected samples were rerun in duplicate to confirm

significant changes in auto-antibody levels. Binding events were displayed as MFI. For each

sample, MFI values for "bare bead" IDs were subtracted from the MFI values for each antigen-

conjugated bead ID. To normalize across samples, the median MFI values for the 4 control IgG

analytes (anti-human IgG [H+L], anti-human IgG F[ab'] fragment–specific, anti-human IgG Fc

fragment–specific, and human IgG from serum) were calculated. For each of the control IgG

analytes, the ratio of the MFI value for each sample to the corresponding median was then calculated. The average of these 4 ratios became the correction factor for all the analytes of that sample in that the MFI of each analyte was divided by the correction factor.

IgG and IgM antibodies to cardiolipin were measured by the CAP/CLIA certified Oklahoma Medical Research Foundation Clinical Immunology Laboratory (58). Antibodies to desmoglein 1 (pemphigus foliaceus) and desmoglein 3 (pemphigus vulgaris) were measured by commercial ELISA (Euroimmun, Mountain Lakes NJ) using serial dilutions of serum samples within the linear range of standard controls (59, 60). Corrected index values were calculated by multiplying index values by the dilution factor.

Statistical analyses

Descriptive statistics (frequency distribution for categorical variables and mean, standard deviation [SD], median, interquartile range, minimum, and maximum for continuous variables) were calculated.

Anti-spike IgG antibodies: Wilcoxon Signed Rank test was used to determine whether there was a change in anti-spike IgG between Post V1 and Post V2 visits or Pre B and Post B1 visits. The Mann-Whitney test or Kruskal-Wallis ANOVA was used to determine whether there was a difference in the change in anti-spike IgG from Pre V to Post V1 visits, Post V1 to Post V2 visits, or Pre B to Post B1 visits among autoimmune or HC. Dwass, Steel, Critchlow-Fligner (DSCF) adjustment was performed to adjust for multiple comparisons. Kruskal-Wallis ANOVA

was used to determine whether there was a difference in the change in anti-spike IgG between anti-NC-, anti-NC Acq, and anti-NC+ subjects at each visit.

T cell assays: Univariable linear mixed regression was used to screen variables (age, gender, race, ethnicity) with a p-value criterion of $p < 0.05$ for entry into the model selection procedure. Backward selection was used with variable entry and retention criteria of $p < 0.05$ to select the final multivariable model. Linear mixed model was performed to determine whether there was a change in CD4 or CD8 response between Pre V and Post V1 visits, a difference in CD4 or CD8 response between anti-NC- and anti-NC+ subjects, a correlation between CD4/8 responses and anti-spike IgG responses at Post V1 among the treatment groups in all subjects and in anti-NC+ or anti-NC- subjects, or a difference in CD4 or CD8 response among groups. Log transformation was applied to meet the required assumptions of the regression model. Interaction between treatment groups and SARS-CoV-2 exposure status was examined. Tukey's exact procedure was performed to adjust for multiple comparisons. Additional regression analyses were performed using Prism 9.0.

Autoantibodies: Differences between autoantibody MFI at the Pre V and Post V1 or Post B1 timepoints were analyzed by t-test for 2 timepoints or linear mixed model for 3 time points.

Unbiased predictive models of anti-Spike antibody and T cell response: Linear regression modeling was used to determine correlations of anti-spike IgG values, CD4 T cell % AIM, or CD8 T cell % AIM at the Post V1 visit with other serological and clinical variables. See **Supplementary Materials** for extended method.

STUDY APPROVAL

This human subjects' research was approved by each center's institutional review board and written informed consent was obtained from each subject prior to performance of any study procedures.

DATA AVAILABILITY

Data are available in the Supporting data value file and the Datasets file.

AUTHOR CONTRIBUTIONS

Designed research studies: EA, MP, GM, SN, MM, BD, TPZ, AK, IS, ETLP, AB, JJ, JB, AP, PJU, CA, AD

Recruited subjects: GM, SN, PKG, MM, AK, AB, JJ, AP, CA, AD

Conducted experiments: MP, EY, AK, RM, AC, CE, NCO, EJC, CA, GP, JG, JJ, AP, PJU, JB

Acquired data: MP, EY, AK, NCO, EJC, JJ, PJU, JB, AD

Analyzed data: EA, MP, EY, AK, TuL, CS, RS, JZ, ToL, TPZ, AP, PJU, JB, AD

Provided reagents: PKG, JJ, AP, PJU, JB

Provided scripts: RS, TuL, CS, ToL, TPZ

Wrote the manuscript: EA, MP, EY, RS, TuL,ToL, CA, AD

Edited the manuscript: BD, ETLP, AB, JJ, PJU, JB

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TABLE 1: Subject demographics

