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Autoantibody signatures in SARS-CoV-2 infected and vaccinated individuals

Kirsten Heiss[1], Yuvaraj Mahendran[1], Fiordiligie Casilag[1], Renate Sekul[1], and Volker Stadler[1]

[1]PEPperPRINT GmbH, Heidelberg, Germany

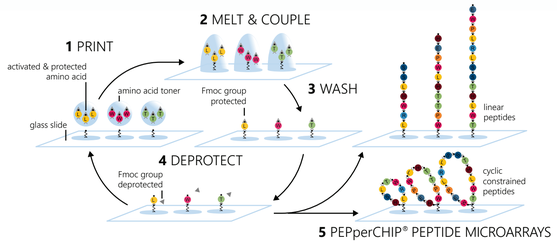
Presented at the 31st Annual Meeting of the Society for Virology (GfV) 2022

KEY FINDINGS

- numerous IgG and/or IgA-specific autoantibody reactivities in severe COVID-19
- several epitopes can be assigned to nuclear autoantigens, others may be summarized as tissue-associated autoantigens
- few antibody responses to the selected 5-14mer peptides shared between SARS-CoV-2 and human proteins in infected individuals
- no antibody responses against most of the selected 5-14mer peptides shared between SARS-CoV-2 spike and human proteins detected after COVID-19 vaccination
- infection versus vaccination: cross-reactive antibodies against peptides shared between SARS-CoV-2 spike and human proteins were detected more frequently after infection

PEPperCHIP® Peptide Microarray Platform Technology

High-density PEPperCHIP® peptide microarrays are generated by digital laser printing on standard glass slides using a proprietary laser printer with 24 individual amino acid toners. For array production, amino acid toners are simultaneously printed with high precision on their respective positions on the glass slides.



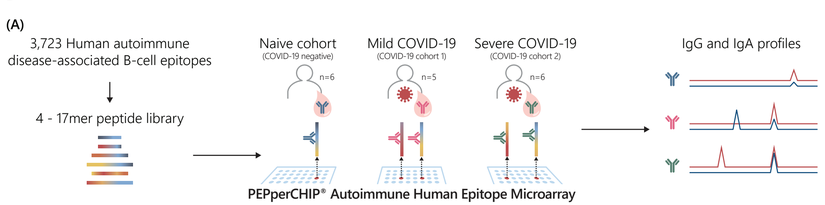
Benefits

- high spot density/high content (1,200 peptides/cm²)
- digital printing flexibility
- high peptide quality with routine double couplings
- very low material consumption

Fig. 1. The PEPperCHIP® Peptide Microarray printing process.

Screening of COVID-19 patient sera against human autoimmune disease-associated linear epitopes

Antibody responses are crucial to combat SARS-CoV-2 infection. However, there is considerable evidence indicating that dysregulated humoral immunity might contribute to the immunopathology of COVID-19. Intriguingly, autoimmune diseases and COVID-19 show common immunological features. Several studies reported the detection of autoantibodies in COVID-19 patients significantly correlating with the disease outcome. Here, we examined epitope-specific autoantibody profiles in patients with a mild versus severe COVID-19 disease progression.



