Identifying COVID-19 Vaccine Epitopes Using Peptide Based T cell Activation Assays

Application Note

GenScript
Make Research Easy

Identifying COVID-19 Vaccine Epitopes Using Peptide Based T cell Activation Assays

I. The Devastating Mark COVID-19 Has Made on the World

Since the onset of its awareness in December, 2019, the coronavirus has disrupted normal way of life all over the world. On top of the more than 506,000 deaths and 10 million reported cases world-wide[1], SARS-CoV-2 infection has halted global normalcies, such as healthcare, work, entertainment, and interacting with one another. The reason for this disruption, is because not only is COVID-19 highly contagious, but can be deadly for the over 10 million immuno-compromised individuals[2], those suffering from reduced lung capacity , and 50 million elderly citizens just within the United States[3].

The SARS-CoV-2 virus is a positive-sense single stranded RNA virus belonging to the family Coronaviridae, named due to its crown like shape. Viruses within the family Coronaviridae are known as coronaviruses, and they are actually much more common than many think. In fact, it is estimated that 15-35% of all common colds are caused by human coronaviruses, so what makes SARS-CoV-2 so deadly? As assumed by its name, SARS-CoV-2 is a mutated version of the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) zoonotic coronaviruses, Bering almost 80% genetic homology to SARS-CoV[4]. Similar to these other deadly viruses, SARS-CoV-2 cause's severe upper respiratory tract distress in the form of lymphopenia and pneumonia, as well as high levels of inflammatory cytokines known as a cytokine storm, all resulting in the high death toll of the disease[4]. Despite the severe infection which comes with the genetic similarity of COVID-19 to SARS-CoV and MERS, because clinicians have treated patients with these diseases in the past, generating a vaccine and treating SARS-CoV-2 is slightly easier, since pharmaceutical companies can simply pick up where they left off from investigating these previous similar viruses.

II. The Structure of Coronaviruses of the Past Could Lead to the Treatment of the Future

Like SARS-CoV and MERS-CoV, SARS-CoV-2 belongs to the genus betacoronavirus within the family Coronoviridea. All of these viruses contain a 30 kilobase single stranded RNA genome which encodes for structural and nonstructural proteins important for viral docking and subsequent infection. There are various structural components of the proteome, but the most important to infection are the Spike (S), the envelope (E), the membrane (M), and the nucleocapsid (N) proteins[5] (Figure 1).

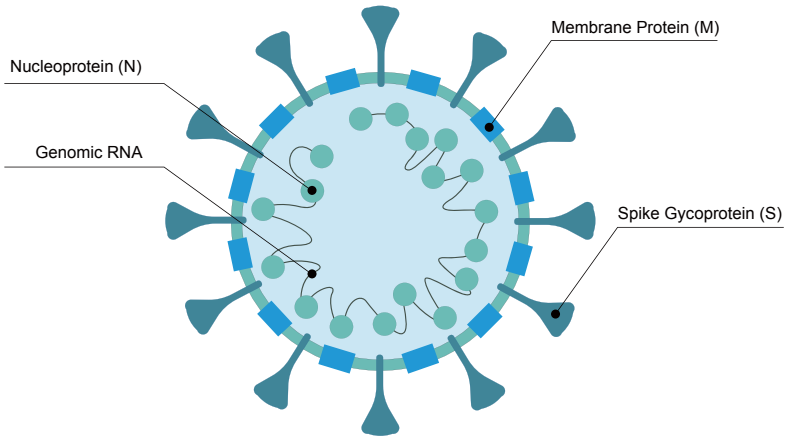

Figure 1. Structure of SARS-CoV-2.[6] Coronaviruses have enveloped virions that measure approximately 120 nm in diameter. On the outside of the virion, are club-shaped glycoprotein spikes in the envelope which give the viruses its crown like shape.

Recent genomic studies have indicated that not only does CoV-2 show similar structure to SARS and MERS, but also similar docking and infection mechanisms through the connection between the Receptor Binding Domain (RBD) of the S protein and the angiotensin-converting enzyme 2 (ACE2) receptor[7, 8, 10] (Figure 2)[8]. Since SARS-CoV-2 contains the same structural components and utilizes an almost identical infectious mechanism to SARS-CoV and MERS, researchers utilize previously defined immunogenicity of these components to design a vaccine against SARS-COV-2. Specifically, previous research indicated that natural immune responses to the original SARS-CoV virus were against the Spike (S), and Nucleocapsid (N) regions[1]. Since the SARS-CoV-2 virus also contains these elements, initial vaccine research has been directed towards these structural components.

