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SARS-CoV-2 may affect the immune response via direct inhibition of T cell receptor: Mechanistic hypothesis and rationale

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ABSTRACT

During co-evolution with their hosts, many viruses have evolved a membrane fusion mechanism to facilitate host cell entry. Examples are human immunodeficiency virus type 1 (HIV-1) and severe acute respiratory syndrome coronaviruses 1 and 2 (SARS-CoV-1 and SARS-CoV-2). These viruses can also infect immune cells (e.g., T cells), providing one of the possible mechanisms for the T cell lymphopenia observed in patients with these infections. Previously, we hypothesized and confirmed *in vivo* that like HIV-1, SARS-CoV-1 can use its fusion domain not only to enter the T cell but also to directly inhibit T cell receptor signaling. Here, based on the analysis of available structural and clinical data, we hypothesize that SARS-CoV-2 may use a similar "disarm the alarm" strategy to suppress immune responses. We also discuss the implications of this hypothesis for better understanding coronavirus disease 2019 (COVID-19) pathology, developing effective COVID-19 vaccines and improving clinical outcomes for COVID-19 patients.

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1. Introduction

Coronavirus disease 2019 (COVID-19), the ongoing pandemic with over 200 million confirmed cases, including near 5 million deaths globally as of October 22, 2021, is the disease caused by severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2). The remarkably variable clinical course of COVID-19 ranges from mild symptoms in most patients to severe illness in about 5% of patients, resulting in nearly a 2% overall fatality rate [1]. A comprehensive understanding of SARS-CoV-2 pathogenesis, and especially, the interaction of this virus with the human immune system, is crucial in providing the scientific and clinical rationale for developing safe and effective COVID-19 therapies and vaccine candidates.

Recent studies revealed that SARS-CoV-2 may affect T lymphocytes, including $CD4^+$ T cells [2–4], which are the main target immune cells for human immunodeficiency virus type 1 (HIV-1) [5] and SARS coronavirus 1 (SARS-CoV-1) [6]. The SARS-CoV-1 viral spike protein 2 (S2), being a class I viral fusion protein, plays a key role in mediating SARS-CoV-1 fusion with and entry into the host cell. Functional domains of the SARS-CoV-1 S2 protein include the so-called fusion peptide (FP) that drives fusion between the viral and target T cell membranes [7,8]. Previously, we uncovered the molecular mechanisms of an inhibitory effect of HIV-1 FP on antigen-specific T cell activation *in vitro* [9] reported by Quintana et al. in 2005 [10]. Using these mechanisms and structural analysis data, we then hypothesized that HIV-1, SARS-CoV-1 and many other viruses can employ their fusion machinery not only to enter the T cell but also to directly inhibit T cell receptor (TCR) signaling, thus "disarming the alarm" of the T cell immune responses [11,12]. Our recent research demonstrated a specific inhibitory activity of the SARS-CoV-1 FP *in vivo*, further supporting this hypothesis [13]. Lymphopenia, which is observed in most patients with HIV-1 [5] and SARS-CoV-1 [14] infections, has been recently found to correlate with morbidity and mortality and have a prognostic value for COVID-19 severity [2,15]. Infection of T cells by SARS-CoV-2 can contribute to lymphopenia and provide a plausible explanation for the dysregulated inflammatory response observed in severe COVID-19 patients [2–4] as well as the reduced T cell immune activation found in COVID-19 patients with persistent SARS-CoV-2 infection [16]. These clinical observations, along with the close resemblance between SARS-CoV-2 and its predecessor SARS-CoV-1 [17], prompted us to analyze the structural features of SARS-CoV-2 in light of its interaction with human T cells. The results led us to

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conclude that like HIV-1, SARS-CoV-1 and probably, many other viruses [10,11,13], SARS-CoV-2 can use its S2 protein and, more specifically, the FP domain to directly inhibit TCR, a key player in the generation of adaptive immune responses.

