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Commentary

Artificial intelligence and clinical data suggest the T cell-mediated SARS-CoV-2 nonstructural protein intranasal vaccines for global COVID-19 immunity

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ABSTRACT

Advanced computational methodologies suggested SARS-CoV-2, nonstructural proteins ORF1AB, ORF3a, as the source of immunodominant peptides for T cell presentation. T cell immunity is long-lasting and compatible with COVID-19 pathology. Based on the supporting clinical data, nonstructural SARS-CoV-2 protein vaccines could provide global immunity against COVID-19.

Coronavirus disease 2019 (COVID-19) pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is ongoing. Vaccines as emergency countermeasures licensed in Europe and North America within 11 months [17]. The adenovirus or lipid nanoparticles vaccines administer intramuscular to deliver SARS-CoV-2 spike protein mRNA [25,35]. COVID-19 mRNA vaccine induces humoral immunity, e.g., neutralizing antibodies, and cellular immunity, e.g., CD4+ and CD8+ T cell responses [35]. After the epidemic from 2002 to 2004, T cell immunity endured 17 years in SARS-CoV patients [24]. In contrast, the humoral immunity components such as transient antibody levels and memory B cells waned rapidly in the SARS-CoV patients [21,24,48].

Therefore, the advanced computational methodologies constructed the SARS-CoV-2 T cell epitope map to identify immunodominant peptides for vaccine development [1,8,13,24,29,34,46]. In a Monte Carlo-based simulation, ensemble machine-learning, and Artificial intelligence (AI) predicted the nonstructural ORF1AB, ORF3a proteins as more effective vaccine epitopes to the spike protein [24]. In an Immunoinformatics approach, TepiTool predicted ORF1, ORF3, ORF9 epitopes CD8+ T cell SARS-CoV-2 epitopes [46]. The Machine learning-based Vaxign-ML reverse vaccinology tools predicted SARS-CoV-2 S, nsp3, and nsp8 proteins as highly antigenic T cell epitopes [29]. In an Immunoinformatics approach with the tools such as CoronaVR, ToxinPred, AllerTOP v. 2.0, Support Vector Machine predicted NSP2, NSP3, NSP4, NSP16, S, M, and ORF7b CD8C T cell SARS-CoV-2 epitopes [13]. In an Immunoinformatics approach with the tools such as TepiTool, NetMHCpan 4.0, Artificial neural network, and Stabilized matrix method of the IEDB predicted SARS-CoV-2 Orf1ab proteins nsp7, nsp8, nsp9, nsp10, nsp12, and nsp14 as T cell epitopes with higher binding affinity compared to the structural proteins [34]. In an Immunoinformatics approach with the tools such as, the IEDB, VaxiJen 2.0, ToxinPred, AlgPred, and ProtParam, predicted the SARS-CoV-2 T cell epitopes as from 7 epitopes from structural proteins and 12 epitopes ORF1ab [1]. In an Immunoinformatics approach using tools such as EpiMiner, Immune Epitope Database (IEDB) server ORF1ab had the highest predicted SARS-CoV-2 cell epitopes [8].

T-cells are essential for protection from severe COVID-19. Thus, in several clinical studies, a higher number of polyfunctional SARS-CoV-2 specific CD4+ and CD8+ T cells with the antiviral cytokine IFN- γ, TNF, and IL-2 secretion was associated with the mild to moderate COVID-19 [12,22,26,31,40,43]. However, in severe COVID-19, the T cell response is delayed and significantly lower SARS-CoV-2–specific T cells activity without the polyfunctional

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antiviral capacity [12,31,39,40]. As modeled by Sette and Crotty, in moderate COVID-19 cases virus-specific CD4+ and CD8+ T cells surge 2–4 after the disease manifestation and clear the primary infections in the airways [22,40,43]. However, in severe COVID-19 cases, the T cell responses are late with low T cell number [40].

In a bronchoalveolar lavage study, in moderate COVID-19 patients, CD8+ T cells had higher replication, with the peculiar expression pattern of activation, migration, and cytokine and tissue-residence markers compared to severe cases [22]. In a human peripheral blood mononuclear cells (PBMCs) study with 206 subjects, including the uninfected family members of the COVID-19 patients, the number of CD4+ and CD8+ T cell numbers and frequency was significantly lower in severe cases [39]. The SARS-CoV-2-specific CD8+ T cell populations showed significant differentiation through the infection [39]. In the early stages of the disease, T cells had the expression of immune activation molecules (CD38, HLA-DR, and Ki-67), inhibitory receptors (PD-1 and TIM-3), and cytotoxic molecules (granzyme B and perforin) [39].