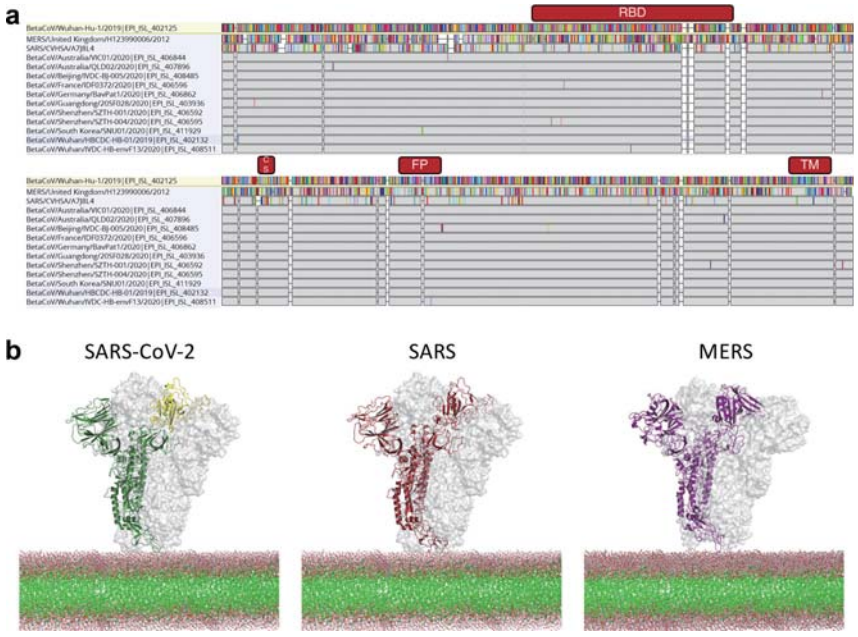

Figure 2. The alignment of SARS-CoV-2 with SARS-CoV and MERS viruses[8]. Structural models for SARS-CoV-2, SARS and MERS spike glycoproteins with one chain represented as cartoon and two chains represented as surface. RBD of SARS-CoV-2 is colored yellow.

III. T Cell Activation Assays Confirm Immunity in COVID-19 Patients

Coronaviruses have adapted an intelligent way of evading the immune system by reducing the body's natural defenses to viral infection. This artifact makes it difficult to design a vaccine against these viruses, as targeting any aspect of the viral infection and transcriptional machinery could simply be for nothing. Therefore, in order to identify top epitope candidates for vaccine development, researchers have first decided to turn to investigating the natural immune response made in patients who have been affected and have fully recovered from COIVD-19 infection using T cell activation assays rather than simply relying on the immunogenic peptides of past sequences.

T cell activation assays are in vitro experiments in which patient samples are exposed to viral peptides in order to measure possible immune responses. This can be done through a variety of different experiments, such as EliSpots, ELISA's, Flow cytometry, cytotoxicity assays, and additional T cell breadth and cytokine analysis (Figure 3).

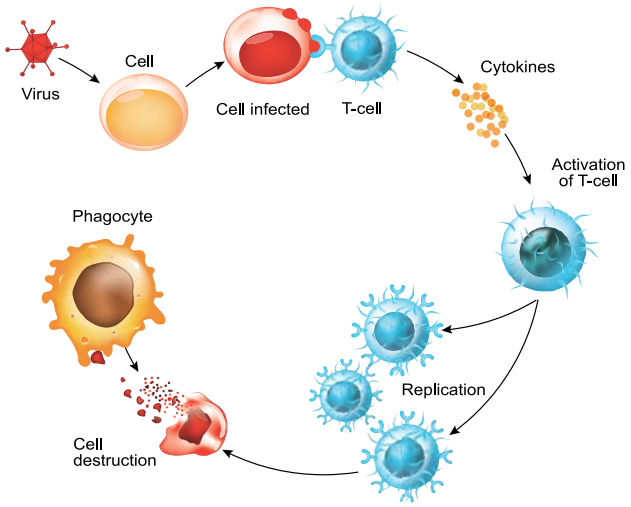

Figure 3. T Cell Activation. T cells can be analyzed by quantifying antigen specific T-cells, T cell proliferation, immunophenotyping, cytokine analysis, and cytotoxic potential. All of these experiments will be done after PBMC's are incubated with peptides representing targeted vaccine epitopes in order to stimulate T cells and induce anti-tumor killing.