Here, we discuss this hypothesis and its implications to better understand COVID-19 pathogenesis and to develop safer and more effective COVID-19 vaccines to improve clinical outcomes. In addition, having an advanced molecular understanding of viral interactions with host immune cells should help strengthen our strategy to fight not only SARS-CoV-2 but also currently unknown viruses that could emerge in the future with pandemic causing potential.

2. Viruses and T cell receptor: The use of molecular mimicry to escape the host immune response

Evolved as a complex structure to provide diverse, versatile and highly target-specific immune signaling, TCR is a member of the family of the so-called multichain immune recognition receptors (MIRRs) [18,19]. As such, the TCR complex is comprised of separate protein chains that are responsible either for antigen recognition and binding outside the cell (TCRα and TCRβ) or signaling inside the cell (ζ, CD3ε, CD3δ and CD3γ) (Fig. 1a). The TCR composite integrity is kept in the cell membrane by electrostatic interactions that occur between positively and negatively charged amino acid residues located in the transmembrane domains (TMDs) of TCR chains. Two positively charged residues of TCRα TMD interact with the negatively charged residues of ζζ and CD3εδ dimers (Fig. 1a) while one positively charged residue of TCRβ TMD interacts with the negatively charged residues of CD3εγ dimer (Fig. 1a). First reported in 2004 [19], the so-called Signaling Chain HOmoOLigomerization (SCHOOL) mechanisms of transmembrane signal transduction, which are typical for all members of the MIRR family including TCR, suggest an important role of these intramembrane interactions not only in keeping the integrity of TCR, but also in TCR signaling.

In 2009 [11], we analyzed the primary sequences of FPs of multiple, seemingly unrelated viruses including HIV-1 and SARS-CoV-1 in light of the SCHOOL mechanisms of TCR signaling and concluded that these FPs mimic TCRα TMD. Functionally, the resemblance of FPs to TCRα TMD leads to disruption of the intramembrane interactions between the TCRα, TCR ζζ and CD3εδ signaling subunits, thus blocking TCR signaling (Fig. 1b) [11]. This allowed us not only to explain the TCR-targeted immunosuppressive activity *in vitro* reported for HIV-1 FP located at the N terminus of the HIV-1 envelope glycoprotein 41 (gp41) [10,20] but also to predict similar activity for other viruses that utilize FPs for immunosuppression [11]. Example is SARS-CoV-1 with its FP (aa 770–788), identified at the N terminus of the SARS-CoV-1 S2 subunit [7,8] (Fig. 1c). Later, we proved this hypothesis for SARS-CoV-1 and demonstrated strong immunosuppressive activity of its FP in the collagen-induced arthritis (CIA) mouse model [13]. Importantly, the immunosuppressive effect was specific as administration of a control peptide containing two positively charged amino acids (K772 and K777) substituted for neutral amino acids had no effect [13].

The striking similarity is observed in the amino acid patterns of FPs from SARS-CoV-1, SARS-CoV-2 and other coronaviruses, on the one hand, and TCRα TMD, on the other hand, in that they are all characterized by two positively charged amino acid residues spaced by 4 neutral residues (Fig. 1c). This strongly suggests that like HIV-1 and SARS-CoV-1, SARS-CoV-2 can use its fusion machinery to directly inhibit TCR signaling, thus contributing to the lymphopenia and T cell immunosuppression observed in patients with COVID-19 [2,15,16]. Further studies are urgently required to test this hypothesis as well as to explore whether this TCR inhibitory activity always accompanies SARS-CoV-2 infection of T cells and vice versa.

According to our hypothesis, like FPs of HIV-1, SARS-CoV-1 and many other viruses [10–13,20], SARS-CoV-2 FP acts inside the T cell membrane (Fig. 1b), downstream of TCR engagement, which suggests its antigen-independent mechanism of receptor inhibition. Thus, SARS-CoV-2 FP-inhibited TCR can still bind to its cognate ligand but cannot transduce the signal across the cell membrane (Fig. 1b). This may contribute to impaired T cell responses to multiple viral epitopes presented by the major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs) in COVID-19 patients. Importantly, due to the antigen-independent mechanism of TCR inhibition (Fig. 1b), this may also impair T cell responses to other, virus-unrelated antigens (eg, tumor-specific antigens).

