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Epitope Discovery & Validation of Vaccination-Induced Antibody Responses with PEPperCHIP® Peptide Microarrays and a Bead-Based Multiplex Assay

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INTRODUCTION

Analyzing vaccine-induced immune responses is important to determine the efficacy of a given vaccine. With respect to humoral responses to COVID-19 vaccination, both the induction and the titers of different classes of virus-specific antibodies, especially neutralizing antibodies, are of interest. High-density peptide microarrays are a powerful tool to simultaneously screen tens of thousands of peptides against serum antibodies in a high-throughput context. Antibody responses to epitopes can rapidly be analyzed yielding high and low immunogenic epitopes with amino acid resolution. Multiplexed bead-based technologies emerged as a valuable approach for the simultaneous screening of up to 500 targets immobilized on color-coded beads. The beads are ideally suited for analyzing serological antibody responses to immobilized antigens.

OBJECTIVE & METHODS

Here we describe an experimental workflow for exploring humoral responses on epitope level using peptide microarrays and subsequent validation of antibody reactivities against identified epitopes/peptides applying a multiplexed bead-based technology (Figure 1).

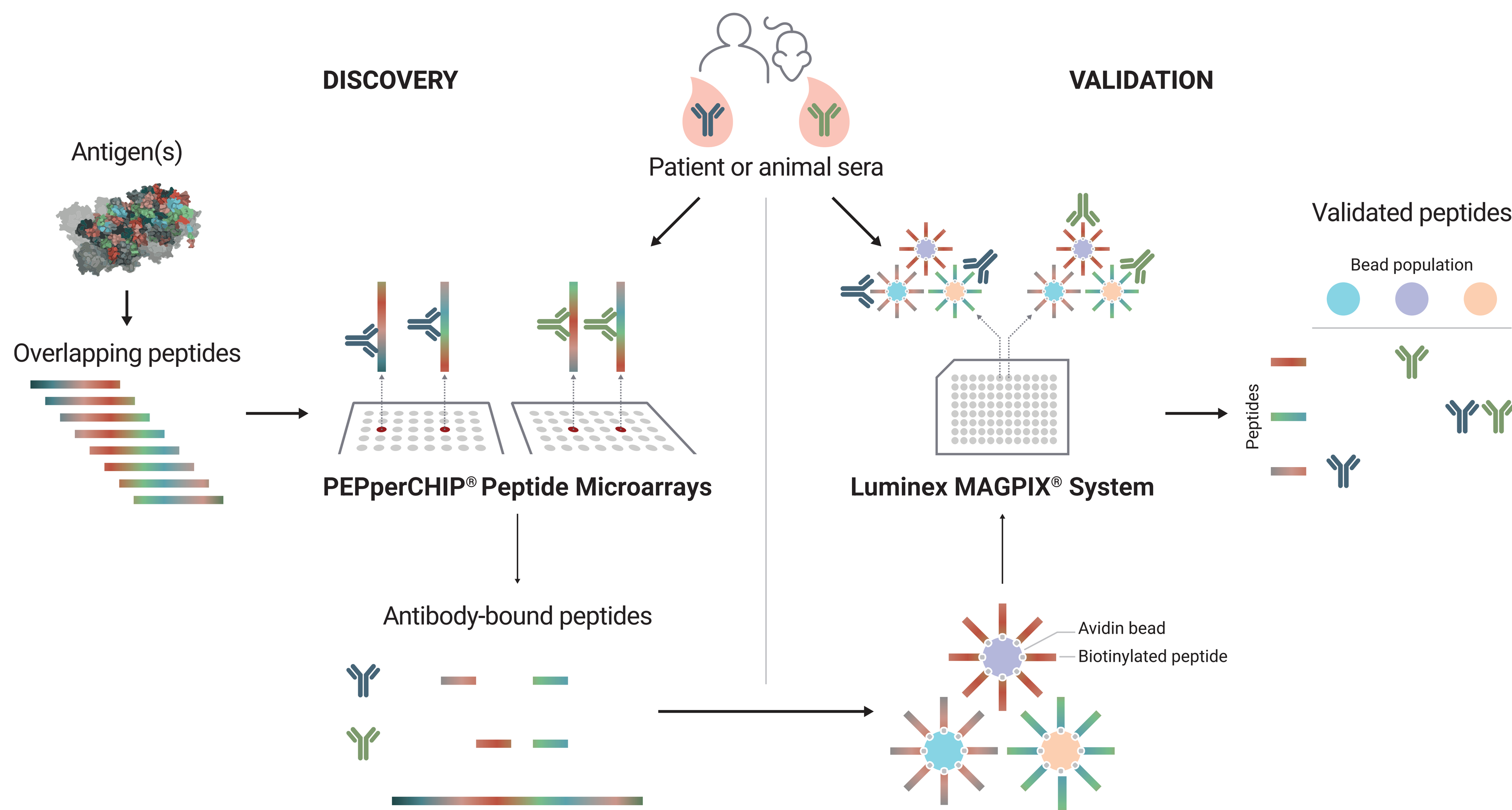

Figure 1. Workflow for epitope discovery and validation. Serum samples are initially screened with PEPperCHIP® Peptide Microarrays displaying peptide libraries of up to 70,000 peptides. Identified hit candidates are further validated applying a multiplexed bead-based immunoassay.

RESULTS

We mapped the humoral responses of COVID-19 vaccine recipients via screening sera before and after vaccination on PEPperCHIP® Pan-Corona Spike Protein Microarrays covering the spike proteins of seven coronaviruses as overlapping linear 15 amino acid peptides (Figure 2). The resulting peptide microarrays comprise 4,564 peptides printed in duplicates. For the epitope fingerprint analysis, sera of six individuals were used. 4 subjects received an mRNA-based vaccination, one a vector-based vaccination, and one underwent a heterologous vaccination with a vector-based vaccine and an mRNA-based vaccine.