Detection of natural immune responses in convalescent patients using T-cell activation assays has shown interest all over the globe. A recent paper in Immunity, authored by Dr. Ling Ni from the Institute for Immunology and School of medicine at Tsinghua University in China detailed the humoral and cellular immune response in COVID-19 convalescent patients[4]. In the study, researchers collected blood samples from recent COVID-19 survivors, and therefore would still contain the natural immune response against the illness. The authors then analyzed antibody responses, number of virus specific T cells, and virus induced pro-inflammatory cytokines.

Within the article, the authors describe using various T cell activation assays in order to determine the epitopes of COV-19 which produce strong natural immune responses. In order to accomplish this, they first isolated peripheral blood mononucleocytic cells (PBMC) from the blood of convalescent SARS-CoV-2 patients. These PBMCs were incubated with peptide components of full length Nucleocapsid, main protease, and Spike-RBD proteins, as these components where shown to be highly immunogenic in previously analyzed Coronaviruses. The incubated PBMC's were then used for IFN-γ EliSpot analysis in comparison with healthy donors. Interferon gamma (IFN- γ) is a type II class soluble cytokine which is required for both innate and adaptive immune response against numerous types of invaders, but particularly viruses. It becomes activated by various types of innate and adaptive T cells post infection. It then, through it's downstream regulatory affects, it is able to activate macrophages and MHCII expression required for epitope driven T cell responses, as well as directly interfere with viral infection. Therefore, IFN- γ will be overexpressed in response to viral peptides which the immune system recognizes as "foreign invaders", through its memory immune response, making it a great marker of immuno-stimulation against vaccine

epitope candidates. In Dr. Ni's research, the IFN- γ EliSpot revealed twice as many IFN- γ secreting cells in stimulated PBMC's from convalescent patients compared with healthy controls, indicating that each viral component analyzed would make a good vaccine candidate.

To further narrow down specific regions of these immuno-stimulating viral proteins, researchers at La Jolla institute for Allergy and Immunology broke each predicted immunogenic proteins up into small 15mer overlapping peptide "megapools" and performed similar T-cell stimulation assays on convalescent patient derived PBMCs[1]. Dr. Alba Grifoni and her co-authors first used these megapools in T cell receptor (TCR) dependent activation induced marker (AIM) assays in order to identify and quantify SARS-CoV-2 specific $CD4^+$ T cells in convalescent patients compared with uninfected controls[1]. The reason they chose this assay, was to identify epitope specific T cell responses in order to identify where the immune responses were coming from and decide on proper vaccine candidates. The results of this assay revealed that the spike protein megapool, as well as other structural components, lead to $CD4^+$ specific immune responses in 100% of convalescent patients with a strong P value compared to unexposed controls. In order to analyze the cytokine induction of these vaccine candidates, T cell activation megapool cytokine bead assays were then used to confirm the presence of IL-2 and IFN- γ T_H1 induced cytokines. The research team them employed similar methods to analyze $CD8^+$ T cells, utilizing a megapool AIM assay as well as intracellular cytokine staining (ICS) assay. Overall, $CD8^+$ responses were detected in 70% of COVID patients through AIM while the ICS assays confirmed the presence of IFN- γ in in the majority of COVID-19 $CD8^+$ cell populations.

Cumulatively, these two papers, as well as many others, indicate that patients who have recovered from COVID-19 do indeed maintain their B and T cell immunities against specific components of the virus, such as the peptide sequences within the Spike and Nucelocapsid proteins. These studies confirm that it is possible to create a vaccine against SARS-CoV-2 against these regions, which share commonality with other, even deadlier, coronaviruses, such as SARS-CoV and MERS. Without the use of these T cell activation assays, we would not have the knowledge that we do today that these particular regions have the potential to make amazing vaccines to target and kill COVID-19.

IV. Using T-Cell Activation Assays to Determine Epitopes for Vaccine Development Against COVID-19

By combining the knowledge gained from generating vaccines against previous betacoronaviruses, such as SARS and MERS, and the insight from the immune response of COVID-19 convalescent patients, researchers began generating vaccines to fight against SARS-CoV-2 within weeks of it becoming a global pandemic. There are now over 115 different vaccine candidates with 73 of them currently in preclinical development, and a few even in clinical trials. The three most promising clinical candidates are Moderna's mRNA-1273 vaccine, CanSino Biologicals Ad5-nCoV, and Inovio's DNA based INO-48009. What is interesting about all the these trials, whether pre-clinical or clinical, is the strong diversity in vaccine delivery platform in order to introduce different segments of the same target S, N, E and M, proteins into patients. Despite these different delivery modalities, ranging from nucleic acids (DNA and mRNA) and macromolecules (peptides and proteins) to inactivated viruses, any pre-clinical or clinical based vaccine will require T cell activation assays in order to study its overall specificity, immunogenicity, and efficacy.

