Epitope signatures in COVID-19 patients with mild and severe disease outcome

Kirsten Heiss[1], Tatjana Schwarz[2], Yuvaraj Mahendran[1], Fiordiligie Casilag[1], Florian Kurth[3], Leif Sander[3], Clemens Wendtner[4], Marcel A. Müller[2], Renate Sekul[1], Christian Drosten[2,5], Victor M. Corman[2,5], and Volker Stadler[1]

[1]PEPperPRINT GmbH, Heidelberg, Germany; [2]Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Institute of Virology, Berlin, Germany; [3]Department of Infectious Diseases and Respiratory Medicine, Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin; [4]Klinikum München-Schwabing, Munich, Germany; [5]German Centre for Infection Research, associated partner Charité, Berlin, Germany

The worldwide ongoing transmission of COVID-19 is a major global health concern. The causative agent of this acute respiratory disease is a newly emerged coronavirus named SARS-CoV-2. The virus originated from China in late 2019 and rapidly spread across the globe. The course of the disease ranges from non-symptomatic to mild symptoms such as fever and cough to severe cases with pneumonia, acute respiratory distress and potentially death.

Humoral responses are an important defense mechanism in viral infections. The investigation of antigens and/or epitopes recognized by SARS-CoV-2-specific antibodies is not only crucial for the development of intervention strategies, but also for epidemiological studies, disease prognosis and the identification of novel diagnostic markers. With the aim to decipher SARS-CoV-2-specific humoral immune responses on the epitope level, we screened sera from COVID-19 patients using high-density peptide microarrays covering the entire proteome of SARS-CoV-2 as 15 amino acid peptides with an overlap of 13 amino acids. The high peptide-to-peptide overlap of our SARS-CoV-2 proteome array allowed a high-resolution epitope analysis giving a detailed picture of antibody binding patterns.

In the present study, we describe IgG and IgA-specific immune epitope signatures across the SARS-CoV-2 proteome particularly in the ORF1a/b, Spike protein, ORF3a, and ORF8 regions potentially applicable for early and late COVID-19 disease detection and as biomarkers able to discriminate severe from mild disease courses.

KEY FINDINGS

The determination of SARS-CoV-2 antibody signatures on epitope rather than protein level yielded a more comprehensive picture of antibody responses. ***Our longitudinal study revealed a potential epitope marker in ORF1a/b (NSP15-derived peptide 1) for early and late SARS-CoV-2 infection.*** In addition, ***we have identified several potential IgG and/or IgA-specific epitope biomarkers capable of discriminating severe from mild disease courses***.

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.

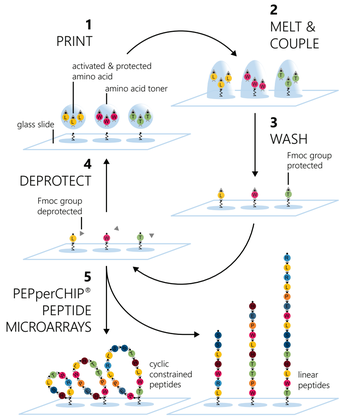


Fig.1. The PEPperCHIP® peptide microarray printing process.

The benefits of this technique are an *unique flexibility* in terms of peptide content, *fast production times*, a *high spot density* with up to 11,000 features per chip, and *low material consumption* enabling the generation of customized peptide array at reasonable costs.

Antibody-peptide interactions are analyzed by immuno-type assays in a high-throughput fashion on peptide microarrays. A variety of microarray formats are available to accomodate different research applications.

	Array copies	Peptide range	Recommended applications
Discovery format	1	< 75,460 per chip (up to ~100 proteins)	Epitope seromarker and target binder discovery
Standard format	1-3	< 11,258 per array copy	Epitope mapping; Infectious disease antigen screening
Mapping format	4-5	< 2,040 per array copy	Multi-sample or multi-replicate analyses
Multiplex format	16	< 273 per array copy	Assay development or hit validation studies

PEPperCHIP® SARS-CoV-2 PROTEOME MICROARRAY

Enabled by PEPperPRINT's unique laser printing technology, a peptide microrray containing the entire proteome of the SARS-CoV-2 (Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1 (GenBank ID: MN908947.3)) was quickly designed and produced.

