# AUTOIMMUNITY IN SARS-COV-2 CONVALESCENT INDIVIDUALS

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## Background and aims

In healthy individuals, polyreactive B cells constitute ~20% of adult blood B cells. They act as the first line of immune defense by binding microbial antigens and play a role in the clearance of dead cells. However, dysregulated polyreactive B cells producing excessive autoantibodies binding to self-antigens can lead to autoimmune diseases.

SARS-CoV-2 infection has been linked with increased autoantibody reactivity with self-antigens. Long COVID symptoms can also be associated with elevated autoantibodies. However, data on SARS-CoV-2 caused autoimmunity remain inconsistent. Some chronic infections, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), *Helicobacter pylori* (HP), and *Toxoplasma gondii* (TG) have also been linked with various autoimmune conditions.

In immunologically naïve individuals, polyreactive antibodies can bind to antigens of SARS-CoV-2 and other pathogens causing non-specific responses and potentially false-positive results in immunoassay tests. Individuals with chronic inflammatory conditions may have higher levels of polyreactive antibodies recognizing microbial antigens.

This study had the following objectives and specific aims:

1. Explore effects of SARS-CoV-2 and selected common chronic infections on autoimmunity:
   - Compare autoantibody responses in convalescent or seropositive individuals vs. seronegative controls.
   - Explore associations between the intensity of serum IgG response to SARS-CoV-2 and other pathogens and autoantibody responses in convalescent and seropositive individuals.
2. Explore effects of autoimmunity on non-specific antibody reactivity with SARS-CoV-2 and other microbial antigens in immunologically naïve and seronegative individuals.

## Methods

**Study design:**

- Cross-sectional serological study in adults (n = 302):
  - 179 samples from convalescent individuals (1st infection) collected 15 to 60 days post-COVID-19 diagnosis in 2020 – early 2021 were acquired from US biobanks.
  - 123 pre-pandemic control samples from participants of previous EPA studies.

**Laboratory tests:**

- In-house multiplex Luminex immunoassay for IgG responses to SARS-CoV-2 recombinant antigens:
  - Recombinant Spike (S) and Receptor Binding Domain (RBD) antigens expressed at EPA, Spike subunit 2 (S2) antigen (ProSci), and nucleocapsid (NC) proteins from AcroBiosystems (AB) and R&D Systems (RD) as described previously [1].
- In-house Luminex assay for total IgG [2].
- Luminex Human Autoimmune Antibody assay (EMD Millipore) for IgG responses to 18 self-antigens (Beta2-GP1,C1q, CENP-B, Jo-1, Ku, MPO, Mi-2, PCNA, PL-12, Pro3, RNP, RNP-Sm, Rib-P, SSA-Ro52, SSA-Ro60, SSB-La, Sm, Scl-70).
- Commercially available ELISA tests for IgG responses to CMV, EBV, *H. pylori*, and *T. gondii*.

**Statistical analysis:**

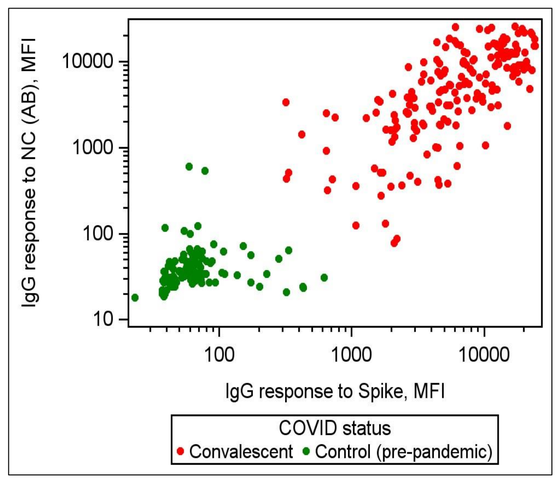
- Total autoantibody reactivity estimated as a sum of IgG responses to self-antigens in Median Fluorescence Intensity (MFI) units.
- All antibody data log-transformed for statistical analysis.
- For correlation analysis, data were stratified as seropositive and seronegative for each infection.
- Regression models for autoimmunity vs. responses to SARS-CoV-2 antigens in convalescent individuals were adjusted for total IgG because it is correlated with both autoantibody and responses to SARS-CoV-2. Results were expressed as fold-changes in autoantibody reactivity per inter-decile range (IDR) increase in anti-SARS-CoV-2 antibody.

