NIH SPONSORED AND HAS OWNER$HIP OF VACCINE NANOTECHNOLOGY FOR A BIOWEAPON

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von Andrian et al.

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(54) **VACCINE NANOTECHNOLOGY**

(58) **Field of Classification Search**

STATEMENT OF GOVERNMENT SUPPORT

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Y is polyalkylene glycol or polyalkylene oxide. In some embodiments, X is PLGA, PLA or PGA. In some embodiments, Z is absent.

In some aspects, a composition comprising a nanocarrier comprising an immunostimulatory agent is provided. In some embodiments, the composition further comprises an antigen and/or a targeting moiety. In some embodiments, at least one of the antigen, targeting moiety, and immunostimulatory agent is conjugated to a water soluble, non-adhesive polymer. In some embodiments, at least one of the antigen, targeting moiety, and immunostimulatory agent is conjugated to a biodegradable polymer. In some embodiments, at least one of the antigen, targeting moiety, and immunostimulatory agent is conjugated to a biocompatible polymer. In some embodiments, the biocompatible polymer is a conjugate of a water soluble, non-adhesive polymer conjugated to a biodegradable polymer. In some embodiments, the antigen is a B cell antigen. In some embodiments, the B cell antigen is not a T cell antigen. In some embodiments, the nanocarrier further comprises a T cell antigen. In some embodiments, the antigen is a T cell antigen.

In some aspects, a composition comprising a nanocarrier comprising a small molecule, an immunostimulatory agent, and a T cell antigen is provided.

In some embodiments, the small molecule is a toxin. In some embodiments, the toxin is from a chemical weapon, an agent of biowarfare, or a hazardous environmental agent.

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[0402] In some embodiments, a therapeutic and/or prophylactic is a cytotoxin, a radioactive ion, a chemotherapeutic, a vaccine, a compound that elicits an immune response, and/or another therapeutic and/or prophylactic. A cytotoxin or cytotoxic agent includes any agent that may be detrimental to cells. Examples include, but are not limited to, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxyanthracinedione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, teracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol, rachelmycin (CC-1065), and analogs or homologs thereof. Radioactive ions include, but are not limited to iodine (*e.g.*, iodine 125 or iodine 131), strontium 89, phosphorous, palladium, cesium,

(57) Abstract: The present disclosure provides methods of producing lipid nanoparticle (LNP) formulations and the produced LNP formulations thereof. The present disclosure also provides therapeutic and diagnostic uses related to the produced LNP formulations.

The tiny tweak behind COVID-19 vaccines

Prepandemic coronavirus research by Jason McLellan and Barney Graham led to a trick for stabilizing the prefusion form of spike proteins

by ***Ryan Cross***

September 29, 2020 | A version of this story appeared in **Volume 98, Issue 38**

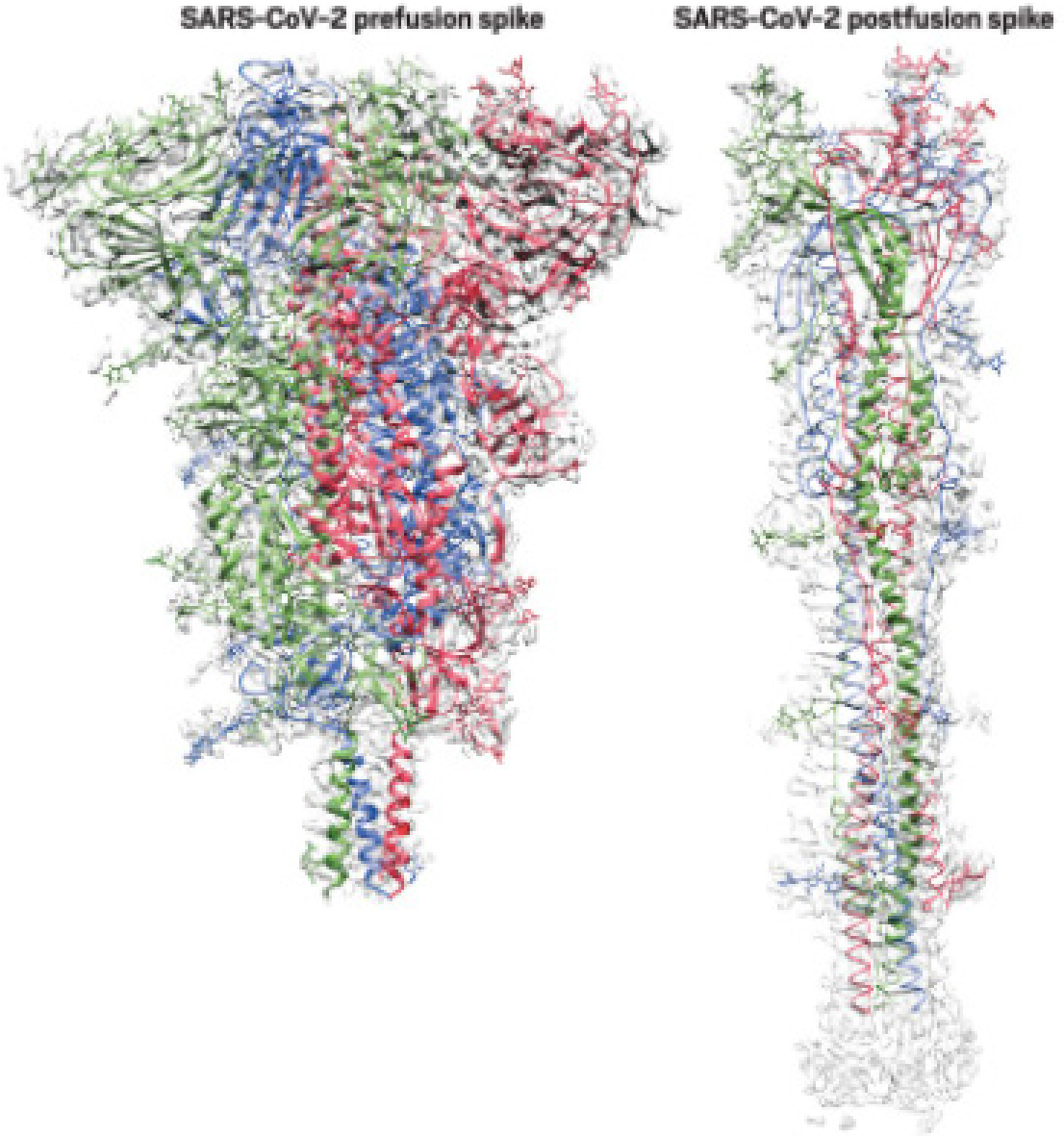
Credit: Ching-Lin Hsieh/Courtesy of Jason McLellan

