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SARS-CoV-2 specific T-cell receptors and insights into Infection, Immunity and Vaccine Design

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ABSTRACT

COVID-19 is a highly contagious novel epidemic that has exploded into the world's worst health problem in modern history. Since its outbreak in late December 2019, COVID-19, a global pandemic caused by the coronavirus SARS-CoV-2, has infected millions of the world population and killed over a quarter of a million people around the globe. NGS (next-generation sequencing) is critical in various fields due to its rapid development. The present state of knowledge on SARS-CoV-2 specific T-cell receptors and their alleged role in infection and immunity leading to the target for therapeutic interventions is summarized in this article. S (Spike) glycoprotein's RBD (receptor-binding domain) is known to initiate viral fusion by attaching to its host receptor, ACE2 (angiotensin-converting enzyme-2). T-cells are critical components of the adaptive anti-viral immune response as they eliminate infected cells and aid in selecting virus-specific antibodies. When severe COVID-19 individuals and healthy controls were differentiated, peripheral T and NK cell frequencies were significantly decreased, particularly for innate-like T-cells and diverse $CD8^+$ T-cell subsets. However, the proportions of several activated $CD4^+$ T-cell subgroups within the T-cell compartment were elevated and clonally dilated, including Th1, Th2, and Th17-like cells. The characterization of COVID-19 TCR (T-cell receptor) groups revealed that $CD8^+$ T-cells recognize SARS-CoV-2 epitopes, including one with immune-dominant characteristics consequent from ORF1ab. These peptides are promising candidates for the development of a COVID-19 vaccine. Numerous prophylactic methods and non-pharmacological intrusions have been used to limit disease spreading, including rigorous infection control, social distancing, and patient isolation. Nevertheless, to limit the ongoing COVID-19 pandemic and prevent its reappearance, vaccines conferring lifelong protection against the pathogenic agent SARS-CoV-2 and the emerging variants related to it must be developed.

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Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus-2; MERS-CoV, the Middle East Respiratory Syndrome Coronavirus; COVID-19, Coronavirus Infected Disease-2019; NGS, Next-generation Sequencing; ACE2: Angiotensin-Converting Enzyme 2; RBD, Receptor-Binding Domain; TCR, T-cell Receptor; BCR: B-cell Receptor; CDR3: Complementarity-Determining Region 3; ORF: Open Reading Frame.

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Introduction

COVID-19 (Coronavirus disease 2019) is produced by a novel beta strain coronavirus named SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). WHO (World Health Organization) has declared it a pandemic. It is a pathological state that leads to severe pneumonia and death [1]. SARS-CoV-2 infects humans via ACE2 (angiotensin-converting enzyme-2), the same receptor-like SARS-CoV [2,3] (Figure 1). SARS-CoV-2 infection elicits a robust immune response via CTLs (cytotoxic T-cells) and Th (helper T-cells), which ultimately results in the death of infected cells [4,5]. Besides cellular immunity, SARS-CoV-2 S protein is efficient in inducing humoral immunity. The adaptive immune system is critical in the etiology of SARS-CoV-2 infections [6-8]. SARS-CoV-2 N protein has also been demonstrated to induce significant T-cell responses in COVID-19 patients, indicating that S and N proteins may serve as primary targets for cellular immunity in COVID-19 [9, 10]. In most instances, T and B-cells respond rapidly to an infection in a coordinated manner, developing a robust memory pool that is detectable months after exposure independent of the severity of the illness [11, 12]. SARS-CoV-2 specific antibodies are difficult to detect within two to three years of infection, although memory T-cells specific for SARS-CoV-2 have been observed 11 years after SARS [13]. Twelve months post-infection, there were detectable memory T-cell responses to SARS-CoV epitopes [14].

Elevated $CD8^+$ T-cell frequency in the lungs is associated with enhanced SARS--CoV-2 development control. Immune memory is proficient in protecting against re-infection with SARS-CoV-2[15]. Interest--ingly it has been found that T-cells are being cross-reactive to common cold coronaviruses as $CD4^+$ cells were detected in COVID-19 patients along with healthy donors [16,17]. Although further demonstration is necessary, the speculations of cross-reactive immunity still await experimental trial to elucidate whether this pre-existing T-cell response is protective. This pre-existing cross-reactive immunity has inclusive inference since it could illuminate the characteristics of different COVID-19 clinical outcomes or influence the effectiveness of COVID-19 candidate vaccines [18]. Recent developments have been accomplished to develop vaccines against COVID-19.

In this review article, we focus on the SARS-CoV-2 specific T-cells, which are at the forefront of all viral immune responses in COVID-19 individuals, their significant role in viral clearance, and the successive formulation of antibody-mediated protection against viral infection. Moreover, we describe, besides antibodies, B- and T-cells arbitrate immunity to COVID-19 and the development of T-cell response against SARS-CoV-2. Therefore, we aim to highlight the critical need to develop T-cells based COVID-19 vaccine in addition to B-cells in this review article.