## Results

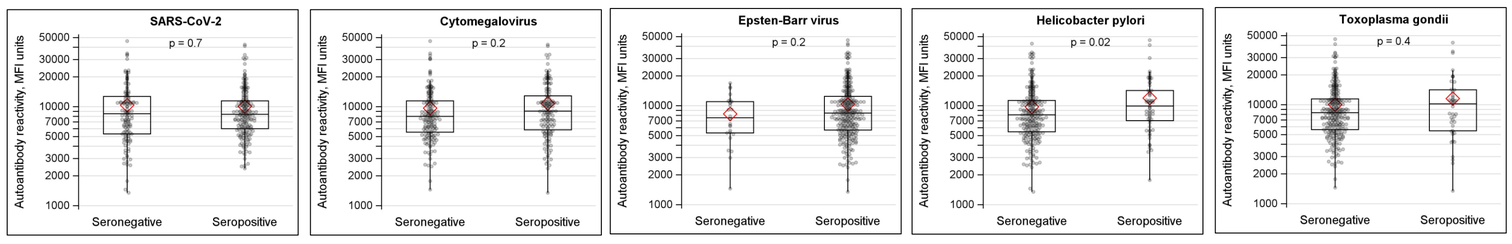
### Table 1. Seropositivity rates of chronic infections

| Categories of samples | Entire study population | Pre-pandemic | COVID-19 convalescent | P value |
|---|---|---|---|---|
| All | 302 (100%) | 123 (100%) | 179 (100%) | NA |
| CMV seropositive | 144 (48%) | 71 (58%) | 73 (41%) | 0.004 |
| EBV seropositive | 276 (91%) | 113 (92%) | 163 (91%) | 0.81 |
| *H. pylori* seropositive | 61 (20%) | 31 (25%) | 30 (17%) | 0.07 |
| *T. gondii* seropositive | 43 (14%) | 22 (18%) | 21 (12%) | 0.13 |

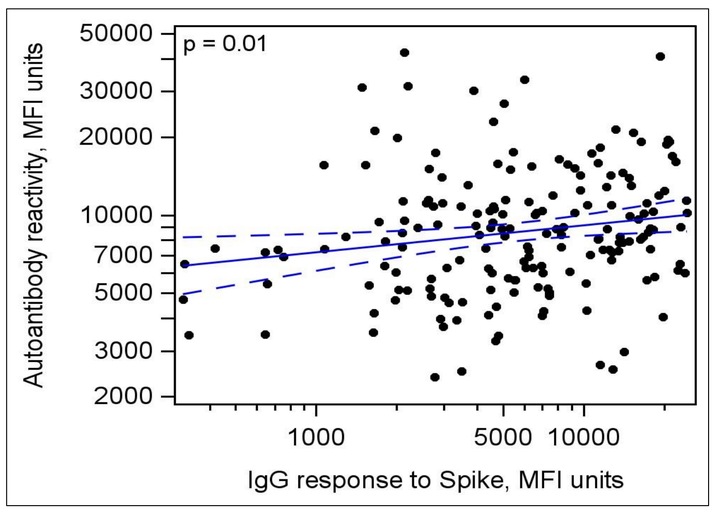
### Fig. 1. IgG responses to SARS-CoV-2 Spike and NC antigens