	Eligible (n = 463)	Ineligible (n = 71)	p
Age (Year, Mean +/- SD*)	50 +/- 14	44 +/- 13 (n=66)	p = 0.006
Gender		n=66	
Male	75 (16%)	17 (26%)	p = 0.055
Female	388 (84%)	49 (74%)	
Ethnicity and Race		n=66	
Hispanic/Latino	117 (25%)	28 (42%)	
Black	23 (20%)	7 (25%)	p = 0.530
White	75 (64%)	14 (50%)	
Asian	1 (1%)	1 (4%)	
American Indian	1 (1%)	0	
Other or No Response	17 (14%)	6 (21%)	
Not Hispanic/Latino	346 (75%)	38 (58%)	
Black	130 (38%)	19 (50%)	p = 0.386
White	167 (48%)	13 (34%)	
Asian	33 (10%)	5 (13%)	
American Indian	8 (2%)	0	
Other or No Response	8 (2%)	1 (3%)	

*SD = standard deviation

Total autoimmune subjects enrolled, N=534

Total healthy controls enrolled, N=112

Ineligible subjects, N=71
(did not receive vaccine or did not have either a Post V1 or matched Pre B, and Post B1 visit)

Initial Vaccine Series, n=69

Booster, n=43

Total subjects included in autoimmune cohort, N=463

Systemic Lupus Erythematosus=192 (41%)   Pemphigus vulgaris=40 (9%)
Rheumatoid Arthritis=63 (14%)   Sjogren's Syndrome=8 (2%)
Multiple Sclerosis=89 (19%)   Other vasculitis=9 (2%)
Inflammatory Myositis=18 (4%)   Other=6 (1%)*
Psoriatic arthritis= 14 (3%)   Scleroderma=5 (1%)
ANCA vasculitis=14 (3%)   Pemphigus foliaceus=2 (0.5%)

Serology (Roche anti-RBD and anti-NC Ig), n=463**

Pre V, n=282
Post V1, n=397
Post V2, n=249
Post V3, n=49
Pre B, n=59
Post B1, n=211

Serology (Roche anti-RBD and anti-NC Ig), n=67

Post V1, n=67
Post V2, n=44

42 subjects with matched Post V 1 and Post V2 samples

Serology (Roche anti-RBD and anti-NC Ig), n=38

Pre B, n=38
Post B1, n=34

29 subjects with matched Pre B and Post B1 samples

T cell assays, n=244

Pre V, n=43
Post V1, n=244
Post B1, n=98

T cell assays, n=21

Pre V, n=10
Post V1, n=11
Matched samples Pre V, Post V1, Post B1, n=5

Autoantibody arrays, n=241

Pre V, n=241
Post V1, n=238
Post B1, n=76
16 positive controls

238 matched   73 matched
Pre V Post V1   Pre V, Post V 1, Post B1

3 matched Pre V, Post B1

Autoantibody arrays, n=8

Pre V, n=8

Note: were performed in a separate cohort of healthy controls; not included in the 112 healthy controls above

Autoantibody ELISAs, n=244

Anti- CL   **Anti-DG**
Pre V, n=244   Pre V, n=23
Post V1, n=240   Post V1, n=23
Post B1, n=77

240 matched   73 matched
Pre V Post V1   Pre V, Post V1, Post B1

4 matched Pre V, Post B1

*Other: Mixed Connective Tissue Disease (2), Common Variable Immunodeficiency, Polymyalgia Rheumatica, Discoid Lupus, Positive ANA