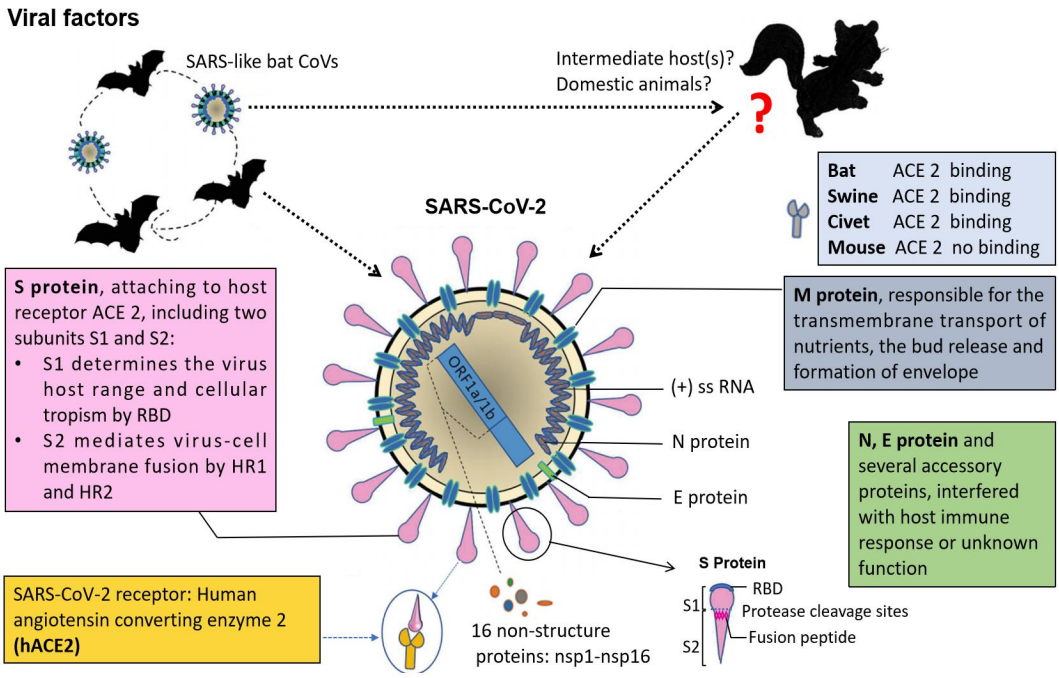


Figure 1: Viral factors that influence the pathogenesis of SARS-CoV-2.

Bats are the reservoirs for the broad range of coronaviruses, including those associated with SARS-CoV. SARS-CoV-2 might have originated in bats or other anonymous intermediate hosts and then infected humans. Viral RNA encodes 16 NSPs (non-structural proteins) primarily located in the ORF1a/b, four critical structural proteins, including S (spike) glycoprotein, M (matrix) protein, N (nucleocapsid) protein, and E (envelop) protein, along with accessory proteins. SARS-CoV-2 S protein adheres to ACE2 (angiotensin-converting enzyme 2) receptors on the host cell, vital in the virus entry process. Additional virus proteins may play a role in pathogenesis. *Adapted from Guo et al. 2020, Military Medical Research, 7(1), 1-10.*

Immune Response against SARS-CoV-2

The immune system is the best defense source since it reinforces the body's inherent capability to protect from pathogens (bacteria, protozoa, and viruses) and resist invasion. While the immune system is working correctly, infections are un-scrutinized. The three categories of immunity include an innate or non-specific immune response (slow response), the adaptive or non-self-pathogens immune response (rapid response), and passive immunity (artificial immunity: received from medicine and natural immunity: received from the maternal side). Adaptive immunity is critical for SARS-CoV-2 infection clearance and directly impacts clinical outcomes. An appropriate and precise

adaptive or acquired immunity can effectively eradicate the virus, most likely to occur in mild and asymptomatic cases. While in severe cases, a weak immune response results in viral infection and, if left unchecked, destroy infected organs, particularly the lungs, which exhibit a high level of ACE2 [19]. The tissue damage triggers the innate immunological and inflammatory responses in the lungs, typically accompanied by acute respiratory failure with life-threatening consequences in extreme instances[20, 21].

Based on several analyses of patients from different world regions[5, 22], it was reported that patients with severe infections had elevated TNF-α (tumor necrosis factor-α), c-reactive protein, and IL-6 (serum interleukin-6). However, patients have an unusually increased number of neutrophils in extreme situations and a substantial drop in total lymphocytes[5]. While infections with SARS-CoV-2 result in antibody production, antibody levels vary according to disease severity and virus inoculum. Antibodies (IgG, IgM) have been found in asymptomatic individuals with SARS-CoV-2 but significantly lower than in COVID-19 patients[23]. Antibodies directed against the spike protein and RBD serve as the primary target of neutralizing antibodies because they inhibit the virus from adhering to airway epithelial cells via ACE2 (the host receptor). In hospitalized COVID-19 patients, strong neutralizing antibody responses have been observed, and mAbs (human monoclonal antibodies) produced from such patients target the numerous epitopes of spike protein[24]. We still do not know how long the SARS-CoV-2 induced antibodies will protect against re-infection.

Contrary to circulating antibodies, generating SARS-CoV-2 memory B-cells and T-cells are critical for long-term protection. The respiratory, immune characteristics affiliated with the COVID-19 severity remain unknown. Recent studies have used single-cell RNA sequencing to characterize immune cells isolated from broncho-alveolar lavage fluid (BALF) of patients with varying disease severity and healthy individuals. BALF contains microenvironment information on bronch--ioles and lung alveoli. In severe COVID-19 individuals, it is characterized by high levels of pro-inflammatory monocyte-derived macrophages, whereas in moderate cases, patients have high clonally expanded $CD8^+$ T-cells[25]. Helper T-cells orchestrate the adaptive immune response, and the cytotoxic T-cells are critically involved in the clearance of virus-infected cells[26].