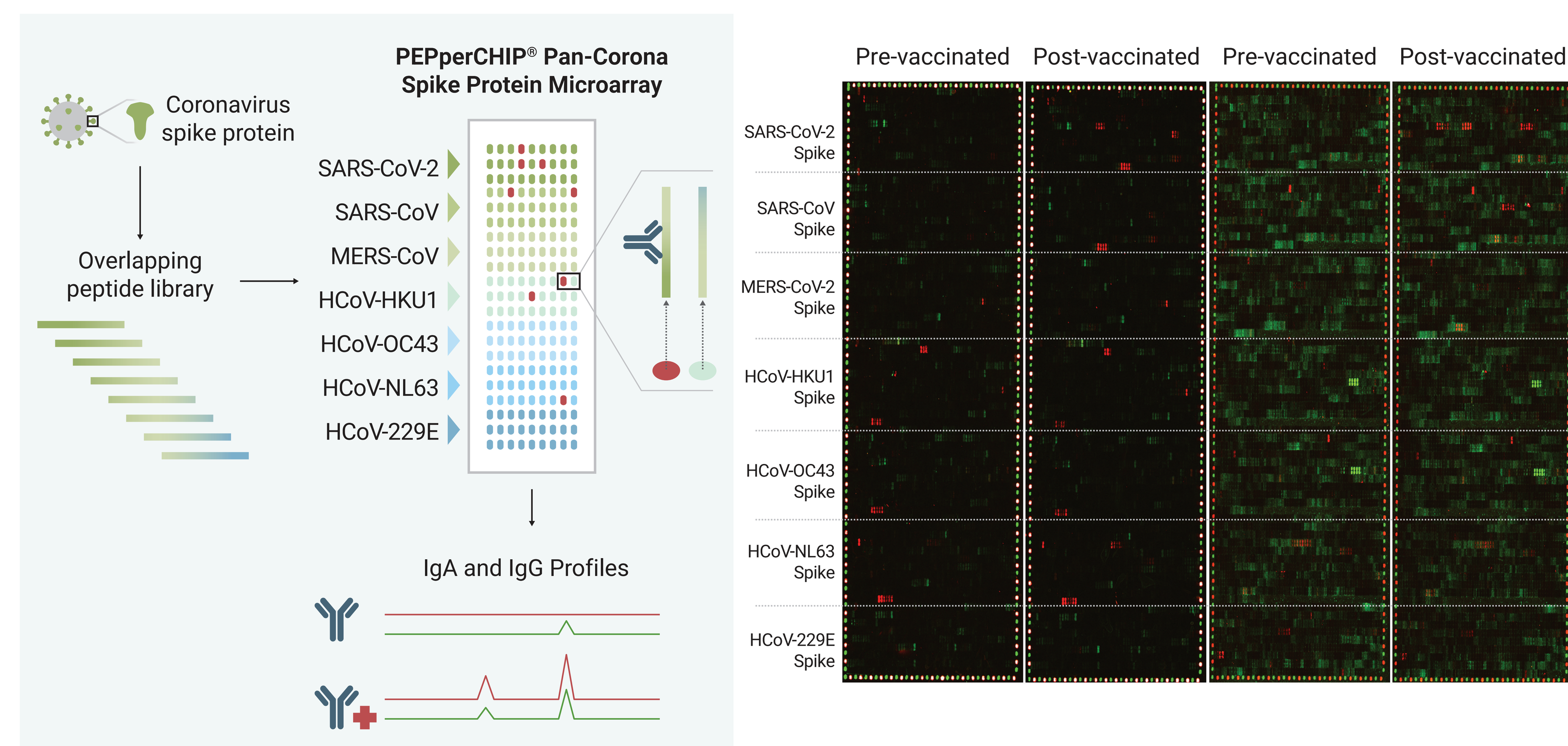

Figure 2. Epitope mapping of COVID-19 vaccine-induced antibody responses. Left side: Experimental workflow. Pre- and post-vaccination sera were screened on PEPperCHIP® Pan-Corona Spike Protein Microarrays comprising spike proteins from seven coronaviruses as overlapping peptides. A dual isotype read-out was performed to analyze IgG and IgA responses simultaneously. Right side: Microarray scans from two vaccine recipients. Sera were diluted 1:200 and incubated over night at 4°C. Detection was performed using secondary anti-human IgG DyLight680 and anti-human IgA DyLight800 antibodies. Mouse mOBJECTIVE &onoclonal anti-HA DyLight800 was added to stain the HA peptide control frame. Red spots= IgG responses, green spots = IgA responses. For the control frame: Red spots = Polio responses from serum; green spots = HA signal.

A comparison of the reactivity profile of sera highlighted epitope-specific IgG antibody responses against spike protein post-vaccination (Figure 2 & Figure 3). For IgA, no relevant epitope recognition was observed. The screening further revealed pre-existing IgG responses against spike protein sequences derived from the four endemic human coronaviruses with no major differences between pre- and post-vaccination sera (Figure 2 & Figure 3A).