** Timing of post-vaccine sample collections relative to immunization
Post V1   4-14 weeks
Post V2   24 +/- 8 weeks
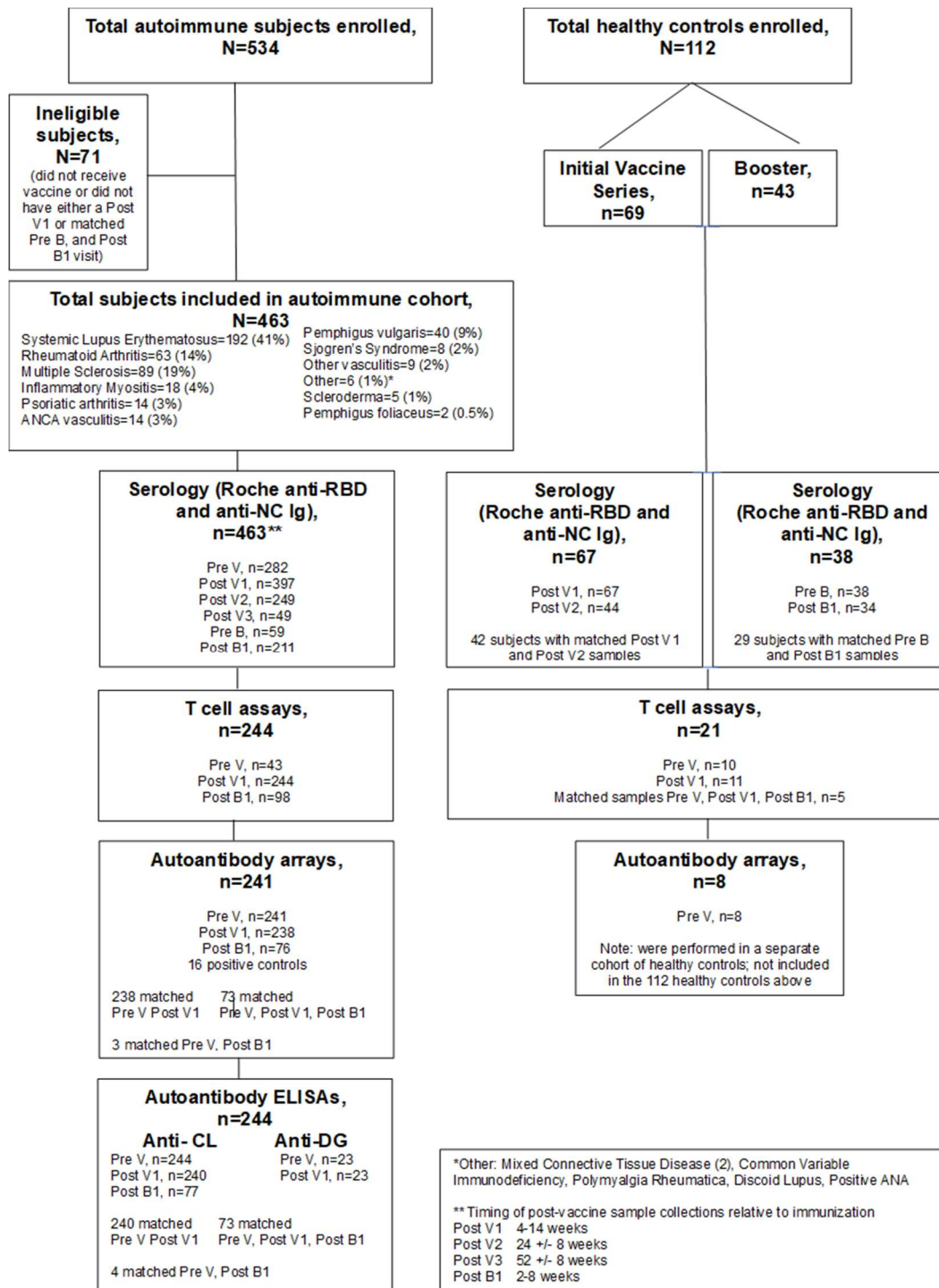Post V3   52 +/- 8 weeks
Post B1   2-8 weeks