T-cells and their role in SARS-CoV-2 Immune Response

Airway viruses have been diversified in numerous mechanisms to escape or counterpoise the innate immune response. Consequently, adaptive immunity (T and B cells) must be activated to respond appropriately. T-cells (T lymphocytes), the major contributor to the immune system, are designed to combat infections they have not encountered[27]. When activated in the thymus, they multiply and differentiate into helper T-cells ($CD4^+$), which secrete cytokines to attract cytotoxic T-cells and macrophages to the infected area, regulatory or cytotoxic T-cells ($CD8^+$). The primary job is to kill toxic/target T-cells and memory T-cells (long-lived lymphocytes) capable of responding to antigens upon reintroduction. When familiar antigens are encountered, effector T-cells proliferate in large numbers [28]. Bone marrow-derived B-cells generate antibodies to bind virus fragments in blood and other mucosal surfaces, thereby limiting the growth of infection.

In contrast, evidence combined from prior coronaviruses (Middle East Respiratory Syndrome Coronavirus and Severe Acute Respiratory Syndrome Coronavirus 1) and the new SARS-CoV-2 advocates that T-cell responses are crucial for initial clearance of virus conversing defense via memory T-cells for a prolonged period[29]. Thus, investi--gating the SARS-CoV-2 specific T-cell immune response assists in a deeper understanding of COVID-19. Substantial efforts are in progress to develop serological tests to determine an individual's anti-viral antibody level.

TCR and clonal expansion

Two prime T-cells subsets contribute in distinct ways to the viral immune response. The activated $CD8^+$ T-cells destroy infected cells directly. In contrast, the $CD4^+$ T-cell subpopulations generate signaling molecules to modulate myeloid cell behavior, initiate and assist the CD8 response along with memory development, and participate in antigen-specific B- cell selection and affinity maturation, resulting in the generation of neutralizing antibodies. Along with the significant MHC-I molecules (histocompati--bility class-I) exhibited on the surface of T-cells, TCR is critical for pMHC (peptide--MHC) identification and cellular activation [30]. Upon antigen recognition, T-cells become activated and sustain a procedure called 'clonal expansion,' The activated T-cells multiply rapidly, generating substantial T-cells with uniform TCRs and hence similar antigen recognition [31]. $CD8^+$ T-cells expressing anti-viral TCRs lyse virus-infected cells directly by inducing apoptosis via Fas ligand and secreting pro-inflammatory mediators such as inter--feron (IFN). While antibody-based immu--nity has received much attention, mounting evidence indicates that T-cells play a crucial role in the clearance of COVID-19[32, 33].

TCR Sequencing and development of T-cell response against SARS-CoV-2

The breakthrough in sequencing methods in recent years has encouraged researchers to explore the TCR repertoire in the area of health and disease. Due to the technology (TCR-Seq), significant novel insights have been revealed into the human TCR repertoire with several individuals' precise approxim--ation of repertoire size and TCRs existence. In contrast to traditional sequencing, this approach involves the construction of a library via selective amplification of TCR gene products, conceding for capturing the repertoire with minimum financial, reagent, and data storage costs. Numerous approaches for analyzing the TCR repertoire utilizing NGS have been developed using genomic RNA (gRNA) and DNA (gDNA) input. The most frequently utilized TCR-Seq techniques are target enrichment, multiplex PCR, and 5′-RACE (RNA only).

