SARS-CoV-2 Vaccine Effectiveness for Immunosuppressed Populations

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Background

Immunosuppressed patient populations have been receiving vaccinations for decades with unclear documentation as to their efficacy from an immunological perspective. Due to the pressures of the SARS-CoV-2 pandemic, research has become prioritized in examining this topic. The Falk Lab specifically investigated the effectiveness of the SARS-CoV-2 vaccine in patients with either kidney disease of rheumatologic disease taking immunosuppressant medications. Patient samples were collected every 3 months following each vaccination and then analyzed to compare the immune responses of patient cells and healthy control populations. This study primarily focused on T cell responses to the receptor binding domain (RBD) of the Spike protein.

Hypothesis

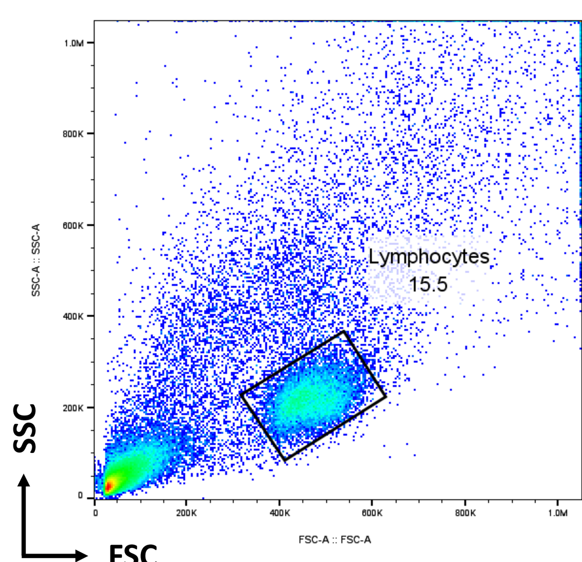
Immunosuppressed patients have lower protection against SARS-CoV-2 than healthy populations.

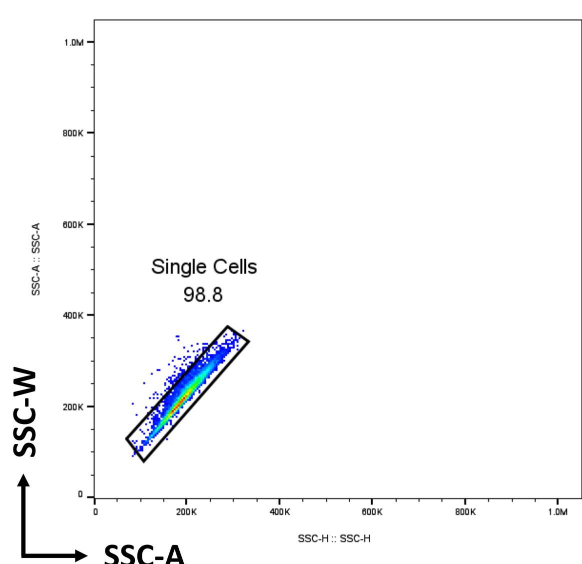
Methods

Phase 1 of the study used **ELISpot** assays to determine if antigen stimulation would elicit an immune response in the form of IFN-gamma release. Peripheral blood mononuclear cells (PBMCs) were stimulated under 3 different conditions or unstimulated as control. Conditions included: and anti-CD3 mAb for assay positive control, a CEFX peptide mix to determine the immune fitness of a patient (CEFX contains peptides from common vaccines and microbes the general population is exposed to) and a second well containing an RBD peptide mix was used to simulate a SARS-CoV-2 exposure. PBMCs were incubated overnight. The plate was then washed, a secondary antibody (anti-INFg) added for incubation, washed again, and then spots were developed by addition of substrate.