In a PBMCs study with 20 subjects, SARS-CoV-2 epitopes from structural and non-structural proteins such as nsp1, nsp3, nsp4, nsp6, nsp12, and ORF3a, and ORF8 stimulated CD4+ T and CD8+ T cell IL-2 or IFN-γ- production [12]. Additionally, the CD4+ T cell response was significant in moderate disease cases compared to severe cases [12].

Another piece of evidence suggesting the importance of the T cells in the immune response is the Lymphopenia associated with severe COVID-19 cases [6,43,47]. In a PBMC study with 522 subjects, the highest T cell number in moderate cases was 652, while the lowest T cell number in severe patients was 64.3 [6]. There was a negative correlation between the T cell numbers and inflammatory cytokines such as IL-6, IL-10, and TNF-α [6]. Moreover, in severe cases, exhausted T cells expressed immune-inhibitory factors such as PD-1 and Tim-3 [6].

Several clinical studies have supported predictions of the computational studies that the immune presentation of the SARS-CoV-2 nonstructural protein peptides [8,10,13,29,34,46]. In a Denmark cohort study, in 18 COVID-19 patients, SARS-CoV-2-reactive T cell content reached 27% of all the CD8+ T cells count [36]. SARS-CoV-2-derived immunodominant peptides presented through HLAs were from ORF3 and ORF1ab, not the spike protein [36]. In a United States cohort study, SARS-CoV-2-derived immunodominant peptides presented were majorly from ORF1ab and, 10% of the HLA epitopes were spike protein [9]. In another cohort study, ORF1ab showed immunodominant CD8+ T cell epitopes compared to epitopes deriving from the spike protein with a shorter duration of T cell immunity [10]. In a Spanish cohort study, SARS-CoV-2 specific CD4+ and CD8+ T cells localized in the respiratory tract suggested limiting the infection progression and airway re-infections [11]. In that study, SARS-CoV-2-specific CD4+ and CD8+ T cells expressed interferon γ, CD107a, interleukin-4, and interleukin-10 [11]. Thus, the CD8+ T cells prevent airways infections in the airways and suppress systemic inflammation [11].

Moreover, in numerous animal models, the intranasal vaccination stimulated the T cell responses and disease prevention. In mice, SARS-CoV-2 S protein mRNA in adenovirus vaccine was administered intranasally stimulated T cells and disease protection [2,14]. Similarly, in hamster-modified vaccinia virus Ankara vectored SARS-CoV-2 S protein intranasal mRNA vaccine programmed T cells and prevented the disease development [4]. In another study on mice, S and N proteins of SARS-CoV-2 delivered with intranasal adenovirus and DNA plasmid vectors provided T cell immunity and disease prevention [19].

In another approach in mice, intranasally delivered recombinant RBD domian of SARS-CoV-2 S protein, stimulated T cell immunity and protection against the SARS-CoV-2 including omicron variant [21].

There are several studies supporting the protective capacity of SARS-CoV-2 ORF-specific T cells. Many studies showed SARS-CoV-2 ORF protein peptides T cell stimulation of IL-2, IFN-γ, TNF-α and infection target cell clearance [10,12,20,23,27,30,31,36,43]. There are commercial SARS-CoV-2 peptide pools with ORF3a peptide to monitor the T cell response [23,31]. In a PBMC study with 136 subjects, S protein, Orf3, and Orf7 reactive T cells with IL-2 and IFN- γ were detected for up to 15 months [23].

In another PBMC study with 42 subjects, T cell memory responses were high in moderate cases and low in severe cases [31]. In convalescent COVID-19, 7 of the 41 peptides of SARS-CoV-2 T cell epitopes were ORF proteins such as ORF3 and ORF8 proteins that CD8+ T cells had the memory cell markers indicating the long-term protection capacity [31].

Ferretti et al. used an unbiased, T-scan genome-wide screening and NetMHC4.0 tools to determine all possible specific, high-affinity SARS-CoV-2 epitopes effective in 78 convalescent patients' memory CD8+ T cells [9]. The major immunoreactive peptides were from nonstructural proteins ORF1ab and ORF3 [9]. The selected peptides stimulated IFN-γ secretion and CD137 upregulation in vitro on the CD8+ T cells obtained from the HLA-A ∗ 02:01 COVID-19 patients [9]. Moreover, the T cell number was lower in severe cases due to the protective role of the T cell memory. Additionally, T cell numbers were lower in the aged patients [9].