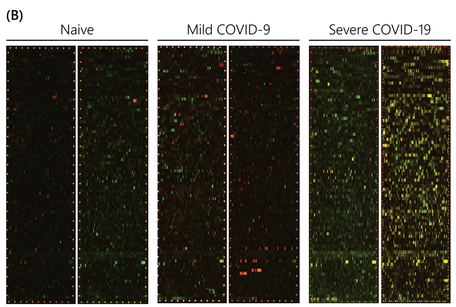


Fig. 2. Autoantibody screening of COVID-19 patient sera. **(A) Study outline.** Sera from COVID-19 patients and SARS-CoV-2 negative subjects (naive cohort) were screened on PEPperCHIP® Autoimmune Human Epitope Microarrays covering 3,723 human B-cell epitopes associated with autoimmune diseases including epitopes with post translational modifications described in autoimmunity. IgG and IgA responses were analyzed simultaneously. The severe cohort comprised of patients with acute respiratory distress syndrome (ARDS). **(B) Strong autoantibody reactivity in severe COVID-19.** Representative staining images of COVID-19 patient sera (mild and severe disease outcome) and SARS-CoV-2 negative subjects (healthy control). PEPperCHIP® Autoimmune Human Epitope Microarrays were incubated with respective sera (1:100) overnight at 4°C. Detection of antibody binding was done using anti-human IgG DL680 (red) and anti-human IgA DL800 (green). Polio peptide spots served as control frame.

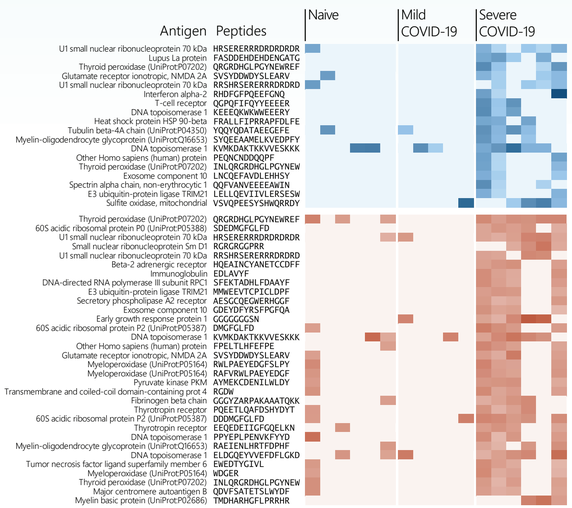
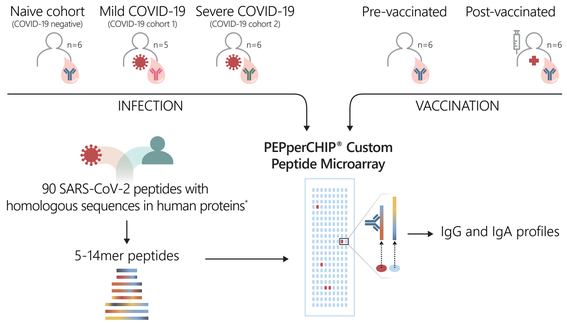


Fig. 3. Most differentially recognized epitopes that show a strong reactivity in severe COVID-19 patients compared to mild COVID-19 and healthy subjects. Heat maps show VSN normalized fluorescence intensities for each peptide (vertical) and individual patient sera (horizontal). The color coding correspond to low (light) to high signal intensities (IgG: blue; IgA: orange). ***Several epitopes can be assigned to nuclear autoantigens, others may be summarized as tissue-associated autoantigens (e.g. from thyroid glands, CNS).***

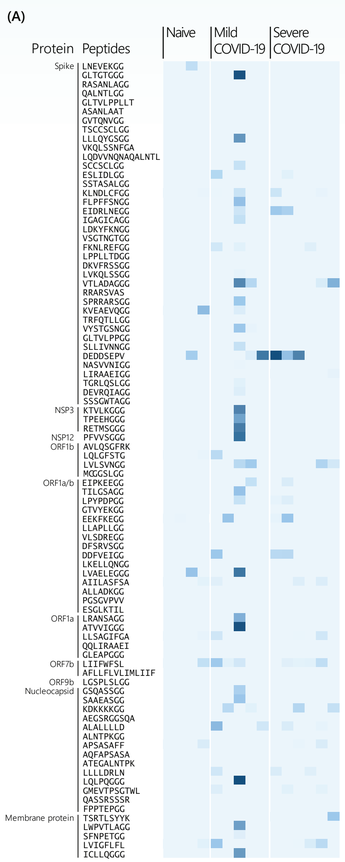
Antibody responses against epitopes shared between SARS-CoV-2 and human proteins in infection and vaccination

Molecular mimicry is one mechanism described for viral-induced autoimmunity. It has been suggested that the immune response induced to fight against SARS-CoV-2 may cross-react with human proteins sharing epitope sequences with viral antigens. In this context, we investigated the epitope recognition in infected and vaccinated individuals, respectively.