Phase 2 used **flow cytometry** to examine both CD4+ and CD8+ T cell populations and their cytokine profile in response to the same peptide mixes. in patient's PBMCs. Our panel examined three different types of cytokine profiles: Th1 (IL-2, TNF-alpha, IFN-gamma), Th17 (IL17-A, IL-17F, IL-22, and Tfh (IL-10, IL-21). PBMCs were incubated with 3 different conditions or unstimulated as a control. Conditions included: PMA/ionomycin as positive control, CEFX peptide mix, and RBD peptide mix. PBMCs were incubated for 4 hrs with the addition of GolgiPlug to retain cytokines in the cytoplasm. Cells were then washed, blocked, and stained with surface antibodies (CD3 and CD4). Cells were washed again, permeabilized and fixed, and then stained for intracellular cytokine antibodies. Cells were then analyzed on the Attune NxT and data analyzed by FlowJo.

Flow Cytometry Gating Model





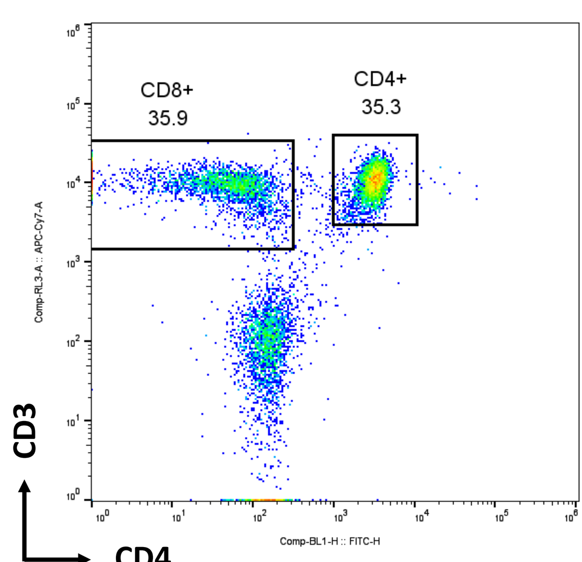


Figure 1. Display of flow cytometry data processed from a healthy control model. We gated first for lymphocytes based on forward and side scatter, then for singlet data points, and lastly gated for cytotoxic T cell populations (CD8+) and helper T cell populations (CD4+). From these last two T cell gates, fluorescence was measured for PE+, AF647+, and PacBlue+ in both populations, respectively as all cytokine antibodies were labeled with these fluorophores.