A recent paper from Dr. Trevor Smith and his team at Inovio Pharmaceuticals details their pre-clinical research into developing a DNA based vaccine against SARS-CoV-2. In the article, they describe how they utilize their prior experience generating a vaccine for MERS against the S protein, in order to engineer a novel vaccine for COVID-19 targeting the same protein. After immunization with INO-4800, mice and guinea pigs showed substantial immune protection against the virus, including antigen specific-T cell responses, neutralizing antibodies, and anti-Spike

protein antibodies within the lungs. Within the paper, the authors utilize T cell activation studies multiple times in order to confirm that INO-4800 would indeed make a great clinical candidate. Firstly, they measure T cell responses against not only SARS-CoV-2, but also against SARS-CoV and MERS-CoV S regions via IFN-γ Elispot in order to determine if vaccine peptides induce cross-reactive immune responses against all three betacoronaviruses. In order to accomplish this, researchers isolated splenocytes from and immunized BALB/c mice and stimulated them with peptide pools of overlapping 15mers spanning the Spike protein from each individual virus. Interestingly, not only did the SARS-CoV-2 peptide pools lead to a strong cytokine response, but so did SARS-CoV just in a lesser context, with no cross reactivity with MERS (Figure 4).

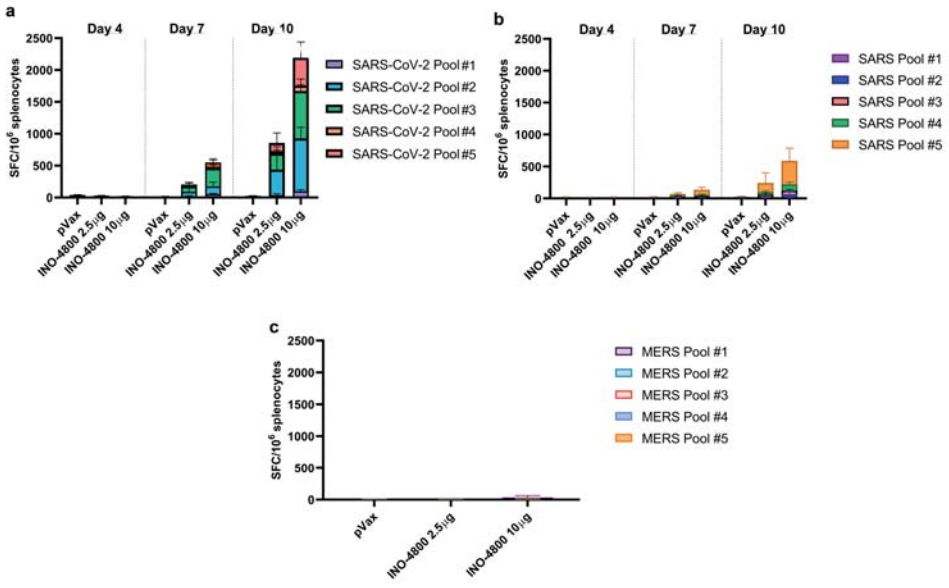

Figure 4[8]. IFN-γ EliSpot results from cross reactivity analysis. BALB/c mice (*n* = 5/group) were immunized with 2.5 or 10 µg INO-4800. T cell responses were analyzed in the animals on days 4, 7, 10 for plots a&b, and day 14 for plot c. T cell responses were measured by IFN- γ ELISpot in splenocytes stimulated for 20h with overlapping peptide pools spanning the SARS-CoV-2 (**a**), SARS-CoV (**b**), or MERS-CoV (**c**) Spike proteins. Bars represent the mean + SD.

In order to identify specific epitopes within the SARS-CoV-2 peptide pools which would make the best vaccine candidate, researchers next performed epitope mapping using a similar T cell activation approach. Thirty peptide pools spanning the SARS-CoV-2 Spike protein were used to stimulate splenocytes isolated from iand immunized mice. Positive immune responses were detected in multiple peptide pools (Figure 5). From there, bioinformatics analysis was used to identify that the most immunogenic peptides came directly from the receptor binding domain, as previously seen in results from convalescent human patients. These results eventually led to the current clinical trial utilizing INO-4800 in human patients which has shown promising results through its phase 1 analysis.