However, in another study in silico selected S protein peptides of SARS-CoV-2 failed to elicit a meaningful response which supports the prediction of the computational methods [30]. The peptides were selected with direct elution and detection of MHC binding [30]. The peptide's T cell stimulation was evaluated with the tandem mass spectrometry characterization [30]. Most of the peptides such as nsp13 elicited strong CD8+ T cell immune response in the blood samples of different HLAs such as (HLA-A0101, HLA-A0201, HLA-A0301, and HLA-A2402) [30]. Moreover, peptides IFN-γ and TNF-α secretion and target cell lyse capacity of CD8+ T cells was evaluated [30].

In a PAMB study with 180 subjects from the most common HLA allotypes to cover the world population up to 91.7% were selected. The CD4 and CD8 binding were predicted using SYFPEITHI and NetMHCpan algorithms covering all of the SARS-CoV-2 genome. Based on IFN-γ ELISPOT assays the selected peptides including from the non-structural proteins ORF1, ORF3, ORF6, ORF7, ORF8. Nelde et al., suggested the use of the multiple epitopes to improve disease protection in severe cases with low T cell count [27]. In a PBMCs study, with 18 COVID-19 patients and 38 control, the CD8 + T cell immunity against SARS-CoV-2 was evaluated. The 3141 MHC binding peptides from the SARS-CoV-2 genome were predicted on ten HLA molecules with NetMHCpan 4.1 algorithm. The immunodominant 122 peptides were mostly from the ORF1 and ORF3 using DNA-barcoded peptide-MHC complex (pMHC) multimers on T cells. The SARS-CoV-2 peptide-induced secretion of IFN-γ and TNF-α was detected in all tested patients. However, the severe cases unlike many studies showed a higher amount of T cell activity which was considered to be related due to the immunosuppressive medication or anti-IL6 antibody therapy [36]. In a PBMCs study with 36 individuals, T cell effectivity was evaluated after recovery from the COVID-19. The peptides were unbiased method without using an algorithm with the peptide selection was limited to the N protein and non-structural proteins NSP7 and NSP13 of ORF1 regions of SARS-CoV-2. Based on IFNγ ELISpot assay, the NSP7 and NSP13 response was detected only in 12 out of 36 COVID-19-convalescent individuals tested [20]. SARS-CoV- 2-specific T cells were functionally superior in asymptomatic individuals compared with symptomatic COVID-19 patients [20]. For example, T cells secreted higher levels of IFN-γ and IL-2 and a well-coordinated production of pro-inflammatory (IL-6, TNF-α, IL-1β) and regulatory cytokines (IL-10) than T cells

from symptomatic COVID-19 patients. In their study, Tan et al., evaluated the temporal reactivity of the different peptides from N, M, ORF7ab, ORF8, ORF3a, the NSP7 and NSP13 of ORF1ab on the PBMC of 12 patients with moderate and severe COVID-19 [43]. The moderate disease progression had rapid and early reaction by the non-strucutural protein specific T cell actitivity [43]. In the moderate cases the T cells collected in early stage of the infection were reactive to ORF7 and ORF8 but not other proteins [43]. The T cells secreted IFN-γ in vitro upon the exposure to the ORF [43]. Additionally, throughout the course of the infection, N, ORF7, ORF8, ORF3a, M, and S protein reactive polyfunctional T cell numbers were statistically corelative with the moderate and shorter disease [43]. Thus this study supports the concept of the prevention of early disease, and intracellular infestation of the SARS-CoV-2 of the lung tissue with nsp responsive TRM which, could be programmed with vaccination.

SARS-CoV-2 as a pandemic-grade contagious virus has an unprecedented capacity to pass the first line of defense of lung epithelium glycocalyx mucus and antiviral peptides with its flat sialic-acid binding domain [28,41]. The second line of defense is based on the elicitation of the epithelium receptors that SARS-CoV-2 manipulates as a viral escape entry [28,41]. Therefore, SARS-CoV-2 rapid entry overruns third line of the defense that is innate immune cells such as dendritic cells, alveolar macrophages, natural killer cells, and neutrophils [28,41].Internalized SARS-CoV-2 replicates several cycles with its nonstructural proteins manipulating host translation and suppress immunity [38]. The SARS-CoV-2 infection progresses within the tissues forming the spike protein mediated membrane fusion of the cells into syncytia [5]. Therefore, COVID-19 progression is dependent on the intracellular innate immune response and T cell-mediated apoptosis of the infected cells [36,38]. That is likely that the dendritic cells and other antigen-presenting cells detect the first replication and release of the SARS-CoV-2 and stimulate the issue-resident memory T cells (TRM) located beneath the alveoli sac attached to the integrins in a dormant state [15]. Inactive TRMs upon the elicitation with antigen, could migrate into the airways, pulmonary capillaries, alveolar space, and bronchial mucosa [15]. The programmed and reactive memory CD8+ T cells, T cell receptor TCR recognize infected cells MHC presenting peptide and regulates the rapid cleaning of the infected cells with several mechanisms in 5 min [16]. Upon TCR-MHA I recognition, T cells release lytic granules such as perforin that lyse the infected cells with pores formed on the lipid bilayer of the membranes [16]. The perforin-mediated pores causes membrane disintegration rapid cell death [16]. Another group of cytosolic proteins released by the T cells is the serine proteases granzymes, which cleave the CPP-32 caspase protein that further activates nuclease caspase-activated deoxyribonuclease (CAD), leads to apoptosis [16].