*Lucchese et al., 2020; Gamazza et al., 2020; Kanduc et al., 2020; Ehrenfeld et al., 2020

Fig. 4. Study outline for assessing antibody responses against epitopes shared between SARS-CoV-2 and human proteins in COVID-19 infection and vaccination. Antibody responses against described cross-reactive epitopes present in SARS-CoV-2 & human proteins were investigated. For this, a customized peptide microarray was designed displaying 5-14mer peptides described in the literature as shared sequences between SARS-CoV-2 and human proteins. In total, the peptide microarray display 90 peptide sequences shared between SARS-CoV-2 and human proteins. Dual isotype read-out was performed (anti-human IgG and anti-human IgA).



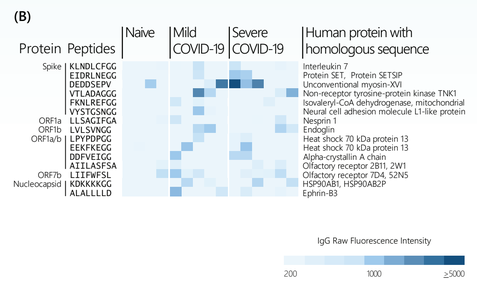


Fig. 5. IgG responses against shared epitopes in SARS-CoV-2 and human proteins in COVID-19 patient sera. Shown is the IgG reactivity pattern for (A) all epitopes printed on the peptide microarray and **(B)** the most frequently recognized epitopes in infected individuals. Data are presented as heat maps showing the raw fluorescence intensities for each epitope and individual patient serum. The color coding correspond to low (light blue) to high signal intensities (dark blue). ***Few antibody responses to the selected 5-14mer peptides shared between SARS-CoV-2 and human proteins.***

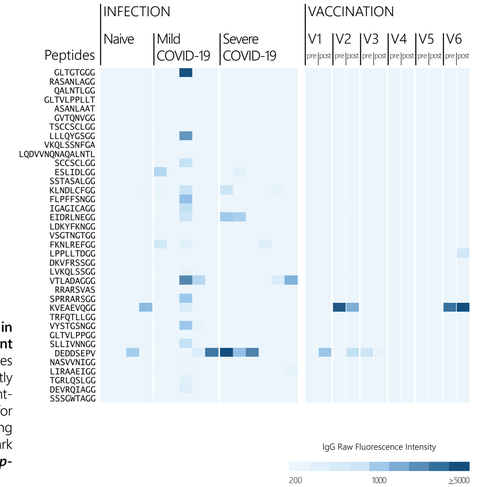


Fig. 6. Side-by-side comparison of IgG responses against shared epitopes in SARS-CoV-2 spike and human proteins after COVID-19 infection or vaccination. Shown is the IgG reactivity pattern for shared epitopes between the vaccine candidate spike and human proteins in the context of COVID-19 vaccination (pre and post vaccination sera) and COVID-19 infection. Data are presented as heat maps showing the raw fluorescence intensities for each epitope and individual patient serum. The color coding correspond to low (light blue) to high signal intensities (dark blue). ***In vaccinated individuals, no antibody responses against most of the epitopes were detected. Comparing responses in the context of vaccination and infection, more frequent cross-reactivity in infected than in vaccinated subjects were observed.***

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PEPperPRINT GmbH
Tullastr. 2
69126 Heidelberg
Germany

www.pepperprint.com
info@pepperprint.com
+49-6221-7264489

pepperprint-gmbh @pepperchip PEPperPRINTGmbH PEPperPRINT