Bulk TCR-sequencing and Single-cell TCR-sequencing

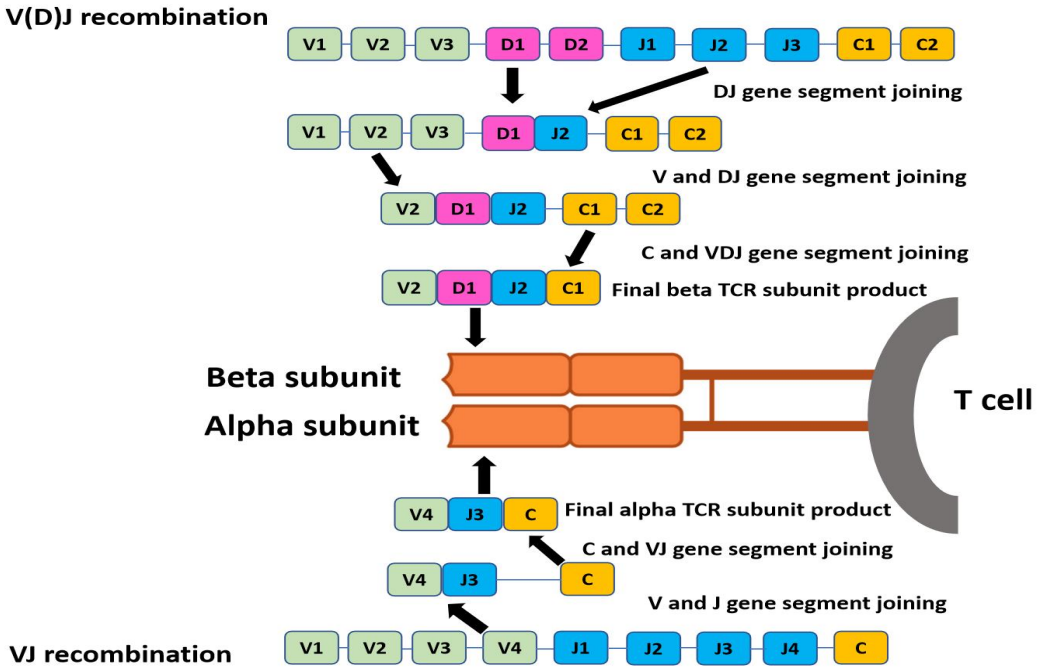
Previously stated methodologies assist bulk TCR-Seq technology to yield information regarding the dynamics, evolution, and TCR repertoire architecture during SARS-CoV-2 infections. The bulk TCR-Seq technology cannot be utilized to investigate the specific TCRs involved in anti-viral responses (speculations based on clone frequency changes)[34]. scTCR (Single-cell T-cell receptor) sequencing in conjunction with flow cytometry arrange--ment enables the precise identification of T-cells specific for SARS-CoV-2. scTCR investigation is constrained by its capacity and depth to apprehend a small number of clones. Regardless of which technology is used, both produce thousands of TCR sequences in a single run and allow the portrayal of the COVID-19 patient TCR repertoire [35]. Examining the TCR repertoire can divulge public TCRs, enabling the traceability of infection and a better understanding of its immune response. Numerous efforts are being made to analyze TCRs in COVID-19 patients. After several mild episodes of COVID-19 infection, a longitudinal high-throughput TCR-Seq study followed alterations in the T-cell repertoire. [36]. This strategy resulted in statistically confident antigen targeting and served as a guide for designing an efficient mRNA vaccination.

T-cell development and specificity

Single-cell and bulk TCR analyses revealed that the SARS-CoV-2 epitope is engrossed in cross-reactive public CDR3s (complementarity-determining region 3) and distinctive CDR3s not previously analogous to known viral antigens endorsing the use of specific VJ gene[37]. The distinguishing attribute of T-cell growth is the congregation of TCR genes from components V (variable), D (diversity), and J (joining) gene components by the process of V(D)J recombination. The T-cell's specificity for

viral antigens is determined by their TCR, shaped by V(D)J recombination for any given antigen [36-38]. The TCR comprises α (alpha) and β (beta). TCR α is formed by V and J genes segments, whereas β TCR consists of V, D, and J gene segments and undergoes random nucleotide addition and removal to constitute the mature TCR gene (Figure 2) [38]. The CDR3 of each TCR determines its antigen specificity, positioned at the V(D)J intersection.

Consequently, each T-cell clone is uniquely recognized by the CDR3 portion and by the specificity of its antigens.



Figrue 2: Schematic Diagram of the V(D)J Somatic Recombination to Generate T-cell Receptor (TCR).

The TCR is comprised of α (alpha) and β (beta) subunits. The loci encoding TCRs α and β-chains are reorganized during T-cell development. Green, pink, blue, and yellow rectangles indicate V (Variable), D (Diversity), J (joining), and C (constant) gene segments, respectively. The β-chain diversity is initially established by the combination of D and J segments, pursued by the V segments, and ultimately one of the two C segments. The α-chain variety is generated by recombining the V and J segments, tracked by a single C segment. Additionally, the procedure implies the insertion and deletion of nucleotides at the V-D, D-J, and V-J junctions for the β and α chains, thereby increasing the potential diversity of the TCR repertoire. *Adapted from Gutierrez, Beckford and Alachkar, 2020, Trends in Pharmacological Science, 41 (8), 518-530.*

A diverse TCR repertoire generated through TCR recombination efficiently identifies various antigens, resulting in potent anti--viral immunity [38, 39]. The configuration and diversity of the TCR repertoire alter the infections, both acute and chronic, cancer, aging, and various internal and external influences, enhancing its dynamic nature. With the development of high-throughput sequencing (TCR-Seq), it is now well established that the diversity of TCR repertoires falls with age, from 60-120 million to 8-57 million in adults over the age of 70 during the first two decades of life span [40-43].

Naïve and memory cells shape the human adaptive immune system and express either BCRs (B-cell receptors) or TCRs on their cell surfaces. In viral infections, $CD4^+$ T-cells (Tfh) stimulate B-cells to assemble high-affinity antibodies to neutralize pathogens, and $CD8^+$ T-cells kill cells infected by a pathogen. The naive TCR collection characterizes the population of antigen-experienced T-cells, implying that a small number of cells will exist with TCRs capable of recognizing a novel pathogen such as SARS-CoV-2. Memory TCR repertoires are the T-cells with prior antigen experience created by an individual's antigen exposure history and can be effectively employed if the same pathogen is reinforced. Although diverse antigens, such as those elicited against former coronaviruses, have been selected for memory T-cells, they can still generate a response to the novel viruses like SARS-CoV-2 [44]. T-cells (memory) are essential for viral clearance as they are detectable even after a decade of initial infection, as reported in the case of SARS-CoV-1 survivors when the viral-specific antibodies went unnoticeable [45, 46].