Figure 1: Subject recruitment. Flow chart of subjects and analyses. Pre V: Pre-vaccine visit; Post V1:first visit 4 to 14 weeks after completion of first vaccine series; Post V2: second visit 24 +/- 8 weeks after completion of first vaccine series; Post V3: third visit 52+/- 8 weeks after completion of first vaccine series; Pre-B: day of first booster; Post B1: visit 2-8 weeks after first booster.
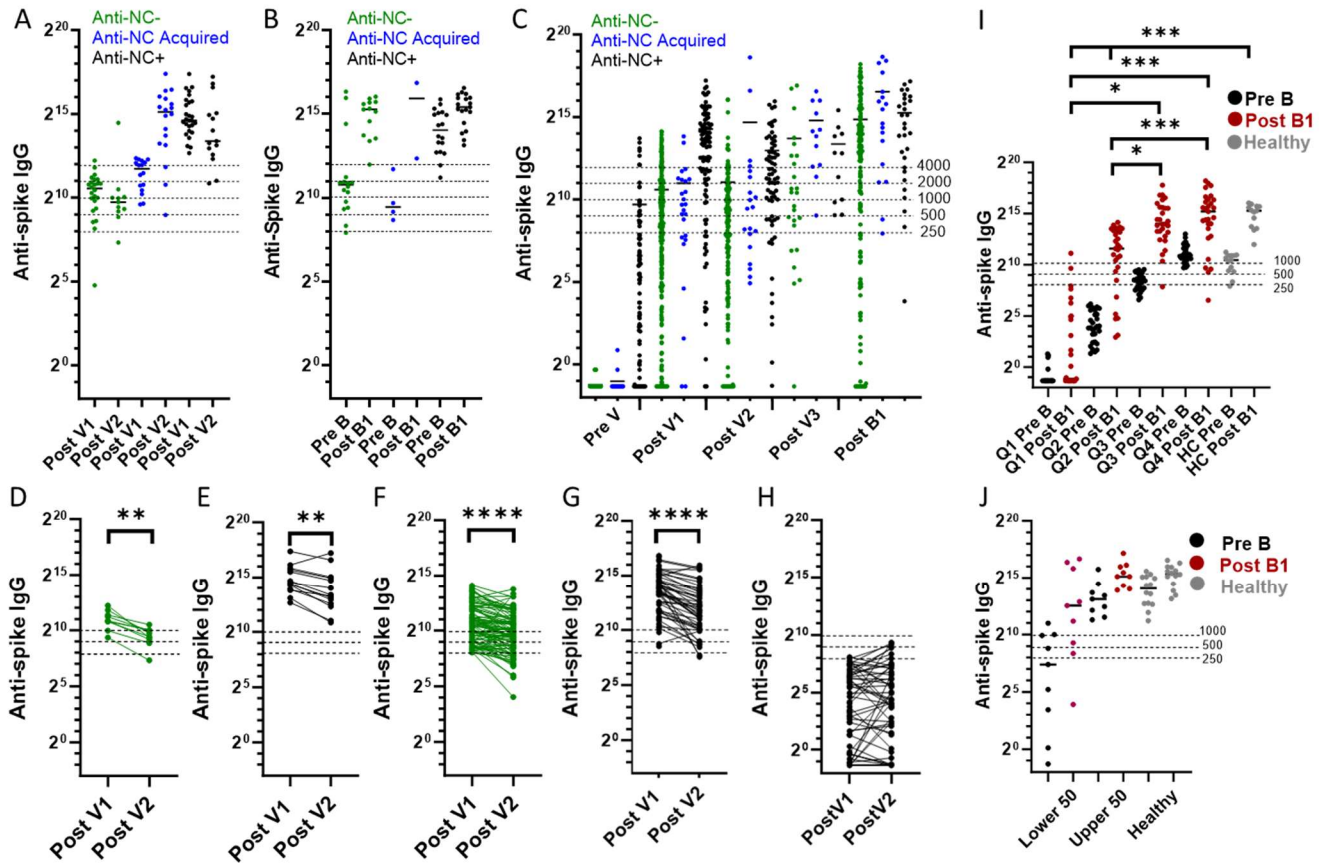
Figure 2: Serological response to SARS-CoV-2 vaccination in autoimmune subjects versus healthy controls according to SARS-CoV-2 exposure status. Subjects are divided into anti-nucleocapsid (NC) IgG- (green: no SARS-CoV-2 infection documented throughout the study), anti-NC Acquired (Acq, blue: SARS-CoV-2 infection documented at or after Post V2) and anti-NC+ (black: SARS-CoV-2 infection documented before initial vaccination) groups; see results for extended definitions. A, B: Anti-Spike IgG levels (U/mL) in HC at each visit after the initial (A) and booster vaccination (B). Anti-Spike IgG levels in autoimmune subjects at each visit before and after the initial vaccination and after booster vaccination (C). Statistical analyses are shown in Supplementary Figure 2. D, E: Trajectory of anti-spike IgG levels after the initial vaccine series in anti-NC- (D) and anti-NC+ (E) HC who responded to the initial vaccination. F, G: Trajectory of anti-spike IgG levels after the initial vaccine