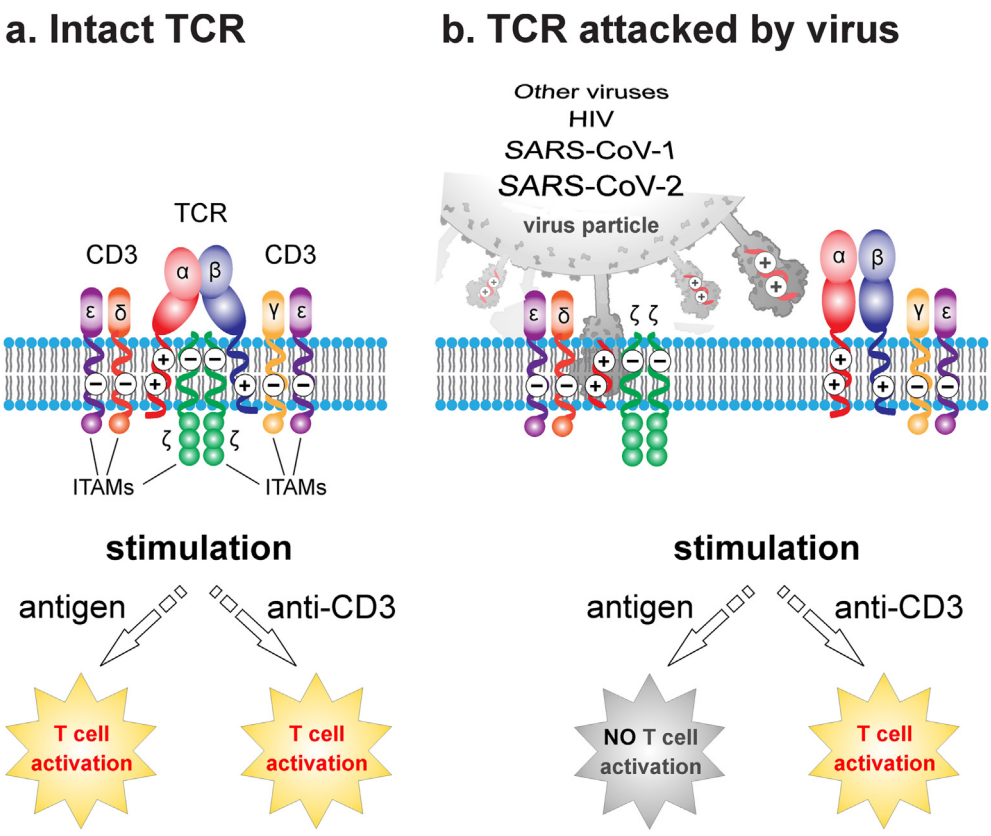
As discussed below, the concept that viruses use ligand-independent mechanisms to sabotage the immune response could significantly contribute to the development of safer and more effective COVID-19 vaccines and therapies. This would also pave the way for the development of a novel class of antiviral drugs that can be efficacious against many viruses including HIV and SARS CoVs as these seemingly unrelated viruses employ the same strategy to disarm the T cell immune responses. Taken together, it can be further concluded that prophylactic and therapeutic strategies developed for SARS-CoV-2 and COVID-19, can be also used to address future viral challenges.