Several studies reported that SARS-CoV-2 specific T-cell response was observed in over 97% of convalescent COVID-19 patients and approximately 40% of unexposed donors [47, 48]. T-cell recognition appears to be generally cross-reactive with the Omicron form as well, while a huge number of mutations within spike render certain epitopes inactive for presentation or recognition [49]. The data from healthy donors may reflect the propagation of common cold coronaviruses (HCoVs). Consequently, memory T-cells generated in response to one pathogen may exhibit cross-reactivity with antigens from other pathogens, instructing previously activated memory T-cells to reactivate in response to a new pathogen. Indeed, a recent study reported that the recognition of conserved epitopes perhaps explains the cross-reactivity of HCoVs [47, 50].

While it is well established that the immune response (humoral) to coronaviruses (CoVs) is varied and frequently transient, the COVID-19 specific responses generated by T-cells may be detectable and extended over time [51]. Because adequate immunological memory persists for decades and often leads

to improved responses, it has been shown that all the modalities of memory T-cell production and response might appear in individuals who contract SARS-CoV-2 naturally [51]. Memory cell (B and T) generation is an objective for vaccine efficacy under development, and it includes several SARS-CoV-2 vaccines presently in clinical trials with humans.

SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T-cell response

SARS-CoV-2 specific T-cells are recruited from a pool of T-cells configured to detect specific viral epitopes that have been randomly generated and pre-constituted. SARS-CoV-2 specific peptides with an improved possibility of being T-cell targets were recently identified by Grifoni et al., 2020[52]. Various research groups demonstr--ated the SARS-CoV-2 specific T-cell reactivity based on peptide pool and associated definite epitopes along with their restriction elements [53-55]. Numerous studies have identified a decline in peripheral blood T-cell counts in patients positive with COVID-19, implying that degradation in both the $CD8^+$ and $CD4^+$ T-cell numbers could be a factor in increased exposure to SARS-CoV-2 infection. The immune cell lineages of individuals with positive COVID-19 revealed impaired effector functions (cytokine production) in bulk T-cells and high level of inhibitory receptor expression than bulk cells from healthy individuals. This condition worsens with the disease stage[56,57]. Previous research indic--ates that cytokine storm is associated with COVID-19 disease severity. It indicates that T-cells are either undergoing acute apoptosis or have exited the circulation. Individuals examined early in infection or at autopsy demonstrate that T-cells have a limited ability to migrate to the lungs and that TCR expression is significantly reduced during the early stages of infection (4-7days) in both moderate and severe cases. Additionally, it was revealed that $CD4^+$ T-cells are the most reactive T-cells towards SARS-CoV-2. Convalescence COVID-19 individuals maintained a significant T-cell ($CD4^+$) response towards SARS-CoV-2 that was functional as evidenced by the production of IL-2 in reaction to spike non-spike mega pool. Approximately 50% of the observed response was conducted against the S protein, remaining towards SARS-CoV-2 ORF. This response is substantial as the spike protein is a critical constituent of most of the COVID-19 vaccine candidates currently being developed. Additionally, the researchers discerned a $CD8^+$ T-cell response specific for SARS-CoV-2 in most recovered COVID-19 patients[58]. A detailed investig--ation of recognized SARS-CoV-2 specific $CD8^+$ T-cell responses indicates inflated NKG2A expression, scarcity in cytokine production, and expression profile in gene sets consistent with the T-cell re-activation. In general, $CD4^+$ and $CD8^+$ T-cell responses were highly analogous [58].

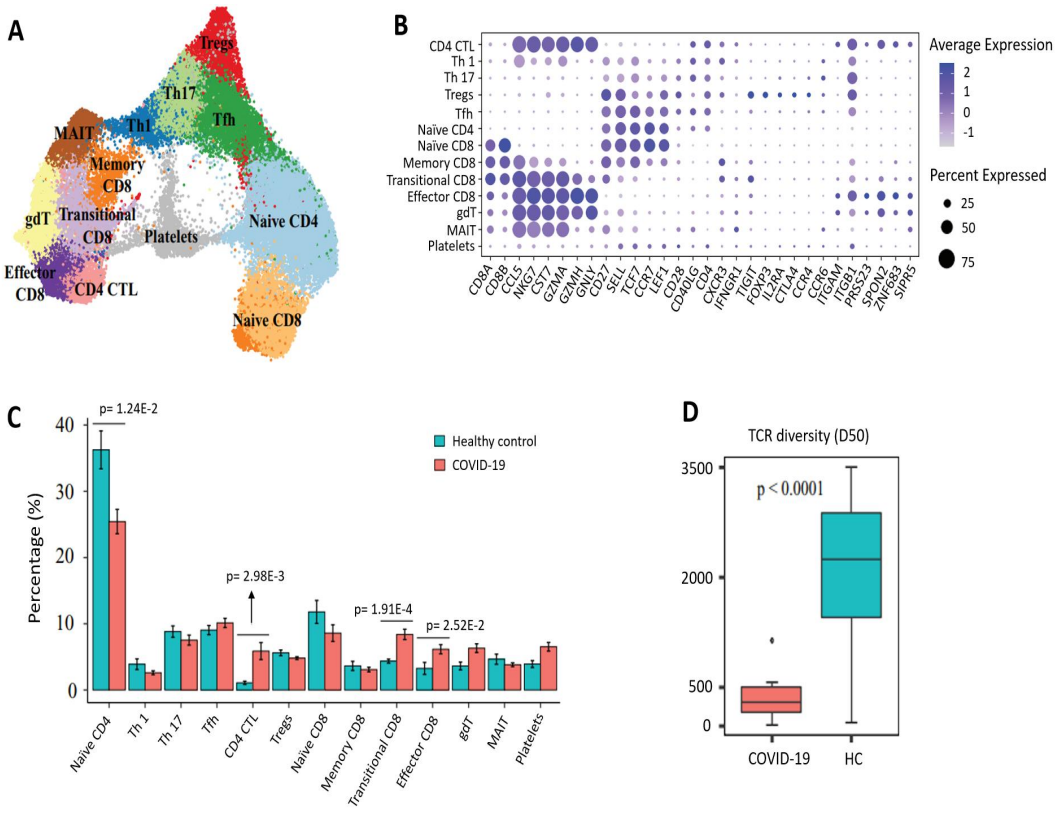
COVID-19 induced changes in T-cell response