Results

ELISpot Plate Model

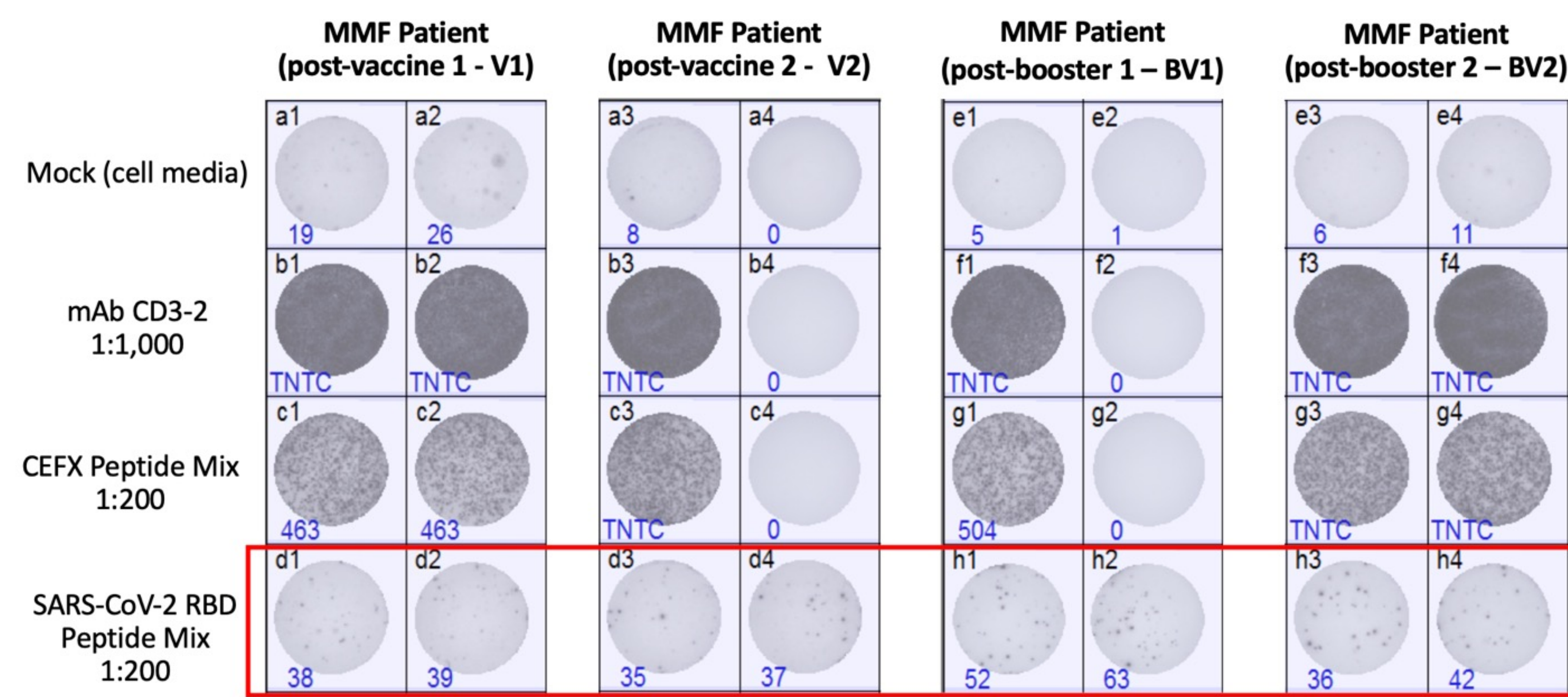