3. Implications for vaccine and drug development

SARS-CoV-2 spike (S) protein that contains SARS-CoV-2 FP (aa 788–806) [21] (Fig. 1c) is the main antigenic target of interest for our own immune responses that is encoded by the current COVID-19 vaccines [22,23]. In light of the potential TCR inhibitory activity of SARS-CoV-2 discussed here, the introduction of SARS-CoV-2 S protein implies that the current vaccines can all possess the inherited capacity to suppress the T cell immune responses to a certain extent. Thus, these considerations should be taken into account when designing future vaccines against SARS-CoV-2 and other viruses that can use molecular mimicry to suppress immune responses. For example, when designing future COVID-19 vaccines, the two positively charged amino acids (K790 and K795) in the SARS-CoV-2 FP region (Fig. 1c) could be substituted for two neutral amino acids to abrogate its TCR inhibitory capacity. Considering that the cytotoxic T lymphocyte (CTL) epitopes predicted for SARS-CoV-2 do not include the corresponding sequence of its FP [17], these substitutions are unlikely to affect the efficacy of the vaccine-induced T cell responses, while simultaneously reducing the risk of their direct inhibitory effect on T cell activation.

To date, no effective therapies are available against SARS-CoV-2. Peptide and peptide-based inhibitors of viral entry including viral fusion inhibitors, have been recently proposed for the inhibition of SARS-CoV-2 infection [24] similarly to the approaches previously used against HIV-1 [25,26] and SARS-CoV-1 [27]. While promising, this strategy would likely be more effective by targeting both fusion-promoting and TCR-inhibiting viral domains. Further studies are needed to test whether therapies affecting viral inhibition of TCR signaling are synergistic with viral fusion-inhibiting approaches.

Further, the recently observed T cell immunosuppression in COVID-19 patients with persistent SARS-CoV-2 infection [16] led the authors to conclude that a combination of antiviral therapies (e.g., remdesivir [28] or convalescent plasma [29]) along with immune activation therapy (anti-PD-L1 or CTLA-4 antibody [30]) should be tested for persistent viral infections, especially in critically ill patients [16]. In this regard, it should be highlighted that the SCHOOL mechanisms of TCR signaling explain an intriguing



c. Protein sequence similarity analysis

T Cell Receptor α-Chain

TCRα Transmembrane Domain	116	VIGF**R**ILLL**K**VAGFNLLMTLR	136
TCRα Core Peptide		GL**R**ILLL**K**V	

CoV Spike Protein S2 Domain

SARS-CoV-1	770	MY**K**TPTL**K**YFGGFNFSQIL	788
SARS-CoV-1*	770	MW**K**TPTL**K**YFGGFNFSQIL	788
SARS-CoV-2	788	IY**K**TPPI**K**DFGGFNFSQIL	806
SARS-CoV *PUMC02*	770	MY**K**TPTL**K**YFGGFNFSQIL	788
SARS-CoV *BJ182-8*	770	MY**K**TPTL**K**YFDGFNFSQIL	788
Bat CoV *279/2005*	756	MY**K**TPAI**K**DFGGFNFSQIL	774
Bat CoV *Rp/Shaanxi2011*	755	MY**K**TPAI**K**DFGGFNFSQIL	773
Bat CoV *Rp/2004*	756	MY**K**TPAI**K**DFGGFNFSQIL	774
Bat CoV *HKU3*	757	MY**K**TPAI**K**DFGGFNFSQIL	775
Bat SARS-like CoV *YNLF_31C*	756	MY**K**TPTL**R**DFGGFNFSQIL	774
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Fig. 1. TCR assembly and mechanism of TCR inhibition by viruses. **(a)** T cell receptor (TCR) assembly is shown. The lipid bilayer of the T cell membrane is shown by the location of lipid head groups (spheres in light blue) and lipid tails (two curved arcs in gray). The TCRα and TCRβ chains that function to recognize an antigen outside the cell are shown in red and blue, respectively. The CD3ε, CD3δ, CD3γ and ζ chains that function to transduce the signal intracellularly are indicated as purple, dark orange, light orange and green, respectively. Immunoreceptor tyrosine-based activation motifs (ITAMs) of signaling chains are shown as spheres colored accordingly to the chain. Electrostatic interactions between basic and acidic amino acids of the TCR recognition and signaling chains bind these chains together in the cell membrane and maintain the receptor integrity. Two basic residues of the TCRα transmembrane domain (TMD) interact with acidic residues of the TMDs of CD3εδ and ζζ: a lysine, which interacts with two aspartic acid residues of the CD3εδ TMDs, and an arginine, which interacts with two aspartic acid residues of the ζζ TMDs. One basic lysine residue of the TCRβ TMD interacts with acidic aspartic and glutamic acid residues of the CD3εγ TMDs. **(b)** Virus fusion peptide that molecularly mimics TCRα TMD inserts in the cell membrane and by competing with TCRα for binding with CD3εδ and ζζ, disrupts electrostatic interactions between TCRα and these signaling chains. **(c)** Primary sequences of TCRα TMD and its core peptide aligned with spike protein S2 fusion domains of several coronaviruses including SARS-CoV-1 and SARS-CoV-2. The analysis shows a similarity in the charge distribution pattern with two essential positively charged residues (shown in bold) spaced apart by four amino acids, suggesting similar mechanisms that are used by diverse coronaviruses in their pathogenesis to inhibit the T cell immune response. *Two sequences of SARS-CoV-1 fusion domains with the single amino acid difference were reported for different strains of this virus and are both shown.