Intend to elucidate changes in T-cell response prompted by COVID-19, a group of researchers examined a healthy cohort (cohort1) using scRNA-Seq profiling and another healthy control group (cohort2) utilizing deep TCR-Seq [59]. Thirteen definite T-cell types (Figure 3A) were identified by employing clustering phenomena, including cytotoxic CD4 CTL ($CD4^+$ T-cells), Th17 (Th17 cells), Th1 (Th1 cells), Tfh (Tfh cells), Tregs (regulatory T-cells), Naïve CD4 (naïve $CD4^+$ T-cells), Memory CD8 (memory $CD8^+$ T-cells), Naïve CD8 (naïve $CD8^+$ T-cells), transitional CD8 (transitional $CD8^+$ T-cells), gdT (gamma-delta T-cells), Effector CD8 (effector $CD8^+$ T-cells), Platelets (platelet-like cells), and MAIT (MAIT-cells). SingleR achieved cell annotations, and different cell types were distinguished using classical marker genes (Figure 3B) [36]. Among all T-cell types identified, the proportions of CD4 CTL, Effector CD8, and Transitional CD8 were significantly up-regulated in patients with COVID-19 than in healthy individuals ($P = 2.98 \times 10^{-3}$, 2.52×10^{-2}, 1.91×10^{-4} and, respectively), while Naïve CD4 proportion was down-regulated ($P = 1.24 \times 10^{-2}$) (Figure 3C). Single-cell repertoire analysis reveals that massive clonotypes exhibit a heterogeneous dispersal of cytotoxic T-cell types, including CD4 CTL, effector CD8, and transitional CD8 [59].

Deep TCR-Seq data indicate that the recovered COVID-19 patients have distinct TCR repertoires from healthy individuals. T-cell diversity was significantly lower in COVID-19 patients than in controls, consonant to clonal expansion over antigen exposure (Figure 3D) [60]. Interestingly, it was found that patients contracted with COVID-19 shared a significant number of TCR subtypes than healthy controls. It indicated that clustering TCRs with similar CDR3s is a constructive strategy for identifying antigen-specific T-cells, as TCRs with similar motifs from different individuals can also share antigen specificity [61-63]. Patient-specific TCR groups have enhanced activated T-cells, explicitly, CD4, effector CD8, and transitional CD8 cell types. Potential antigenic epitopes were revealed from the virus genome to recognize COVID-19 specific TCR groups [62].

Epitope repertoire in SARS-CoV-2

Various studies have identified specific epitopes distinguished by T-cells (CD4, CD8) in the COVID-19 positive cases with confirmed SARS-CoV-2 infections by synthesizing sets of overlapping peptides traversing the whole genome ORF of SARS-CoV-2. Most $CD4^+$ T-cells respond to distinctly expressed SARS-CoV-2 ORFs in the spike, membrane, and nucleoprotein regions. Additionally, the bulk of COVID-19 instances possessed SARS-CoV-2 specific $CD4^+$ T-cells in nsp3, nsp4, and ORF8 [47]. In non-exposed individuals, $CD4^+$ T-cell



Figrue 3: Single cell transcriptome profiling of T cells of patients with COVID-19 and controls.

(A) UMAP (Uniform manifold approximation and projection) plot of T-cells from COVID-19 patients and healthy controls. Clustering phenomena employed by unsupervised k-means with batch effects removed using normalized gene expression values.

(B) The dot plot depicts the average log-normalized expression of marker genes for the various cell types scrutinized in this data. The dot size indicates the percentage of cells in each cluster expressing the gene, and the color code indicates the average expression level post scaling..

(C) Bar plots showing the distribution of T cell types in patients with COVID-19 and healthy controls.

(D) Contrasting the diversity of D50 TCRs in patients with COVID - 19 and healthy controls. A low D50 value designates a lack of diversity besides rapid clonal expansion..

Adapted from Wang, Xu et al 2021, Clinical and Translational medicine, 11(5).