### Fig. 2. Autoantibody reactivity in seronegative vs. convalescent or seropositive individuals



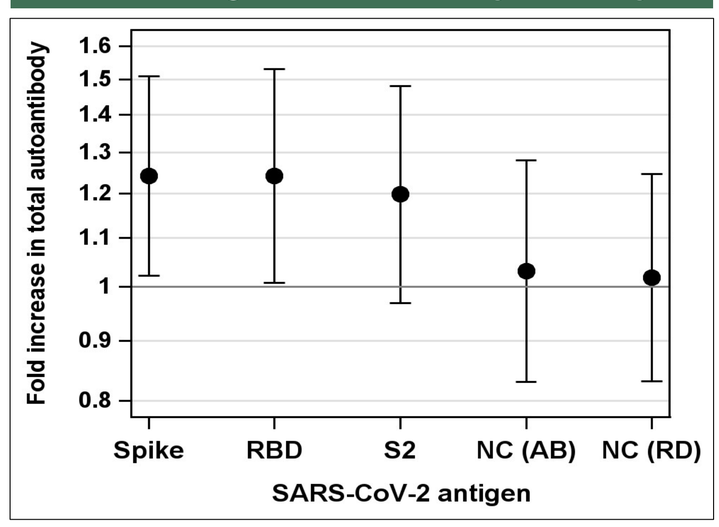

### Fig. 3. Univariate regression of autoreactive IgG on anti-Spike IgG post-COVID




### Table 2. Pearson correlations of IgG responses to microbial antigens with autoreactive IgG

| Antigen | Pre-pandemic/ seronegative | | | Convalescent/ seropositive | | |
|---|---|---|---|---|---|---|
| | n | r | p | n | r | p |
| SARS-CoV-2 Spike | 123 | 0.06 | 0.5 | 179 | 0.18 | 0.016 |
| SARS-CoV-2 RBD | | 0.02 | 0.9 | | 0.16 | 0.03 |
| SARS-CoV-2 Spike-2 | | 0.13 | 0.2 | | 0.13 | 0.08 |
| SARS-CoV-2 NC (AB) | | 0.38 | 0.00002 | | 0.02 | 0.8 |
| SARS-CoV-2 NC (RD) | | 0.37 | 0.00002 | | 0.02 | 0.7 |
| CMV antigen, ratio to cut-off | 158 | 0.23 | 0.003 | 144 | 0.12 | 0.2 |
| EBV antigen, ratio of cut-off | 26 | 0.33 | 0.1 | 276 | 0.04 | 0.5 |
| *H. pylori antigen,* ratio to cut-off | 241 | 0.25 | 0.0001 | 61 | 0.15 | 0.3 |
| *T. gondii* antigen, ratio to cut-off | 259 | 0.22 | 0.0003 | 43 | -0.02 | 0.9 |

### Fig. 4. Effects of an IDR increase in anti-SARS-CoV-2 IgG on autoantibody reactivity




- Seroprevalence rates of chronic infections (Table 1) were consistent with the general US population.
- A combination of serum IgG responses to S and NC antigens of SARS-CoV-2 differentiated seronegative pre-pandemic controls and convalescent (seropositive) individuals (Fig. 1) as described previously [1].
- *H. pylori* seropositivity was associated with greater autoantibody reactivity (Fig. 2); the effects of other infections were not significant.
- In the convalescent group, IgG responses to S and RBD (Table 2 and Fig. 3) were correlated with autoantibodies.
- In seronegative individuals, autoantibodies were correlated with IgG responses to SARS-CoV-2 NC antigen and antigens of chronic infections (Table 2).
- IgG responses to S and RBD antigens remained significantly associated with increased autoantibody reactivity after adjusting for total IgG (Fig. 4).

## Discussion and conclusions

- Antibody responses to the SARS-CoV-2 spike protein and its domains were associated with autoimmunity in convalescent individuals.
- Potential interaction effects of antibody responses to SARS-CoV-2 antigens in convalescent individuals and seropositivity to chronic infections should be explored in a bigger dataset.
- Levels of autoreactive antibodies in SARS-CoV-2 convalescent and pre-pandemic groups did not differ significantly. As sociodemographic data on convalescent blood donors were limited to age and sex, confounding by unmeasured factors could mask the effects of COVID-19.
- Evidence of autoantibodies enhancing non-specific antibody responses to the nucleocapsid protein of SARS-CoV-2 in pre-pandemic individuals as well as antigens of other pathogens in seronegative individuals.
- Further research on the effects of repeated SARS-CoV-2 infections and vaccinations on autoantibodies is warranted.

## Literature

1. Egorov et al. (2021). A multiplex noninvasive salivary antibody assay for SARS-CoV-2 infection and its application in a population-based survey by mail. Microbiology Spectrum. 9(2):e00693-21.
2. Griffin et al. (2011). Development of a multiplex microsphere immunoassay for the quantitation of salivary antibody responses to selected waterborne pathogens. J. Immunol. Methods. 364(1-2):83-93.



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