Peptide Tools to Support the Fight against COVID-19

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To date, the understanding of the immune response to COVID-19 is still incomplete. In this application note we describe innovative peptide-based tools for the assessment of humoral and cellular immunity to SARS-CoV-2. These tools are suitable for immune response target identification and clinical immune monitoring. They can also be used for T-cell and antibody response profiling and will be useful for the development of effective diagnostics, treatments, and vaccines.

Introduction

As the COVID-19 pandemic is still ongoing,[1] the development of effective vaccines is a pressing need. As a result of an unprecedented effort across the scientific community, two vaccine candidates are already approved in the US and EU based on positive phase III data[2,3].

SARS-CoV-2, which is the cause of COVID-19, is a single-stranded RNA virus containing several proteins, including the structural proteins, Spike (S), Nucleocapsid (N), Membrane (M), and Envelope small membrane protein (E), as well as a number of non-structural proteins (Figure 1). The S protein is the target of the vast majority of vaccine candidates. However, other proteins are increasingly attracting attention, *e.g.* for the assessment of SARS-CoV-2-specific immunity or diagnostic test development.

Peptides have proven to be powerful tools for COVID-19 research. Here, we provide a systematic summary on the use of JPT's peptide-based products for the development of potential SARS-CoV-2 diagnostic tests, treatments, and vaccines.

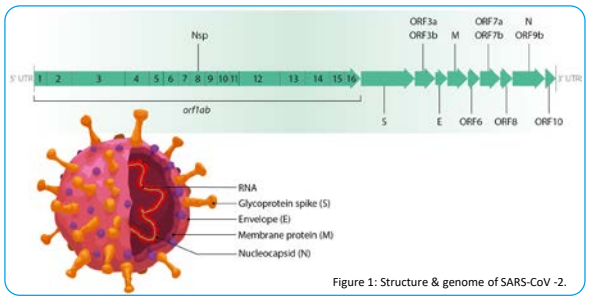

Figure 1: Structure & genome of SARS-CoV -2.

Materials & Methods

Peptide libraries spanning the entire viral proteome were created and are provided in different peptide qualities and quantities as well as in various formats (Figure 2). Additionally, libraries covering the mutational landscape of SARS-CoV-2 and the ubiquitous human common cold corona-viruses were generated.

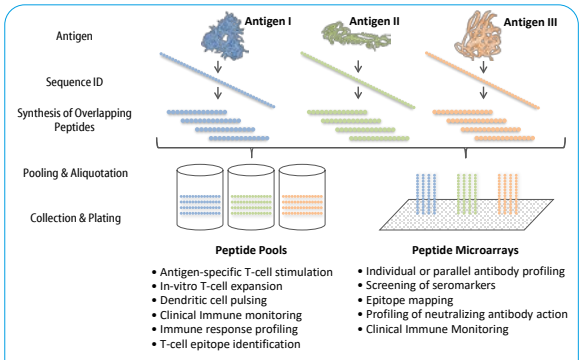

Figure 2: Preparation of peptide pools and peptide microarrays to study cellular and humoral immunity.

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 by flow-cytometry (Figure 2, left).

For B cell epitope discovery and humoral immune monitoring, peptide microarrays[4] represent an efficient technology using overlapping peptides to 'display' the entire virus proteome (Figure 2, right). Following incubation with samples, fluorescently labeled antibodies are used for detecting antibody binding.

Table 1: Overview on JPT's peptide based tools for SARS-CoV-2 and related corona viruses. *Epitope Mapping Peptide Set.

Corona Virus Species	Protein	Cellular Immunity			Humoral Immunity	
		PepMIx	EMPS*	Antigen Peptides	High density Microarray	Multiwell Microarray
SARS-CoV-2	Spike glycoprotein (S)	x	x	x	x	x
	Spike glycoprotein (S) RBD	x	-	x	x	x
	Membrane protein (M)	x	x	x	x	x
	Envelope small membrane protein (E)	x	x	-	x	x
	Nucleocapsid protein (N)	x	x	x	x	x
	Non-structural / ORF proteins /Replicase polyprotein	x	-	-	x	-
	Spike mutations - Updated regularly	x				x
Common Cold Viruses NL63, HKU1, 229E, OC43	Spike glycoprotein (S)	x	x	-	x	-
	Nucleocapsid protein (N)	x	-	-	x	-
	Membrane (M), Envelope (E)	-	-	-	x	-
SARS-CoV-1 & MERS	Spike glycoprotein (S)	x	x	-	x	-
	Membrane (M), Envelope (E), Nucl. (N)	-	-	-	x	-