The CD8+ T cells cause apopotis of the infected cells in 5 minuties with endogenous nucleases that stimulates DNA fragmentation, and viral nucleic acids cleavage to limit its escape [16]. Another mechanism is through the T cell Fas to the membrane Fas ligand of the infected cells that stimulate caspase activity leading to apoptosis [16]. Cytotoxic CD8 T cells secrete cytokines IFN-γ, TNF-α, and TNF-β. IFN-γ directly inhibits viral replication and induces the MHC class I system simultaneously to increase the rate of peptide presentation to other T Cells [16]. Additionally, IFN-γ activates macrophages, recruiting them to sites of infection both as effector cells and as antigen-presenting cells. TNF-α or TNF-β can synergize with IFN-γ and interleukin-2 (IL-2) in phagocytic macrophage activation and killing some target cells through their interaction with TNFR-I [16]. Additionally, T cells stimulate B cell neutralising antibody generation through the MHC II antigen presentation [16].

In COVID-19 patients, survival was correlated with TRM T cells with an active antiviral profile [32,42]. After infection, some of the lung effector T cells phase into TRMs and stay in a dormant state to prevent future infections with rapid reaction [15,32,42]. conducted a study based on the blood and airways of the COVID-19 patients [42]. The CD4+ and CD8+ T cells in COVID-19 airways were predominantly TRM with tissue residence markers CD69, and CD103, in the airways [42]. Additionally, T cells had the activation profiles of surface phenotypes and antiviral molecule expression, such as perforin [42]. In a study, nasal and blood samples of 20 hospitalized COVID-19 patients were analyzed in temporal 2 to 61 d after the infection Roukens et al., 2021. The lymphopenia in the blood samples was not detected in the nasal mucosa in severe cases [32]. In moderate cases, the T cell numbers declined after the infection, and the only prominent T cells were the TRM CD38 + CD8+ suggesting the antigen-specific long-term protective capacity in the airways that could rapidly deter SARS-CoV-2 infection [32].

Possibly, there is already a piece of convincing evidence that SARS-CoV-2 nsp-specific T cells would be the predominant drivers of protective immunity. Thus the SARS-CoV-2 ORF coding nsp are highly conserved with common cold-causing endemic Coronaviruses HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HcoV-229E [12]. Thus, in many studies the cross-reactivity based on coronavirus ORF-specific T cell immunity was detected in up to 81% of the individuals who did not develop COVID-19 [13,20,27].

Clinical and animal studies supporting the ORF peptide epitopes COVID-19 prevention capacity with robust T cell response. In a PBMC study with patients who recovered from COVID-19, the SARS-CoV-2 N protein and CD8+ T cell epitopes were investigated using computational tools [47]. The SARS-CoV-2 N protein peptide pool of was constructed with the PeptGen website [47]. Additionally, the overall 57 potential epitopes from the N protein-peptide pool were selected with the NetMHC website [47]. Amongst the selected peptides the HLAA* 1101 restricted CD8+ T cell specific epitope N25 had the highest IFN-γ stimulation capacity after 10 h of incubation on the T cells collected from the patients [47].

Zhuang et al., 2021 tested the concept of SARS-CoV-2 ORF vaccines on the mice expressing the human receptor (Ad5-hACE2) [48]. After the vaccination with Venezuelan equine encephalitis replicon particles coding the truncated SARS-CoV-2–specific T cell peptides form SARS-CoV-2 S N M and E structural and ORF3a, ORF6, ORF7a, ORF8, ORF9b, and ORF9c non-structural proteins, the reactive CD4 +, and CD8+ T cells were isolated from mice lungs [48]. The CD4+ and CD8+ T cells were collected from the bronchoalveolar lavage fluids, lung tissues, and spleens of the vaccinated mice [48]. The T cells were reactive to ORF3a, N, and S protein peptides and secrete IFN-γ, TNF, IL-10, and IL-2 cytokines and lyse peptide-pulsed target splenocytes cells in vivo [48]. SARS-CoV-2–specific CD4+ T cells and CD8+ T secrete the activation (CD44, etc.) and cytotoxicity markers (such as CD107a/b) in response to the peptide exposure [48]. Interestingly the SARS-CoV- 2–specific T cells number was much higher than T cells in the lungs, DLNs, and spleens as, seen in SARS-CoV and MERS-CoV suggested the reactive capacity of the T cells to invade the airways for viral clearance [48]. SARS-CoV-2–specific CD4+ T cells and CD8+ T had airway migration and localization markers such as adhesion molecules (CD11a and CD49d) and chemokine receptors (CXCR3, CXCR6, and CCR5) [48].