series in anti-NC- (F) and anti-NC+ (G) autoimmune subjects who responded to the initial vaccination. H: Trajectory of anti-spike IgG levels after the initial vaccine series in anti-NC- autoimmune subjects with an inadequate response (<250 U/mL, p = NS). I: Anti-spike IgG levels in anti-NC- autoimmune subjects versus HC before and after booster vaccination according to quartile of pre-booster anti-spike IgG levels. J: Anti-spike IgG levels in anti-NC+ autoimmune subjects versus HC before and after booster vaccination according to the upper and lower 50th percentile of pre-booster anti-spike IgG levels. Each data point represents an individual subject. D-G: Wilcoxon signed rank test; I, J: Kruskal-Wallis ANOVA with DSCF correction for multiple comparisons * p < 0.05, *** p < 0.001, **** p < 0.0001. Timing of sample collections is shown in Figure 1.
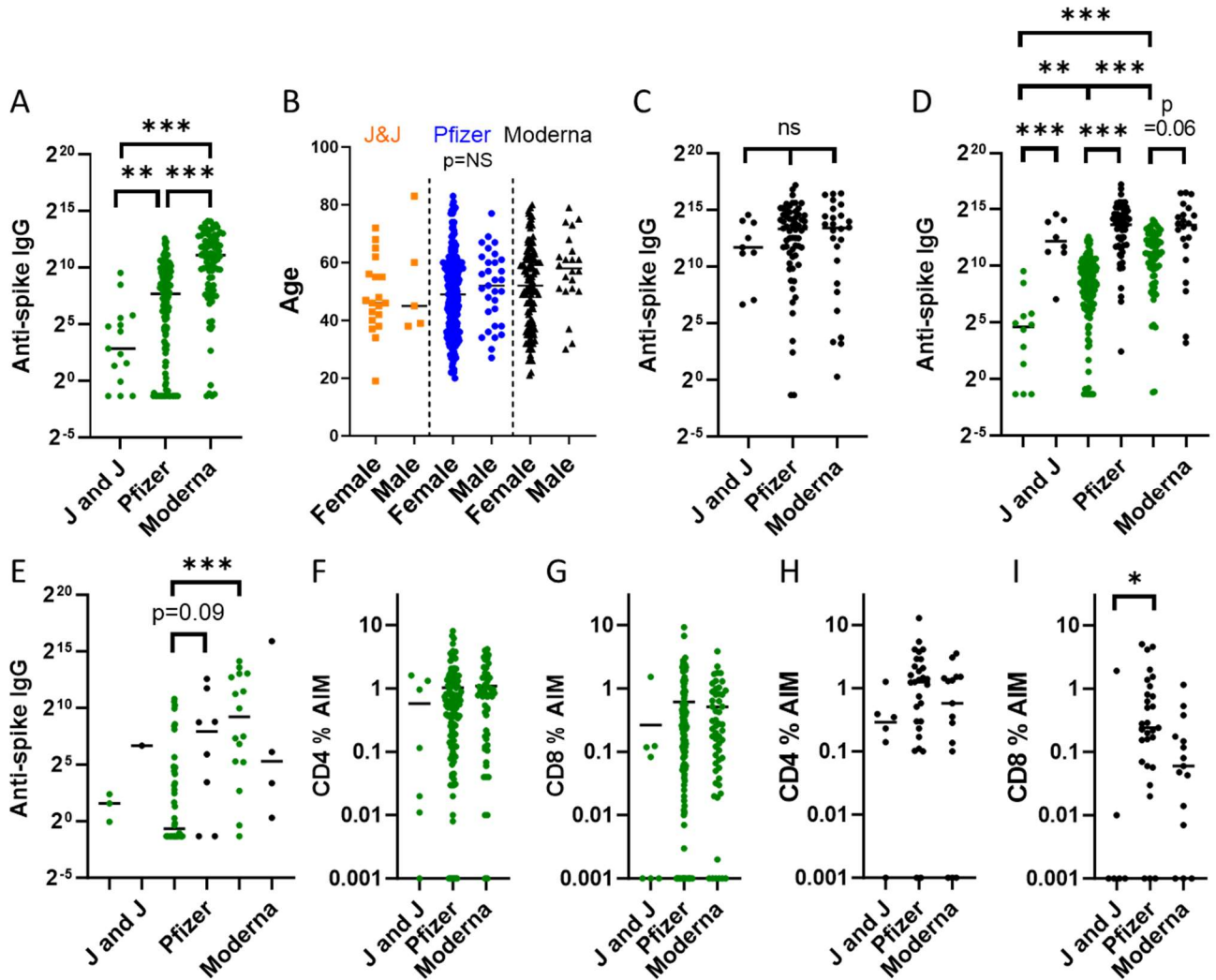
Figure 3: Adaptive immune responses following different SARS-CoV-2 vaccines in anti-NC- (green symbols) and anti-NC+ (black symbols) subjects. A-B: Anti-spike IgG responses to different vaccines in anti-NC- subjects shows a better response to Moderna than to either Pfizer or Johnson and Johnson vaccines (A) that is not associated with differences in age or gender (B). C: no difference in responses to the different vaccines in anti-NC+ subjects. D-E: Differences in anti-NC- subjects occur regardless of whether they were unexposed (D) or exposed (E) to B cell–depleting agents. F-I: CD4 (F, H) and CD8 (G, I) T cell responses to SARS-CoV-2 spike peptides in anti-NC- (F, G) and anti-NC+ (H, I) subjects by vaccine type. Each data point represents an individual subject. Kruskal-Wallis ANOVA with Dunn's correction for multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
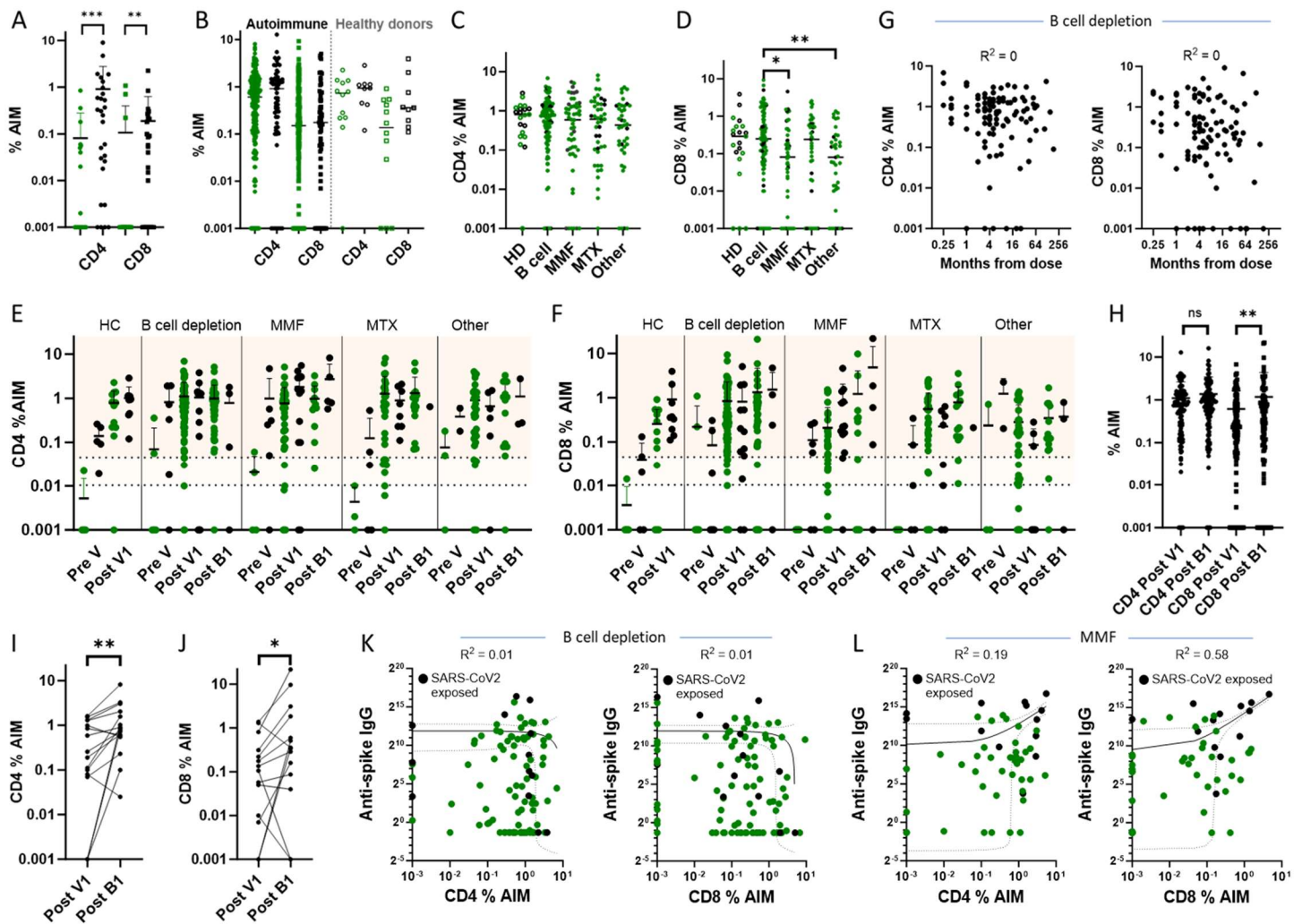
Figure 4: T cell responses to SARS-CoV-2 peptides. A: CD4 and CD8 responses to spike peptides measured by activation-induced marker (AIM) assay in autoimmune subjects before vaccination (A) and in healthy and autoimmune subjects at Post V1 (B) according to prior SARS-CoV-2 exposure. C-D: CD4 (C) and CD8 (D) responses to spike peptides at Post V1 in autoimmune subjects according to medication use. E-F CD4 (E) and CD8 (F) responses to spike peptides at sequential visits according to SARS-CoV-2 exposure and medication use. G: No correlation between T cell responses to spike peptides at Post V1 and time since last dose of B cell depletion. H: Change in CD4 and CD8 response to spike peptides after boosting in matched samples from the whole cohort. I-J: Change in CD4 (I) and CD8 (J) response to spike peptides after boosting in matched samples from subjects who were adherent to MMF treatment. K-L: Correlation between T cell responses at Post V1 to spike peptides and anti-spike IgG values in subjects treated with B cell depletion (K) or MMF (L). Each data point represents an individual subject. Anti-NC- subjects are shown as green symbols. Anti-NC+ subjects are shown as black symbols. A, D: Kruskal-Wallis ANOVA with Dunn's correction for multiple comparisons *p < 0.05, **p < 0.01, **p < 0.001. E-L: See results for statistical analyses using linear mixed regression model.
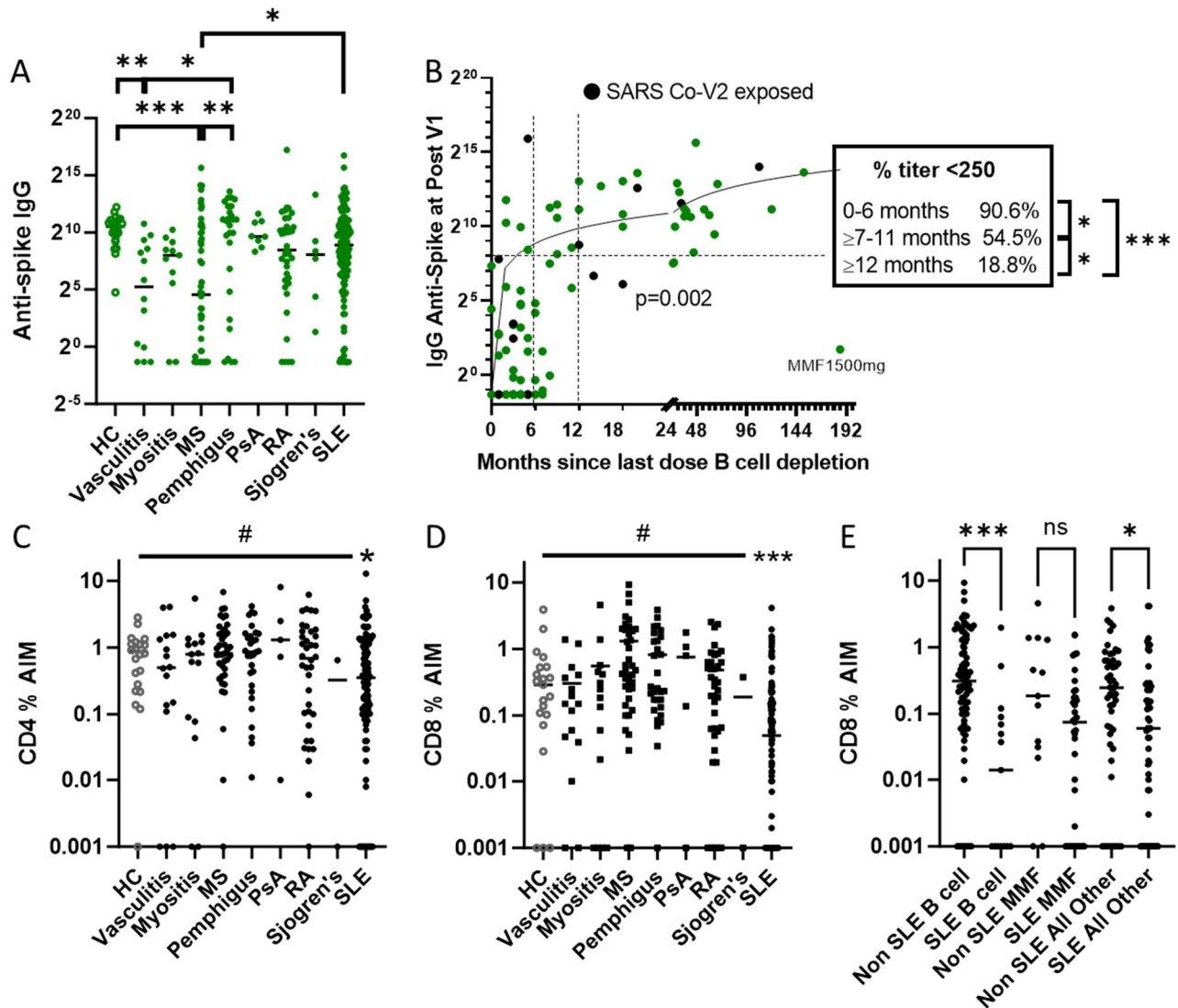
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Figure 5: B and T cell responses to vaccination according to drug and diagnosis. A: Anti-spike IgG values at Post V1 in anti-NC- subjects according to diagnosis. B: Correlation of IgG anti-spike responses (U/mL) with time since last dose of B cell depleting drug. Each data point represents an individual subject. Anti-NC- subjects are in green and anti-NC+ subjects are in black. Simple linear regression p = 0.002. Inset shows % non-responders for each time window. C, D: CD4 (C) and CD8 (D) T cell responses at the Post V1 visit according to diagnosis. Statistics for the linear mixed regression model (#) are shown in the bar. E: Comparison of CD8 % AIM responses in subjects with and without SLE separated by those taking or not taking B cell depleting drugs or MMF. Each data point represents an individual subject. Results of the linear mixed regression model are shown. *p < 0.05, **p <0.01, ***p < 0.001.
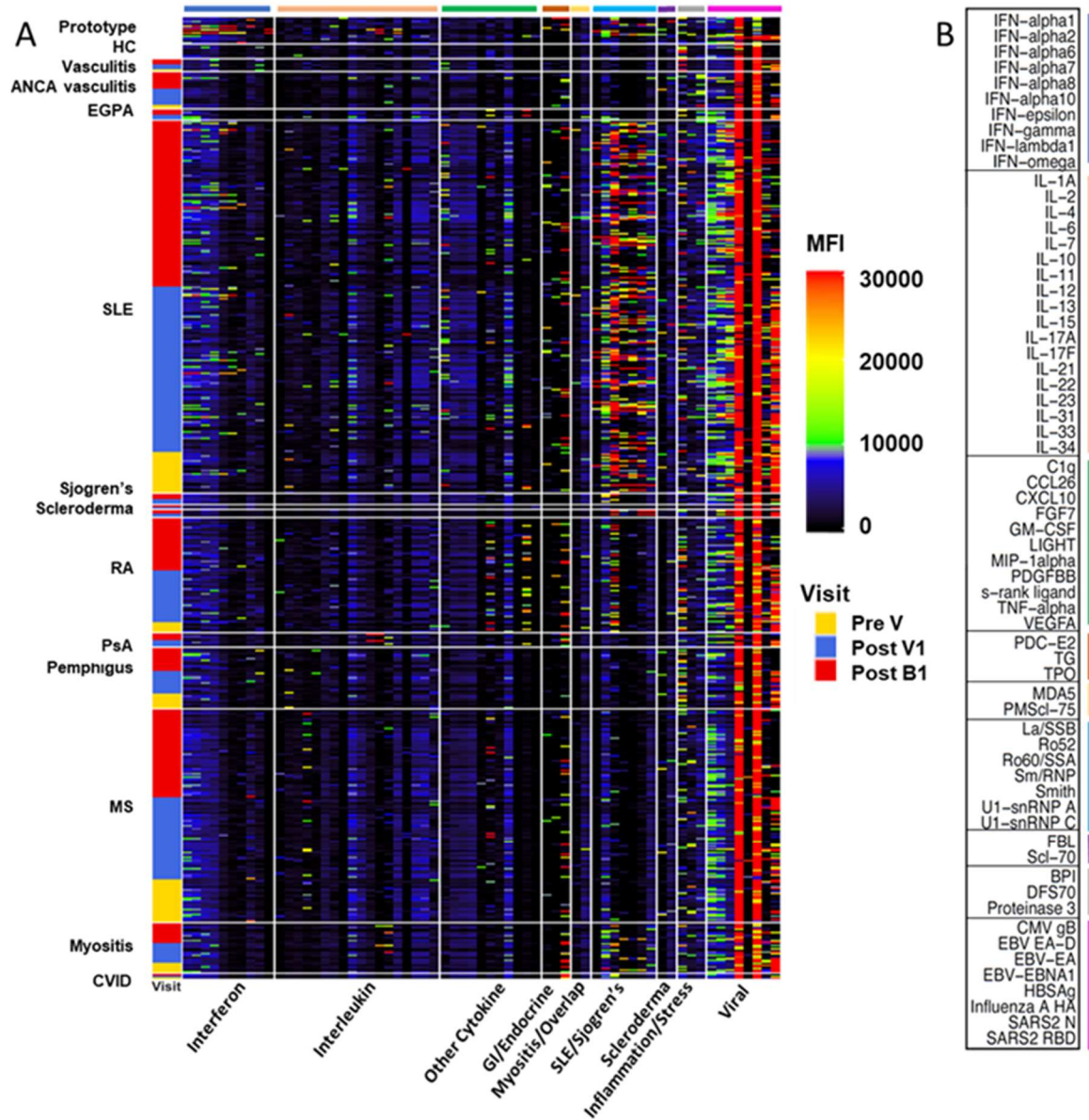