The analysis of the IgG responses resulted in six major immunogenic epitopes, which were observed in at least two post-vaccination sera (Figure 3B). The epitope pattern was independent of the type of vaccine. To validate the epitopes identified by the peptide microarray screening, a bead-based multiplex immunoassay was applied (Luminex xMAP® technology). Peptides with a C-terminal biotin tag were coupled to paramagnetic MagPlex®-Avidin Microspheres. Positive control beads were prepared by coupling a poliovirus peptide (Table 1).

ID	Peptide sequence	MagPlex®-Avidin Microspheres bead regions
SARS-CoV-2 Spike E554-D568	ESN**KKFLPFQQ**FGRD-eBio	012
SARS-CoV-2 Spike E574-T588	DA**VRDPQTLEI**LDIT-eBio	015
SARS-CoV-2 Spike Q1142-F1156	QPELDSFK**EELDKYF**-eBio	025
SARS-CoV-2 Spike P1162-V1176	PD**VDLGDISGI**NASV-eBio	029
SARS-CoV-2 Spike N1178-N1192	NIQKEI**DRLNE**VAKN-eBio	033
SARS-CoV-2 Spike C1250-V1264	CGSCCK**FDEDDSEPV**-eBio	036
Human Poliovirus 3 Polyprotein K615-T628	KEVPALTAVETGAT-GG-eBio	020

Table 1. Peptides used for the bead-based multiplex immunoassay. Bold letters in the peptide sequence highlight the identified epitope (Figure 3B); eBio = Peptides were synthesized with a C-terminal biotin ethylene glycol linker unit.