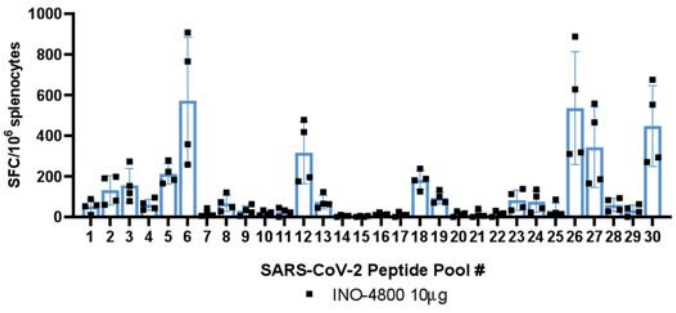

Figure 5[8]. Epitope Mapping of SARS-COV-2. Splenocytes were stimulated for 20 h with SARS-CoV-2 peptide matrix pools. T cell responses following stimulation with matrix mapping SARS-CoV-2 peptide pools. Bars represent the mean + SD of five mice.

V. Conclusion

As a global pandemic, COVID-19 has completely uprooted our way of life due to the devastating effects of its infection. The race to find an efficacious vaccine has begun the top priority for top pharmaceutical companies all over the world. With these daunting studies, it is important that researchers can find reliable peptides for their T-cell activation assays. The fast moving efforts of better understanding immune response to COVID 19 and vaccine development is needed to combat the current crisis. In particular peptide production is a roadblock that can be overcome by a range of different methods including outsourcing and better bioinformatics tools. For examples of how this can be done go to www.genscript.com/neoantigen_peptide_service.html. By working together, researchers and clinicians will find a viable vaccine to prevent SARS-CoV-2 infection so future generations do not have to suffer from this terrible illness.

VI. References

1. Grifonia, Weiskopf, et al. "Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals." Cell, Vol 181, 1-13, 25 June.2020, doi: 10.1016/j.cell.2020.05.015

2. Duncan, Charles. "What Does 'Immunocompromised' Mean, and What Should People at Risk of Coronavirus Do?" Sacbee, The Sacramento Bee, 6 Mar. 2020, www.sacbee.com/news/nation-world/national/article240962946.html#:~:text=There's%20an%20estimated%2010%20million,and%20how%20to%20stay%20safe.

3. Administration of Community Living. "2017 Profile of Older Americans." Acl.gov, 2018. https://acl.gov/sites/default/files/Aging%20and%20Disability%20in%20America/2017OlderAmericansProfile.pdf

4. Ni, Ye, et al. "Detection of SARS-CoV-2-Specific Humoral and Cellulat Immunity in COVID-19 Convalescent Individuals." Immunity, Vol 52, 1-7, 16 June, 2020, doi: 10.1016/j.immuni.2020.04.023

5. Ahmed, Syed Faraz, et al. "Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies." Viruses, vol. 12, no. 254, 25 Feb. 2020, doi:10.1101/2020.02.03.933226.

6. The Editors of Encyclopaedia Britannica. "Coronavirus." Encyclopædia Britannica, Encyclopædia Britannica, Inc., 20 Apr. 2020, www.britannica.com/science/coronavirus-virus-group.

7. Dhama, Kuldeep, et al. "COVID-19, an Emerging Coronavirus Infection: Advances and Prospects in Designing and Developing Vaccines, Immunotherapeutics, and Therapeutics." Human Vaccines & Immunotherapeutics, 2020, pp. 1–7., doi:10.1080/21645515.2020.1735227.

8. Smith, et al. "Immunogenicity of a DNA Vaccine Candidate for COVID-19." Nature Communications, 2020, doi: 10.1038/s41467-020-16505-0

9. Le, Andreadakis, et al. "The COVID-19 Vaccine Development Landscape." Nature Reviews Drug Discovery, Vol 19, May 2020 Pgs 305-306.

10. Prompetchara, Ketloy, and Palaga. "Immune Responses in COVID-19 and Potential Vaccines: Lessons Learned from SARS and MERS Epiodemic. Asain pacific Journal of Allergy and Immunology. 2020;38:1-9 DOI: 0.12932/AP-200220-0772

11. Fadi Saade, et al. "Pushing the frontiers of T-cell vaccines: accurate measurement of human T-cell responses." Expert Reviews Vaccines. 2012 December; 11(12): 1459–1470. doi:10.1586/erv.12.125.

www.GenScript.com

GenScript USA Inc.
860 Centennial Ave.
Piscataway, NJ 08854 USA

Email: orders@genscript.com
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188
Fax: 1-732-210-0262
1-732-885-5878

GenScript
Make Research Easy