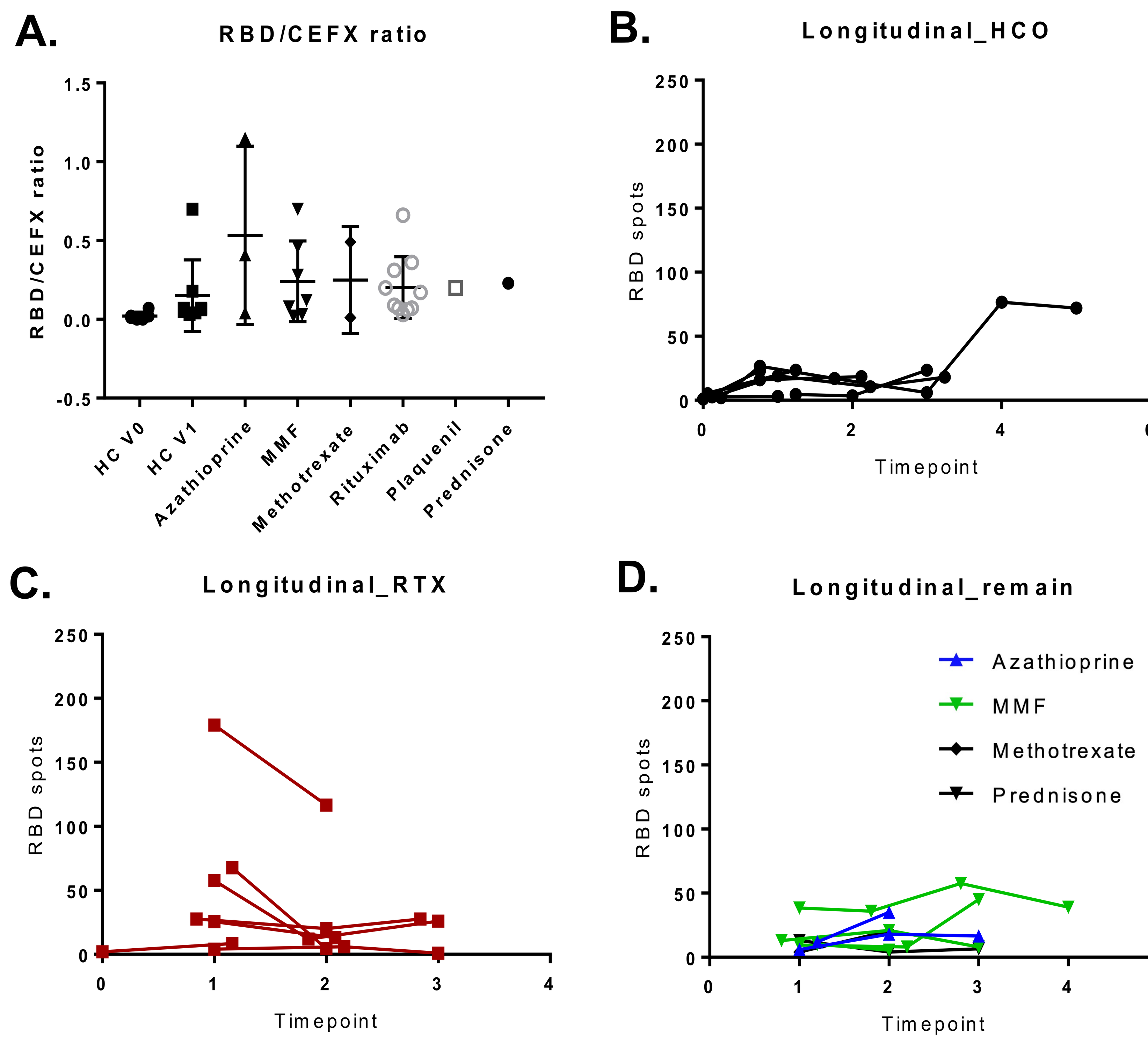