CONCLUSION

We applied a combination of our proprietary peptide microarray technology and a multiplexed bead-based assay to identify and validate targets of humoral responses to COVID-19 vaccination and determined immunogenic linear SARS-CoV-2 epitopes of which two were linked to neutralizing IgG responses.

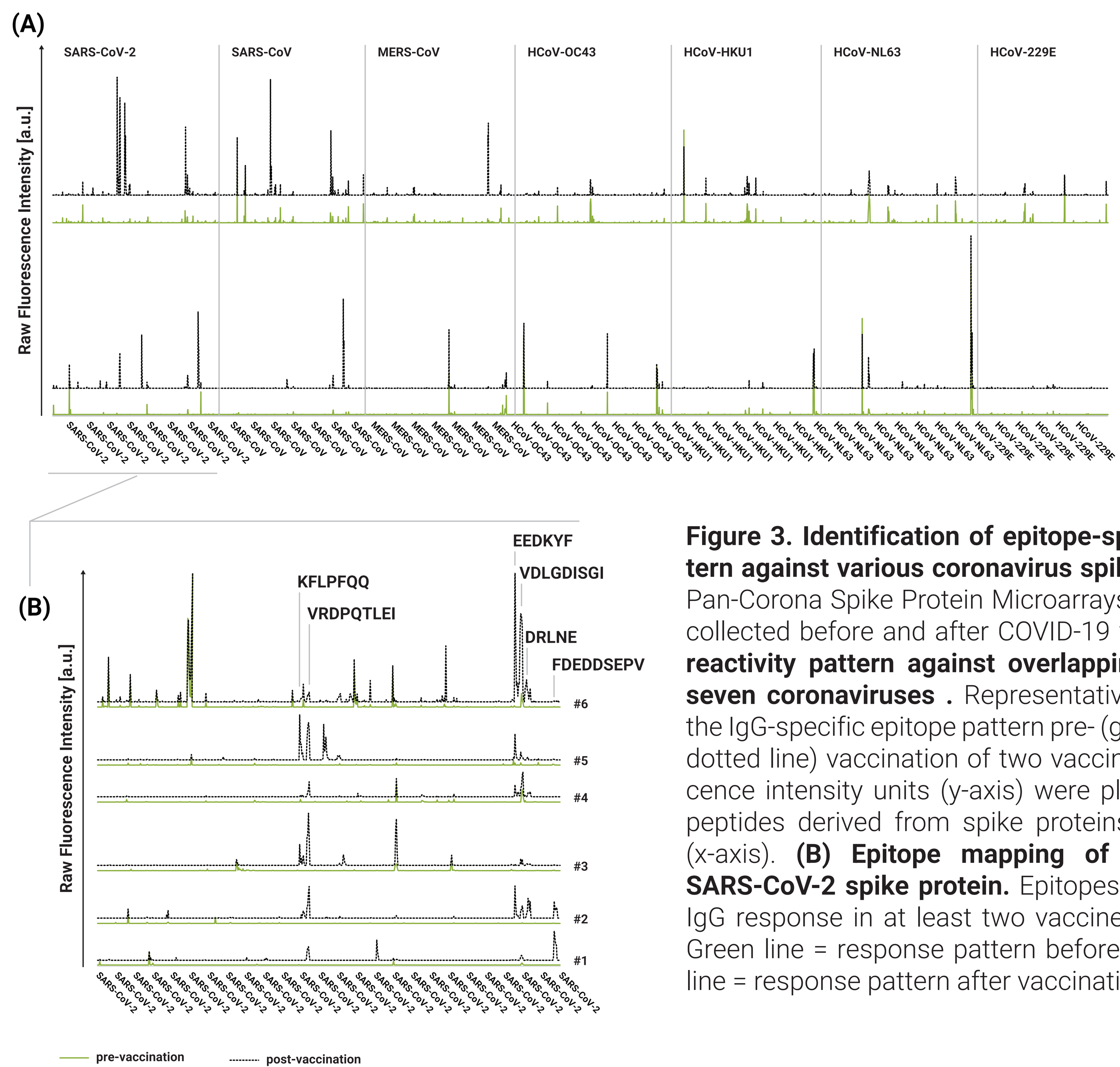

Figure 3. Identification of epitope-specific IgG response pattern against various coronavirus spike proteins. PEPperCHIP® Pan-Corona Spike Protein Microarrays were screened with sera collected before and after COVID-19 vaccination (n=6). **(A) IgG reactivity pattern against overlapping spike peptides from seven coronaviruses .** Representative intensity plots showing the IgG-specific epitope pattern pre- (green line) and post- (black, dotted line) vaccination of two vaccine recipients. Raw fluorescence intensity units (y-axis) were plotted against overlapping peptides derived from spike proteins of seven coronaviruses (x-axis). **(B) Epitope mapping of IgG responses against SARS-CoV-2 spike protein.** Epitopes were annotated when an IgG response in at least two vaccine recipients was detected. Green line = response pattern before vaccination; black dotted line = response pattern after vaccination.

For the majority of the SARS-CoV-2 spike peptides, IgG responses measured by the bead-based immunoassay were in excellent agreement with the microarray data (Figure 4, Table 2). For peptide P1162-V1176, IgG responses could only be partially confirmed. Considerable strong antibody reactivities were confirmed for peptides E554-D568, D574-T588, Q1142-F1156, and N1178-N1192 (Figure 4B). In accordance with the microarray analysis, peptide C1250-V1264 was not observed in most of the subjects (Figure 3B,

