Rapid Development of Peptide Tools to address emerging Viruses: Case Example SARS-CoV-2

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Introduction

The emergence of new pathogenic viruses or variants of a virus poses a major challenge for healthcare systems.[1] Peptide-based tools for the assessment of humoral and cellular immunity can be rapidly adapted to the relevant epidemiologic situation and have been shown to be of great value for the development of effective diagnostics, treatments and vaccines.[2]
Here we present a workflow that we applied to develop peptide tools for studying immune responses to SARS-CoV-2. It combines bioinformatic algorithms and a high-throughput peptide synthesis method alongside high-content assay formats.

Methods

The use of peptide pools has become standard for stimulation of antigen-specific T-cells in functional T-cell assays such as ELISpot, Interferon-gamma release assays (IGRAs) and Intracellular Cytokine Staining combined with Flow Cytometry (ICS-FC).

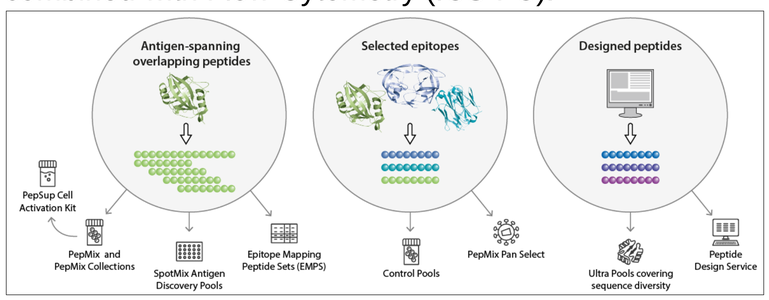


Figure 1: JPT's peptide pool formats used in immune monitoring.

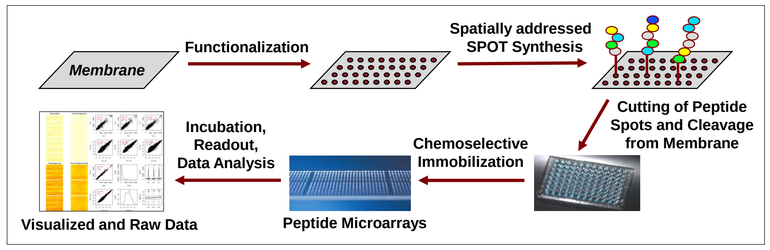
Different library concepts have been developed (Fig. 1). A major challenge in monitoring clinical infection and/or vaccine response is the selection of optimal target antigen sequences to derive efficient therapeutic agents. We address this by a peptide library based workflow that combines:

Improved bioinformatic algorithms

New algorithms for library design were developed. These are based on the scoring of all possible peptides according to their frequency of occurrence across all sequences to provide the most homogenous overall coverage.[3] For instance for the SARS-CoV-2 Spike glycoprotein the sequence diversity of all currently designated Variants of Concern (acc. WHO definition as of Sep-2022) is covered by only 562 peptides that represent a scan (15mers with 11 aa overlap) of the complete antigen.

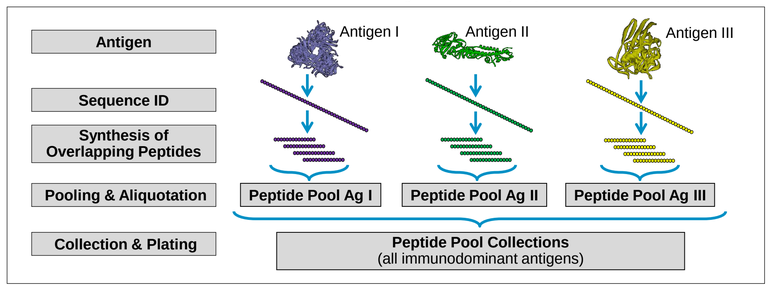
High throughput peptide synthesis, peptide presentation and synergistic assay formats

For B-cell epitope discovery and humoral immune monitoring high density peptide microarrays represent an efficient technology that accommodates vast numbers of sequence variants. Peptides are synthesized by SPOT synthesis[4] and re-immobilized on microarrays[5] in a clean-room environment (Scheme 1). Readout is usually performed by fluorescently labeled secondary antibodies.



Scheme 1: Preparation of peptide microarrays to study humoral immunity.