Figure 2. Model ELISpot plate displaying data from patient taking mycophenolate mofetil (MMF) from V1 to BV2. Four wells were completed per patient time point with each possessing a duplicate. Wells A and E were mock wells containing cell media and isolated PBMCs. Wells B and F were a positive control (anti-CD3) and would elicit an excessive immune response. Wells C and G were the first experimental wells containing the CEFX peptide mix and wells D and H were the second experimental wells containing the RBD peptide mix.

ELISpot Aggregated Data



Time Point Legend:
0=V0, 1=V1, 2=V2, 3=BV0/BV1 some, 4=BV1/BV2 some, 5=BV2

Figure 3. Aggregate data of ELISpot experiments. (A) Ratio of RBD spots to CEFX spots for all treatment groups at timepoint V1. (B) Longitudinal data for RBD responses from all healthy controls. (C) Longitudinal data for RBD responses from all patients who received rituximab. (D) Longitudinal data for RBD responses from patients receiving azathioprine, MMF, methotrexate, or prednisone.

Comparison of IL-2 secretion between Healthy Control and Immunosuppressed Patient

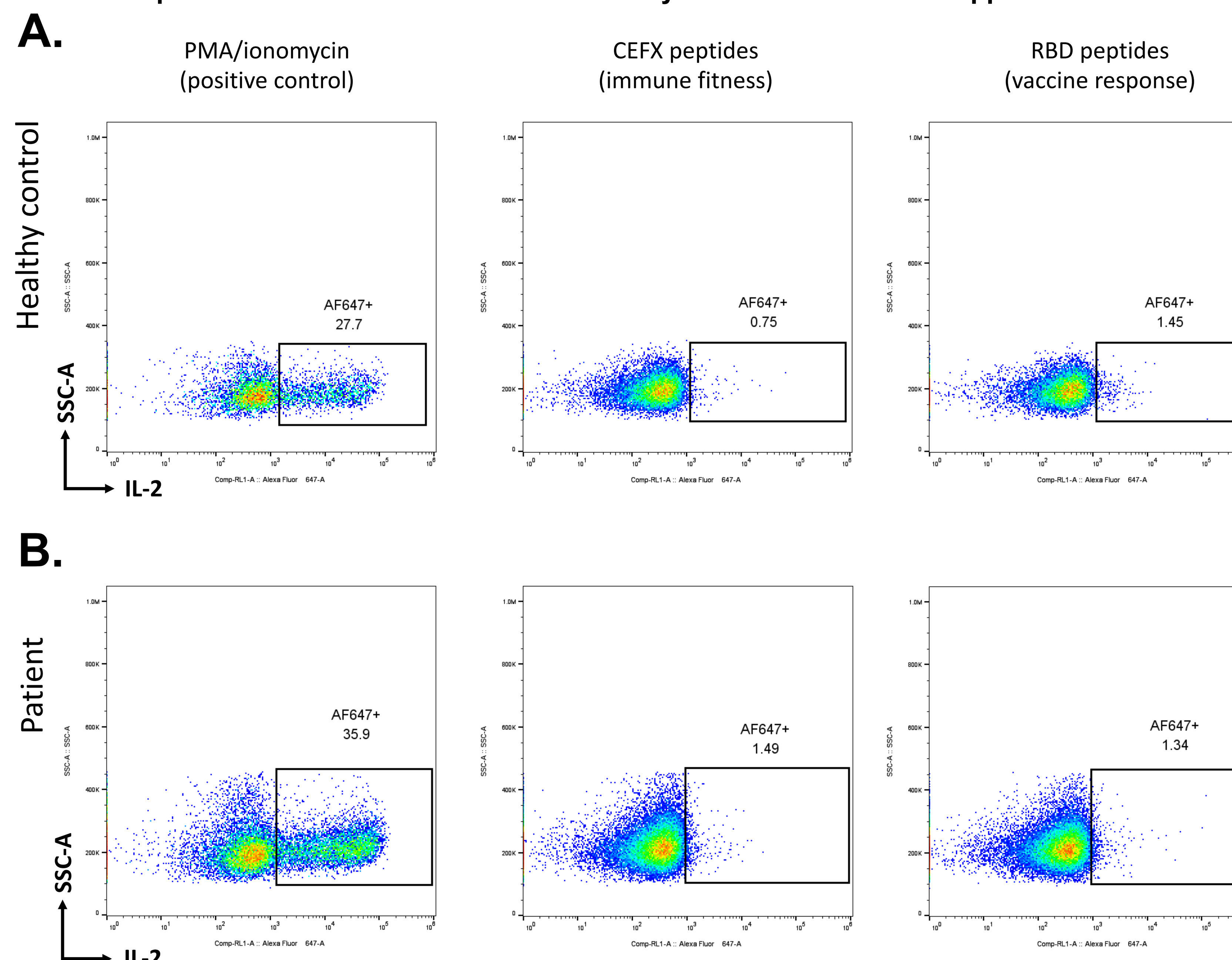


Figure 4. Comparisons of IL-2 secretion with different stimuli between healthy control and a patient (A) Healthy control patient at time point V2. (B) Immunosuppressed patient taking MMF at time point BV1.