The resulting PEPperCHIP® SARS-CoV-2 Proteome Microarray contains 4,883 individual peptides printed in duplicates, consisting of 15-mer overlappng peptides with a peptide-to-peptide overlap of 13 amino acids, and covers the protein sequences of ORF1/ab, Spike protein, ORF3a, Envelope protein, Membrane glycoprotein, ORF6, ORF7a, ORF8, Nucleocapsid phosphoprotein and ORF10.

STUDY OUTLINE

We followed a two-branched approach investigating antibody profiles (i) longitudinally, in COVID-19 patients (n=7) with mild disease symptoms and (ii) in patients with mild (n=9) versus severe (n=7) COVID-19 disease. SARS-CoV-2-negative subjects (n=7) served as healthy/naive control group.

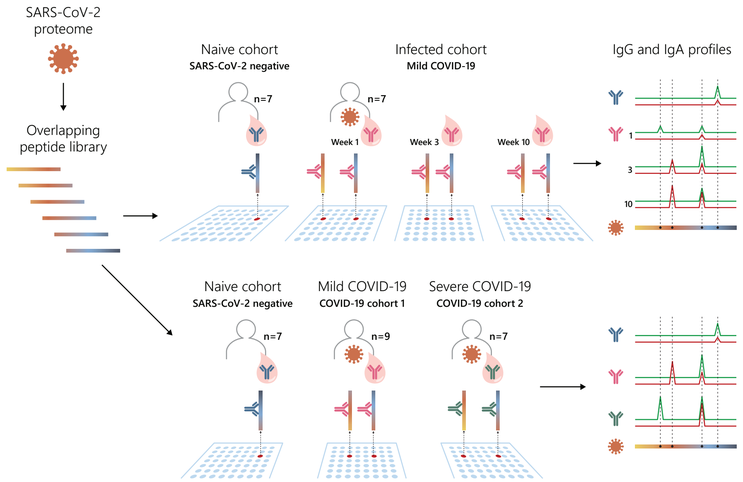


Fig.2. SARS-CoV-2 proteome-wide IgG and IgA epitope mapping. Sera from confirmed COVID-19 patients and SARS-CoV-2-naive individuals were incubated on PEPperCHIP® SARS-CoV-2 Proteome Microarrays. Serum antibody binding was visualized using respective fluorescence labeled secondary antibodies (anti-human IgG and anti-human IgA). Image acquisition and data processing resulted in epitope-specific antibody profiles for SARS-CoV-2.

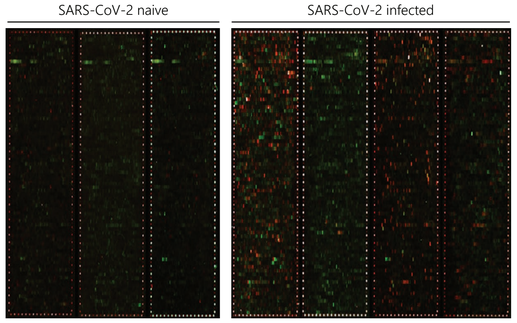


Fig.3. Heterogeneous antibody responses in COVID-19 patients across the SARS-CoV-2 proteome. Representative staining images of SARS-CoV-2-naive and SARS-CoV-2-positive individuals. The PEPperCHIP® SARS-CoV-2 Proteome Microarrays were incubated with respective sera overnight at 4°C. Antibody detection was done using anti-human IgG DyLight680 (red) and anti-human IgA DyLight800 (green). Polio and hemagglutinin peptide spots served as control frame.

LONGITUDINAL ASSESSMENT OF SARS-CoV-2 ANTIBODY RESPONSES ACROSS THE PROTEOME

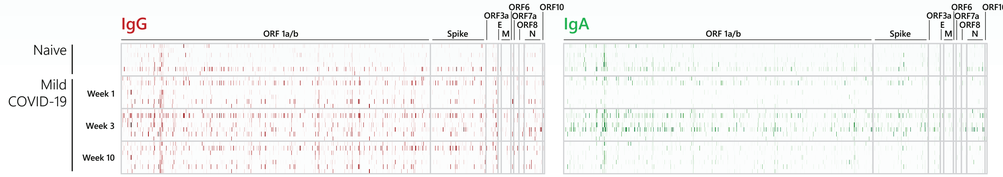


Fig.4. Epitope-specific antibody profiles. Heat maps show raw fluorescence intensities for each peptide detected for each individual in the corresponding cohort.