In several studies the concept of global vaccination, and universal immunity against SARS-CoV-2 was proposed [27,10]. The global immunity concept derives from the use of peptides with high binding capacity to common HLAs and using the mixture of different peptides to cover all of the population with different HLA [10,27].

For instance, Nelde et al., used SYFPEITHI and NetMHCpan, to identify all possible SARS-CoV-2-ORFs T cell spesific peptites [27]. To cover the global population most common HLA classes such as HLA-A*01:01, -A*02:01, -A*03:01, -A*11:01, -A*24:02, -B*07:02, -B*08:01, -B*15:01, -B*40:01 and -C*07:02 for HLA-I and HLA-DRB1*01:01, -DRB1*03:01, -DRB1*04:01, -DRB1*07:01, -DRB1*11:01 and -DRB1*15:01 for HLA-DR were predicted. This

prediction covered up to 91.7% of the world population due to inclusion of at least one allotype [27].

Several factors reduce the T cell numbers and, dysregulate the function such as age and comorbidities of obesity, diabetes, and hypertension which is a limiting factor in T cell-based immune protection strategy in COVID-19 prevention [42,18,45]. TRM cells decline with aging and the majority of lung T cells are tissue-resident memory T cells (TRM) that traffic the pulmonary capillaries and bind to the lung parenchyma through the integrins [15]. Recently in the skin model, the declined integrin expression with aging was associated with the histone mutations which could be the case in airway tissues as well [33]. Similarly, the Dendritic cells (DCs) in the airways with their denticles reach through the alveoli surface and regulate innate immunity in lung tissue [15]. After interaction with the pathogen and activation, mature DCs migrate to the lung draining lymph nodes, and present antigens to naïve T cells [15]. DCs in old aged population has less phagocytosis and migration capacity [3].

Another comorbidity is diabetes where the hyperglycemia-mediated ROS reduces the T cell function of cytokines TNFa and IFNc release, lowering the TRM T cell numbers in the tissues [45]. Thus in several studies, COVID-19 patients with Diabetes mellitus had lower CD4+ and CD8+ T cell numbers [45]. Another comorbidity is obesity that which the excessive fat tissue releases the leptin that dysregulates T cell functions and induces senescence which was seen in different diabetic COVID-19 cases [18].

The lymphocyte malfunction due to the prolonged stimulation is a well-established fact in chronic wounds that the lymphocytes must leave the damaged tissue after the pathogen clearance [7]. However, as seen in the chronic wounds in the severe cases of COVID-19 the T cells differentiated into inflammatory or ineffective stages stall in the infected tissue [7]. Since, as modeled by Sette and Crotty the T cells must have an early protective during COVID-19 infection burst following the decline of its number [40].

For instance, T cells in COVID-19 could phase into different forms with distinct disease outcomes, such as CD127+ T h1 cells were associated with the survival but IL6 + CD8+ T was correlated with the fatality [26]. Additionally, in severe and fatal cases of COVID-19, the bystander lung homing CXCR4+ T cells increased excessively in number without any antiviral effect but harmful with the release of toxic cytokines [26]. Similarly in a PBMCs study in 14 severe cases, the SARS-CoV-2–specific T cells levels were higher compared to convalescent individuals [37]. However, the polyfunctional CD4+ T cells secreting IFN-γ, TNF-α, and IL-2 were lower in severe cases [37]. In severe cases, T cells differentiated to secrete a lower amount of cytokines IFN-γ, IL-2, and TNF-α and increased expression of the markers CTLA-4 and PD-1, and Ki67. [37]. Additionally, CD4 + and CD8+ T cell responses were more significant in severe COVID-19 patients which could be related to the differentiation [44]. However, as stated before the differentiation and malfunction of the T cells cannot be considered a major trait but more due to other background pathologies.

In summary, based on the given evidence from several clinical and in silico studies, intranasal nonstructural SARS-CoV-2 protein mRNA vaccines have potential to provide global immunity against COVID-19.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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