common feature of TCR inhibition by HIV gp41 FP and the so-called TCR core peptide (CP) that represents a 9-mer synthetic peptide derived from the TCRα TMD sequence (Fig. 1c): these peptides both suppress antigen- but not anti-CD3-stimulated T cell activation [10,31] (Fig. 1b). Together with the T cell suppressive effect of SARS-CoV-1 FP observed in the CIA mouse model [13], this strongly suggests that the T cell immunosuppressive effect of SARS-CoV-2 can be driven, at least in part from direct TCR inhibition by viral FP in an antigen-independent manner (Fig. 1b). Considering that this viral inhibition of TCR can be overcome by using agonistic anti-CD3 antibodies (Fig. 1b), one of the potential therapeutic strategies to activate T cell immune responses could be the use of such antibodies. Previously, the use of lymphostimulatory doses of agonistic anti-CD3 has been suggested as immune activation therapy to eradicate highly active antiretroviral therapy-persistent HIV-1 [32–34].

4. Conclusion

Since the dawn of life, in the violent interplay between viral infection and the resultant immune response, a virus is always seeking ways to infect, replicate, and persist in the host while the immune system is responding to quell the infection. Often, the virus and human host coexist symbiotically with the virus in the latent phase. In contrast, new viruses (e.g., SARS-CoV-2) that emerge or cross the species barrier will need to replicate rapidly and efficiently to proliferate as quickly as possible. This clearly poses a serious threat to the immune system often resulting in deaths. Therefore, there is an immediate need to develop novel strategies to target these viruses and treat the resultant diseases.

It would be advantageous to combine strategies that target virus-specific proteins or processes with those that target redundant processes found amongst a number of viruses. An example of such a process is the viral inhibition of TCR, the alarm of the T cell immune response, where the virus uses molecular mimicry of TCRα TMD by its fusion protein to disrupt the link between the recognition and signaling molecules of the TCR machinery. This strategy seems to be used by many seemingly unrelated viruses such as HIV-1, SARS-CoV-1, Lassa virus, lymphocytic choriomeningitis virus, Mopeia virus, Tacaribe virus, and others (reviewed in Ref. [12]) and as hypothesized here, – by SARS-CoV-2. Thus, direct inhibition of TCR may represent an important and previously underestimated mechanism in viral pathogenesis and our improved understanding of this mechanism could result in the development of powerful vaccines and drugs not only for SARS-CoV-2 but also for many other viruses including currently unknown viruses that might become a serious threat to the world population.

Declaration of competing interest

Alexander B. Sigalov is employed by SignaBlok, Inc., a company developing ligand-independent inhibitors of cell surface receptors including TREM-1, TREM-2 and TCR inhibitors.

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