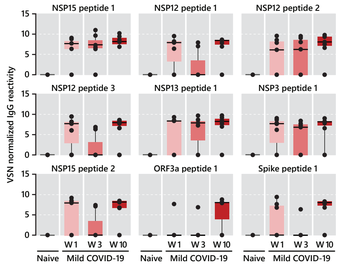


Fig.5. Individual IgG antibody responses to nine peptides significantly identified in COVID-19 patients. Significant differences in epitope recognition was determined by LIMMA analysis comparing COVID-19 patients to naive controls. The analysis identified ***NSP15-derived peptide 1*** as an epitope marker of early and late COVID-19 disease.

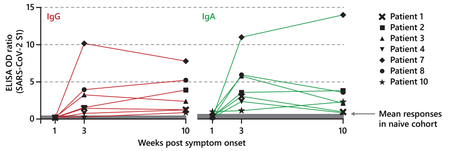


Fig.6. Levels of IgG and IgA antibodies recognizing SARS-CoV-2 Spike S1 domain in COVID-19 patient sera as determined by ELISA.

EPITOPE SIGNATURES FOR MILD AND SEVERE COVID-19 DISEASE

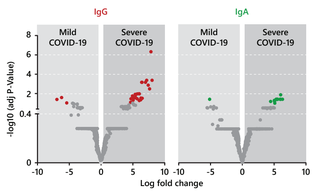
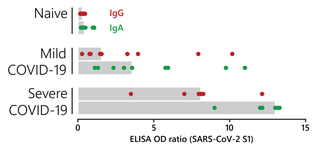


Fig.7. Linear B cell epitopes associated with mild and severe COVID-19. The LIMMA package was used to determine significant differences in epitope recognition in mild vs. severe COVID-19 cases. In each volcano plot, red or green colored data points indicate significantly recognized epitopes (FDR < 0.1).



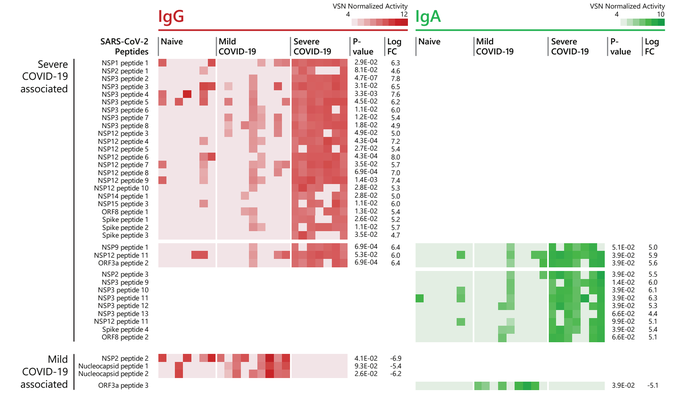


Fig.8. IgG and IgA reactivity patterns showing inidvidual antibody responses to peptides significantly associated with mild and severe COVID-19 disease. Heat maps show VSN normalized values. LogFC = log fold change.

Fig.9 Levels of IgG and IgA antibodies recognizing SARS-CoV-2 Spike S1 domain in COVID-19 patient sera with mild and severe symptoms as determined by ELISA. Bars indicate median values.

Interested in working with our PEPperCHIP® SARS-CoV-2 Proteome Microarray?
Read about its applications, and explore our related peptide microarrays for coronavirus and infectious disease reasearch.
Visit ***bit.ly/covid19chip*** to learn more, or get in touch with Dr. Kirsten Heiss at ***kirsten.heiss@pepperprint.com***.

PEPperPRINT GmbH
Tullastr. 2
69126 Heidelberg
Germany

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

pepperprint-gmbh @pepperchip PEPperPRINTGmbH PEPperPRINT