It has been shown that SARS-CoV-2 elicits strong cellular and humoral immune responses and both B and T cell immunity appear critically important for the control of the virus as well as for the development of effective SARS-CoV-2 vaccines.[5] Therefore, peptide based tools addressing both types of adaptive immunity have been developed (Table 1).

Emphasis was placed on providing complete coverage of all SARS-CoV-2 proteins. This is because of increasing evidence that not only the S protein, but also the other structural proteins (N, M, E, M), and even the non-structural / ORF proteins might substantially contribute to an immune response against SARS-CoV-2. For example, cellular immune responses against 12 different SARS-CoV-2 proteins have been observed in recovered COVID-19 patients.[6] Other studies detected T cells against 21 and 6 different proteins..[7,8] Humoral immune responses to SARS-CoV-2 were found to be mainly focused on the S and N protein.[9] However, significant IgG reactivity has also been observed to peptides derived from the M, AP3A and R1AB proteins. [10]

Clinical Immune Monitoring

Several recent studies describe the use of JPT's PepMix™ peptide pools for immune monitoring of COVID-19 vaccine candidates (Table 2). These include clinical studies with some of the most advanced vaccine candidates to date.

Table 2: Recent publications on the use of JPT's PepMix™ peptide tools for immune monitoring of vaccines against SARS-CoV-2.

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

Cell Therapy Development

With the recent success of novel immunotherapies, including cell-based therapies, the need for clinical grade peptides & peptide pools has grown rapidly. Building on an ISO 9001:2015 certified quality management system we have established an enhanced peptide production environment that adds critical quality measures to the standard production process used for research use only (RuO). The resulting quality levels, referred to as ISO PLUS Peptides and Clinical Grade Peptides (CGP), focus on the more stringent requirements of immunotherapy: for example, cell therapy, clinical immune monitoring, as well as vaccine development. These peptides have been approved for a variety of clinical applications.

Cell-based therapies using peptide pools for preparing expanded antigen specific T-cell populations have been developed for a number of infectious diseases. Examples include pools for the *ex vivo* generation of T cells for HIV[11,12], CMV[13,14], broad-spectrum antiviral (AdV, EBV, CMV, BKV, HHV6)[15] and, very recently, COVID-19 treatment.[16] Several investigational new drug (IND) applications for cell therapies using clinical grade peptides have been submitted, including a program against SARS-CoV-2.

Diagnostic Test Development

Many diagnostic tests for prior SARS-CoV-2 infection, based on the detection of antibodies, have been developed using full-length viral proteins as capture antigens.[17] However, epitope defined analysis of B cell immunity of COVID-19 patients using peptide microarrays showed that significant cross reactivity existed against other corona viruses, i.e. SARS, MERS, and the common cold viruses OC43, HKU1, NL63 and 229E.[10] The discovery of immunodominant epitopes that are specific for SARS-CoV-2[10] will accelerate the development of a sensitive and highly specific test for previous SARS-CoV-2 infection.

Development of T cell based diagnostics for SARS-CoV-2 will have to consider cross reactivity to common cold viruses, as pre-existing SARS-CoV-2 (cross-)reactive T cells have been found in donors not previously exposed to this novel virus.[18] Several T cell based tests are in development, and a discovery assay is already available[19] that builds on a technology that is already in clinical use.[20]

Discussion & Conclusions

To support the fight against the COVID-19 pandemic, we established peptide based tools that combine high throughput peptide synthesis, innovative peptide presentation approaches, and synergistic assay formats. Examples include COVID-19 immunity assessment, target identification, clinical immune monitoring, cell therapy development and diagnostic test development.

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The Company

JPT Peptide Technologies is a ISO 9001 certified integrated provider of innovative peptide solutions for vaccine & immunotherapy development, cellular & humoral immune monitoring, epitope & target discovery, and targeted proteomics.

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