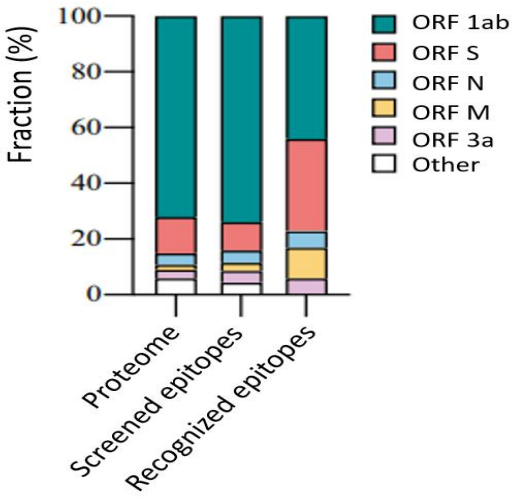
response was relatively prominent against spike. At the same time, marginal reactivity was found against SARS-CoV-2 N (nucleoprotein) and M (matrix) protein [47], indicating that circling 'common cold' coronaviruses and SARS-CoV-2 are cross-reactive. Data analysis of other coronaviruses suggests that the spike protein accounts for particularly two-thirds of the reported reactivity in the case of $CD4^+$ T-cell response. In contrast, M and N accounted for only a small proportion of reported reactivity, and no reactivity was identified in a substantial analysis of the human SARS-CoV-1 responses [64].

$CD8^+$ T-cell responses directed against ORF1ab cross were significantly more potent than those required against ORF3a, spike, M, and N. A recent study reported $CD8^+$ T-cells in COVID-19 positive patients recognized several distinct SARS-CoV-2 epitopes [65]. With few of the SARS-CoV-2 epitopes being unique to SARS-CoV-2, the majority were found to be in common with SARS-CoV-1 and several 'common-cold' coronaviruses. These epitopes originated from various sources, including ORF1ab, S, M, and N proteins. While many of these epitopes originated from ORF1ab, an enrichment for spike protein epitopes identified by $CD8^+$ T-cells was also observed (Figure 4). It has been determined that $CD8^+$ T-cell epitopes derived from SARS-CoV-2 are vastly conserved across virus variants [65, 66]. Grifoni, A. et al. reported different immuno-dominance patterns for SARS--CoV-2 specific $CD8^+$ T-cells, of which ORF1ab encodes a TTD (TTDPSFLGRY) epitope with immuno-dominant landscapes [47, 65]. Strikingly, TTD-specific $CD8^+$ T-cell response was remarkable and substantially higher in magnitude than every other $CD8^+$ T-cell response detected[65]. The immuno--dominance hierarchy is partly calculated by the naïve precursor's frequency [67]. The TCR data related to TTD-specific CD8 T-cells were analyzed while investigating several T-cell clones responsible for the immuno--dominant CD8 T-cell response specific for SARS-CoV-2. Interestingly, the data demonstrated a significant degree of diversity in TCRs, with TCR β chains comprising distinct CDR3 regions, despite similarities between TCR β and α chains within and across patients indicating a tremendous clonal TCR variability for TTD-specific responses generated by $CD8^+$ T-cells[68]. Preceding data revealed the predominance of TCRs with TRBV27 (TCR β chain V27) region contrary to the majority of $CD8^+$ T-cell repertoire, indicating that the TTD epitope-specific TCRs are probably rearranged during the T-cell maturation [69]. Collective data revealed that the epitopes from regions other than the SARS-CoV-2 S protein could influence enormous $CD8^+$ T-cell responses [47, 54, 70]. At the instant, it is uncertain whether any S protein epitopes possess immuno-dominant characteristics during the un-availability of ORF1ab and

whether $CD8^+$ T-cell responds to the S protein only, thus provoking T-cell immunity.

It is critical to identify SARS-CoV-2 specific epitopes predicted by human T-cells to monitor COVID-19 immune responses in laboratories worldwide. Generating a comprehensive picture of the T-cell antigen view utilizing the proteome of the virus in its entireness will provide insight into how precisely the antigens targeted by $CD8^+$ T-cells complement the antigen profile of currently available potential vaccine target. It will help determine whether these vaccines incorporate the immuno-dominant epitopes required for successful vaccine development. Amino acid substitutions and deletions in the SARS-CoV-2 S protein variant (B.1.1.7), also called VoC (a variant of concern), potentially enhance host ACE2 binding and viral immune evasion[71]. Presently, SARS--CoV-2 approved vaccines mainly target the spike protein or RBD, as they reserve the epitopes essential for provoking effective and efficient neutralizing antibody response [72,73]. Another stride was the identification of cross-reactive immunity in coronaviruses strains in a fraction of the human population to determine if it can affect the vulnerability to the COVID-19 pandemic.

Additionally, it is significant for vaccine manufacturing since cross-reactive immunity could affect response toward candidate vaccines[74]. A considerable cross-reactivity was identified between influenza-derived epitope (M1 GILGFVFTL) and SARS--CoV-2 TCRs in both SARS-CoV-2 expressed and non-expressed individuals [75]. For this, peptide-based vaccines are promising for treating pathogenic virulence. They contain minimal components of the infectious microbe and are, therefore, safer than other vaccine types because of their less allergic and less toxic properties [76].