Peptide tools for studying cellular immunity, *e.g.* for T cell epitope discovery and immune monitoring, comprise individual peptides, matrix pools or antigen spanning pools for direct use in T cell assays such as ELISpot and ICS combined with flow cytometry (Scheme 2).



Scheme 2: Preparation of peptide pools to study cellular immunity.

Application Examples

Humoral immune response

To provide guidance for diagnostic test development, n=24 patient and n=12 control samples were screened for IgG reactivity against the entire SARS-CoV-2 proteome using peptide microarrays.[6] While widespread cross-reactivity was revealed across several immuno-dominant regions of S and N (Figure 3), IgG binding to several peptides provided statistically significant discrimination (AUC > 0.9 for five peptides)[6] between infected and controls. Selected target peptides could be used as capture agents for highly specific diagnostic tests.

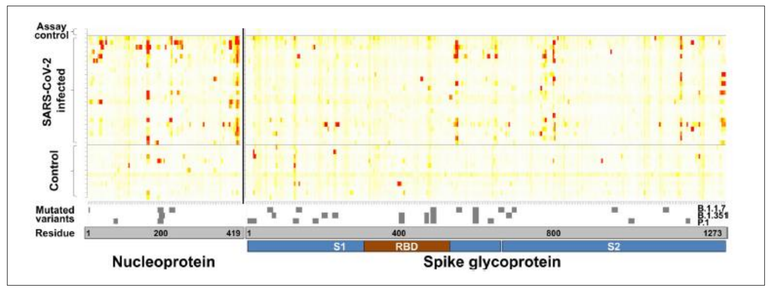


Figure 3: Peptide microarray based analysis of IgG responses to SARS-CoV-2 Nucleoprotein and Spike glycoprotein.

Cellular immune response

T cell immunity appears critically important not only for the control of the virus but also for the development of effective SARS-CoV-2 vaccines.[7] Several studies describe the use of JPT's divers peptide pool formats and quality levels for immune monitoring during vaccine development (Table 1).

Study	T Cell Assay	Reference
Human	ELISpot, ICS	Sahin et al., Nature 2021, 590, E17 Sahin et al., Nature 2021, 595, 572
Human	ICS	Jackson et al., N Engl J Med. 2020, 383, 1920
Human	ELISpot, ICS	Zuo et al., Nat. Immunol. 2021, 22(5), 620
Human	ELISpot	Li et al., Nat. Med. 2021, 27(6), 1062
Rhesus macaque	ELISpot, ICS	Vogel et al., Nature 2021, 592, 283
Rhesus macaque	ICS	Corbett et al., N Engl J Med. 2020, 383, 1544
Rhesus macaque	ELISpot, ICS	Mercado et al., Nature 2020, 586, 583
Rhesus macaque	ELISpot, ICS	Sanchez-Felipe et al., Nature 2021, 590, 320
Rhesus macaque	ELISpot, ICS	Mandolesi et al., Cell Rep. Med. 2021, 2(4), 100252
Baboon	ELISpot, ICS	Tian et al., Nat. Commun. 2021, 12(1), 372
NHP	ELISpot	Kalnin et al., NPJ Vacc 2021, 6(1), 61

Table 1: Publications on the use of JPT's peptide tools for immune monitoring of vaccines against SARS-CoV-2.

Summary

Readily available and tailored peptide formats, as exemplified for SARS-CoV-2, can be important tools that contribute to the fast development and optimization of vaccines and diagnostic tests for emerging viruses of concern.

References

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(2) (a) Sahin, U. et al. *Science* **2018**, *359*, 1355-1360. (b) van de Veerdonk, F.L.. et al. *Nat. Med.* **2022**, *28*, 39–50. (c) Loyal, L. et al. *Science* **2021**, *374*, 6564.
(3) Stephenson, K. et al. *J. Immunol. Meth.* **2015**, *416*, 105-123.
(4) Wenschuh, H. et al. *Biopolymers* **2000**, *55*, 188-206.
(5) (a) Schutkowski, M. et al. *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 2671-2674. (b) Masch, A. et al. *Methods Mol. Biol.* **2010**, *669*, 161-172.
(6) Holenya, P. et al. *Eur J Immunol* **2021**, *51*, 1839-1849.
(7) Moss, P. *Nat Immunol* **2022**, *23*, 186-193.

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