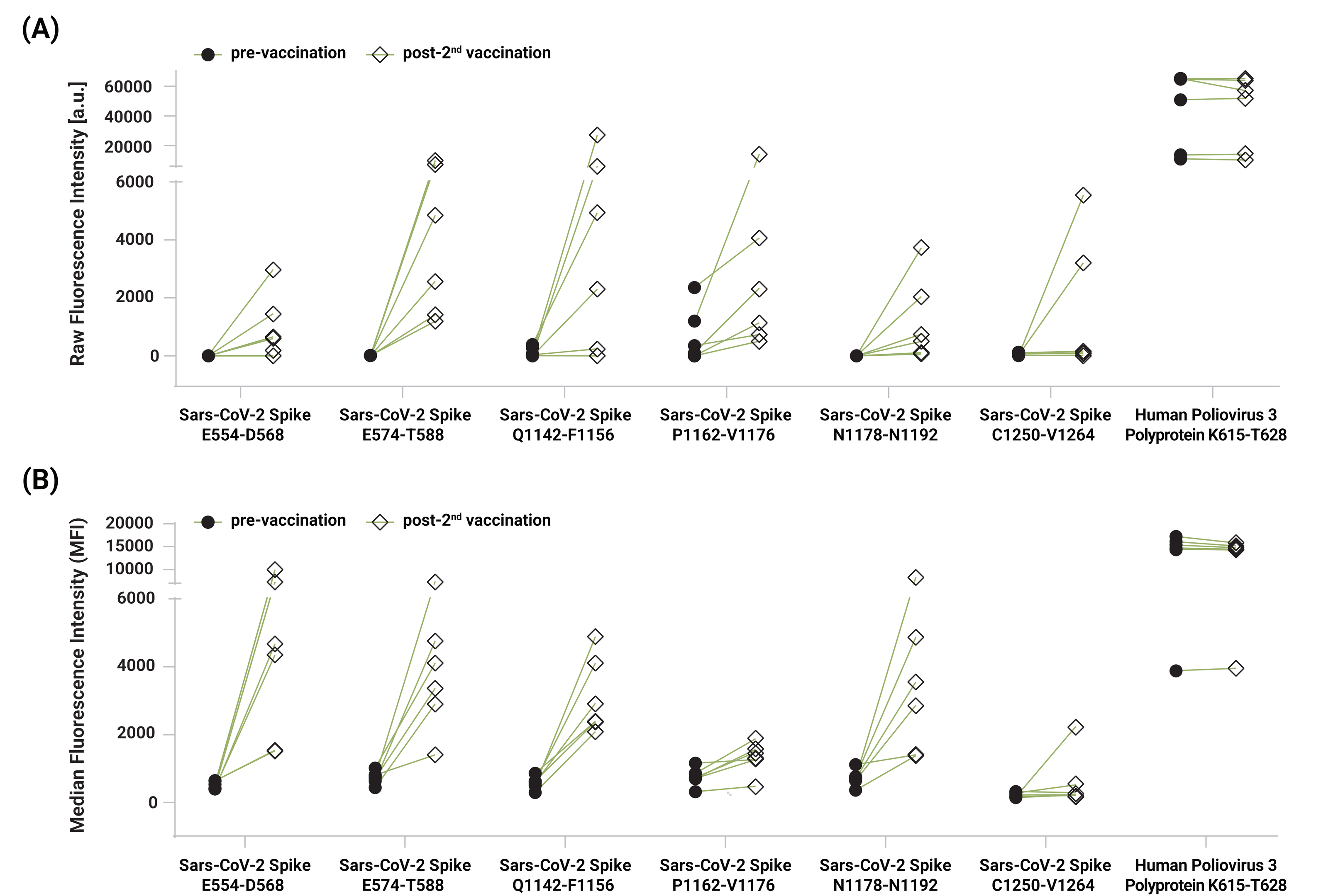

Figure 4. Validation of IgG responses. The microarray screening resulted in the identification of IgG-specific epitopes, which were further re-evaluated with a multiplexed bead-based immunoassay (xMAP® technology, Luminex). **(A) Peptide microarray data** PEPperCHIP® Pan-Corona Spike Protein Microarrays were screened with sera pre and post COVID-19 vaccination (n=6). Epitope mapping resulted in six peptides showing IgG responses in at least two vaccine recipients. A poliovirus epitope served as control. **(B) IgG responses analyzed with the Luminex MAGPIX® system.** For each bead set, 5 µg of biotinylated peptide was coupled to 10^6 MagPlex®-Avidin Microspheres. Peptide-coupled microspheres were combined and incubated with sera, IgG binding was detected with a PE-conjugated anti-human IgG antibody.

	SARS-CoV-2 Spike E554-D568		SARS-CoV-2 Spike E574-T588		SARS-CoV-2 Spike Q1142-F1156		SARS-CoV-2 Spike P1162-V1176		SARS-CoV-2 Spike N1178-N1192		SARS-CoV-2 Spike C1250-V1264	
	A	B	A	B	A	B	A	B	A	B	A	B
#1	-	+	+	+	-	+	+	-	-	-	+	-
#2	+	+	+	+	+	+	+	-	+	+	+	+
#3	+	+	+	+	-	+	+	+	-	+	-	-
#4	-	+	+	+	+	+	-	+	+	+	-	-
#5	+	+	+	+	+	+	+	-	+	+	-	-
#6	+	+	+	-	+	+	+	+	+	+	-	-

Table 2. Side-by-side comparison of IgG responses in post-vaccination sera. Antibody responses were considered as positive when signal intensities in the post-vaccination serum were twofold higher than in the pre-vaccination serum. A = Peptide microarray data; B = Luminex MAGPIX® data; #1-6 = vaccine-recipient (+) = positive IgG response in post-vaccination sera; (-) = no response in post-vaccination sera.

Comparing both technologies, the IgG response pattern against peptides E554-D568, D574-T588, Q1142-F1156, N1178-N1192 and C1250-V1264 matched 80-100% for most of the individuals. In few cases, the vaccine-induced IgG responses analyzed with the Luminex MAGPIX® system did not match with the peptide microarray screening and vice versa.

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