Figrue 4: The bar graph on the left depicts the ORF contribution to the SARS-CoV-2 proteome contrasted with the predicted fragment of epitopes for each ORF in the middle and the contribution of each ORF to the recognized CD8 T-cell epitopes in the right bar. *Adapted from Gangaev, Ketelaars et al. 2021, Nature communications, 12(1), 1-14.*

Current vaccine candidates need to engage T-cells

The critical question is whether the defense against SARS-CoV-2 infections can be achieved through extensive vaccination or recurrent waves of disease over the next few years until approximately 60-70% of the population develops immunity. SARS-CoV-2 is unique to the human population. In contrast to SARS-CoV-1 and MERS-CoV, the genome of SARS-CoV-2 is relatively stable. The vaccination with SARS-CoV-2 S protein prompts an immune response that remarkably reduces hospitalization and death rate, so far even to viral variants like Omicron [77]. Recurrent epidemics bring out excessive-high mortality rates, severe socio-economic disturbance, and significant alterations to daily life. Thereby, to end the current COVID-19 pandemic and prevent a recurrence, an efficacious COVID-19 vaccine will need to address these issues if the virus becomes endemic and causes recurrent seasonal epidemics. A vaccine should induce $CD4^+$ and $CD8^+$ T-cell immunity and neutralize antibody production to accomplish this trouble.

On 16 March 2020, the first COVID-19 vaccine candidate entered unprecedented rapid human clinical testing [78]. In the United States, four vaccines are undergone phase III clinical trials, while the other 18 are being tested across many countries [79]. Two of the four SARS-CoV-2 candidate vaccines that have undergone phase III clinical trials in the United States are mRNA vaccines[79], the very first of their kind. A single dose of mRNA vaccine could rapidly and vigorously strengthen an immune response that has been primed by natural illness[80]. The other two use adenovirus as a vector for genetic data transmission [80]. The degree of spike-specific T-cell induction differs by vaccination subtype, with adenovirus-based platforms eliciting slightly stronger responses in some trials and mRNA-based platforms eliciting higher antibody titers in others[81]. Both types encode the SARS-CoV-2 S protein, the principal prey of neutralizing antibodies produced naturally and therapeutic monoclonal antibodies[82]. Phase III clinical trials demonstrated that mRNA vaccines provided 90–95% protection against COVID-19[82]. In a clinical trial conducted in the UK, mRNA vaccine produced a striking increase in SARS-CoV-2 S protein-specific effector T-cell responses in various age groups at different time intervals[83]. The vaccines induce a significant magnitude of neutralizing antibodies and virus-specific T-cell responses. The existing preliminary trials offer a compelling case for widespread and rapid vaccination; Within a year, more than 150 clinical-stage vaccine studies utilizing a variety of techniques were started, with varying degrees of success[84].

From a vaccine innovation standpoint, the pandemic also facilitated the regulatory approval of the first nucleic acid-based

vaccines after more than 30 years of development[85]. Vaccination against SARS--CoV-2 is quite protective so far, however, booster vaccines are required to maintain efficacy over time. Indeed, whereas many viral VOCs are capable of evading humoral immunity, vaccine-induced cellular responses exhibit significant cross-protection against VOCs, supporting the hypothesis that cellular responses contribute significantly to disease control[86]. While vaccinations have demonstrated tremendous efficacy, they are far from perfect. This not only increases the likelihood of infection by SARS-CoV-2 but also raises the possibility that subsequent infection with SARS-CoV-2 may result in vaccine-associated increased respiratory illness, as has been observed in animal trials for several vaccinations[87]. A quick fix is to improve immunity with booster vaccines [88].

Around the world, there is still a dire need for the production and distribution of the COVID-19 vaccine with excellent efficacy. Sustained masking, hand sanitation, and social distancing will be necessary to intercept viral transmission in the future irrespective of location, demographic stature, or political influences. We cannot rely solely on vaccination to overcome this pandemic. We must maintain a comprehensive outlook for preventing and eliminating the disease.

Conclusion and Future Perspective

Altogether, T-cell responses are crucial for initial eradication of virus conferring defense by memory T-cells for a longer period of time as they remain detectable decades after initial infection. T-cells activated in response to one pathogen may cross-react with antigens from the other pathogens, prompting previously activated memory T-cells to reactivate in response to a new pathogen. While studying T-cell response against SARS-CoV-2, we found that SARS-CoV-2 epitopes distinguished by T-cells (CD4, CD8) and T-cell epitopes share some similarities with other coronaviruses as cross-reactive T-cells can also be detected in a large proportion of unexposed individuals [89]. These epitopes originated from numerous sources, including ORF1ab, S, M, and N proteins, of which, ORF1ab encodes an epitope (TTD) with immunodominant properties. The TCR data for TTD-specific $CD8^+$ T-cells revealed a high degree of TCR diversity and a tremendous clonal TCR variability for TTD-specific responses mediated by $CD8^+$ T-cells. T-cell epitope prediction using computational tools has advanced significantly over the last three decades and has become a vital part of antigen discovery, epitope mapping, and vaccine design programs. Additionally, recent studies have indicated that B- and T-cells are incredibly probable, making them candidates for developing peptide-based COVID-19 vaccines[84]. Furthermore, complete epitope mapping will improve the comprehensive undertaking of T-cell responses specific to human coronavirus in

future studies, aiding vaccine development [90]. Traditional vaccines against viruses are effective, but viruses often evolve rapidly and possess antigens that are mutation prone, avoiding the antibodies recognition[91]. T-cell-mediated immunity may overcome this limitation, recognizing conserved viral HLA peptides expressed on virus-infected cells. Taking into account of the conserved regions of a virus genome, T-cell epitope-based vaccines are less susceptible to mutation and could be effective against multiple virus strains. Such T-cell epitopes can also be helpful in designing m-RNA based vaccines in future.

Competing interests

The authors declare all financial and non-financial competing interests.

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