Results

Differential cytokine responses between patient and immunosuppressed patient

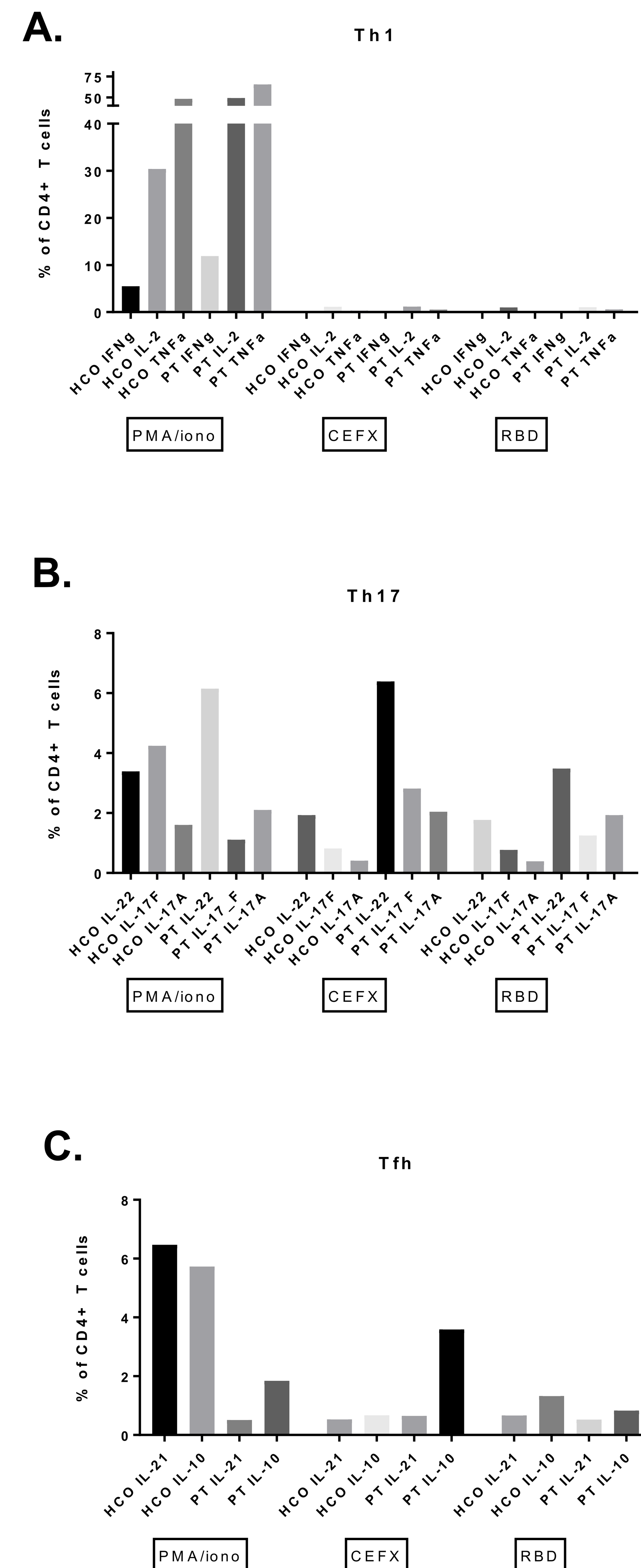


Figure 5. Cytokine responses from a healthy control and patient treated with rituximab at timepoint V1. Cytokines based on total CD4+ T cells after stimulation with either PMA/ionomycin, CEFX peptides, or RBD peptides.. (A) Th1 cytokine family including INF-gamma, IL-2, and TNF-alpha. (B) Th17 cytokine family including IL-22, IL-17F, and IL-17A. (C) Tfh cytokine family including IL-21 and IL-10.

Summary

- Patient RBD/CEFX ratios possess wide confidence intervals compared to their HC counterparts.
- Patients taking immunosuppressant drugs had variable SARS-CoV-2 protection (T cell based) as vaccine number increased compared to the HC population.
- T cell response to RBD peptides in ELISpot assay were similar between patients and healthy controls indicating that T cell immunity may be less influenced by medications.
- SARS-CoV-2 vaccination induced similar Th1 and Tfh cytokine responses between a patient and healthy control.
- However, vaccination did induce higher Th17 responses in the patient which could be a link to risk of relapse.

Future Directions

- Correlate T cell responses with neutralizing antibody titers.
- Continue cytokine analysis on 11 additional patients and 3 additional healthy controls.
- Correlate cytokine responses to risk of contracting COVID-19 and risk of disease relapse.