Figure 6: Autoantibody MFI values remain stable throughout vaccine course, with rare exceptions. A. Heatmap representing serum IgG detected with an 83-plex array of cytokines and chemokines, traditional autoimmune-associated antigens, and viral antigens. Two hundred and forty-one vaccinated subjects are represented and grouped into 1 of 15 different primary diagnoses. Within each diagnosis group, samples are clustered and annotated by the visit at which the sample was taken (Pre V, yellow; Post V1, blue; Post B1, red). Representative data from 16 prototype samples and 8 HC subjects are included. ACE2 and CENPA were excluded from the analyses because of cross-reactivity. Only those analytes with values above 5000 MFI are shown in the heatmap. Analytes in each group of antigens are color coded and individual antigens in each group of antigens are shown in B. p = NS for all comparisons performed by either t-test or linear mixed regression model.
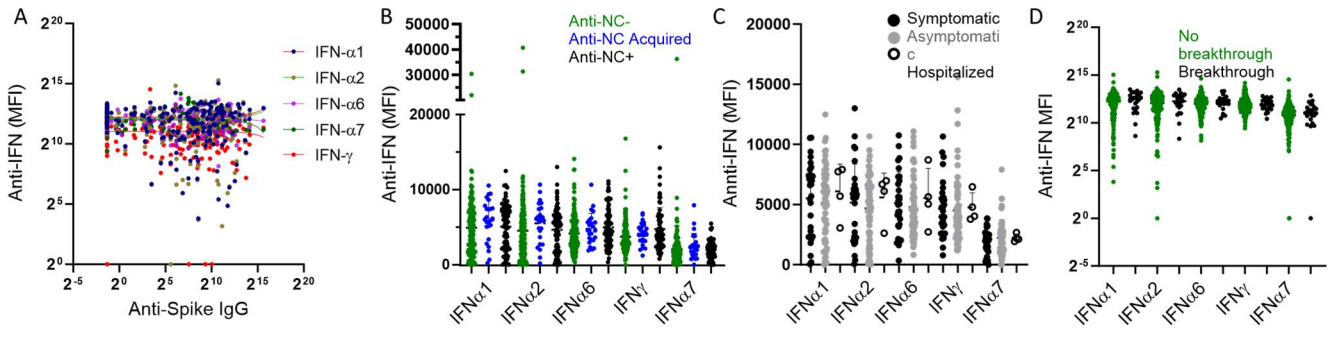
Figure 7. Correlation of autoantibodies to IFNs, measured at Post V1, with anti-spike IgG and SARS-CoV-2 infections. A: No correlation of anti-IFN MFI with anti-spike IgG values. B: No correlation of prior SARS-CoV-2 exposure with anti-IFN MFI. C: No correlation of anti-IFN MFI with severity of pre-vaccination SARS-CoV-2 infections. D: No correlation of anti-IFN MFI with frequency of breakthrough SARS-CoV-2 infections. p = NS for all comparisons performed using Kruskal-Wallis ANOVA.