Jason McLellan holding a 3-D printed structure of the SARS-CoV-2 spike protein

On Jan. 10, Chinese scientists uploaded the genetic sequence of a **novel coronavirus**, later named SARS-CoV-2, to an open-access website, GenBank. The virus had been linked to a growing number of mysterious pneumonia cases, and its rapid spread was beginning to raise alarms. A few hours later, Barney Graham woke up and saw the sequence. Though it was a Saturday, he got right to work.

Days before, his lab at the US National Institute of Allergy and Infectious Diseases (NIAID) had partnered with the biotech company Moderna to design an experimental vaccine for the virus, which causes the disease COVID-19. All they'd needed to start was that sequence.

The 2P mutation might quite literally be the smallest detail that could make or break the first generation of COVID-19 vaccines. It's an easy enough tweak to add during the early stages of vaccine design. And if successful, 2P-based vaccines may herald a new generation of vaccines whose molecular makeup is fine-tuned to craft a safer, stronger immune response.



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(54) **PREFUSION CORONAVIRUS SPIKE PROTEINS AND THEIR USE**

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(57) **ABSTRACT**

Coronavirus S ectodomain trimers stabilized in a prefusion conformation, nucleic acid molecules and vectors encoding these proteins, and methods of their use and production are disclosed. In several embodiments, the coronavirus S ectodomain trimers and/or nucleic acid molecules can be used to generate an immune response to coronavirus in a subject. In additional embodiments, the therapeutically effective amount of the coronavirus S ectodomain trimers and/or nucleic acid molecules can be administered to a subject in a method of treating or preventing coronavirus infection.

Specification includes a Sequence Listing.

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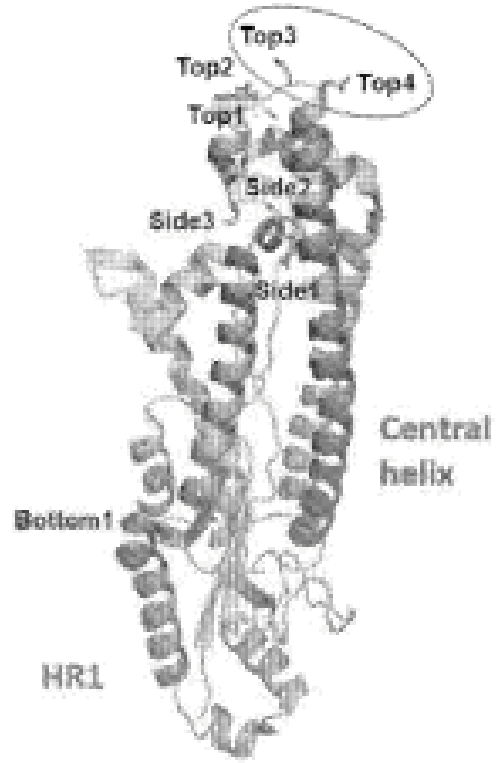

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FIG. 14A

FIG. 14B

FIG. 14C

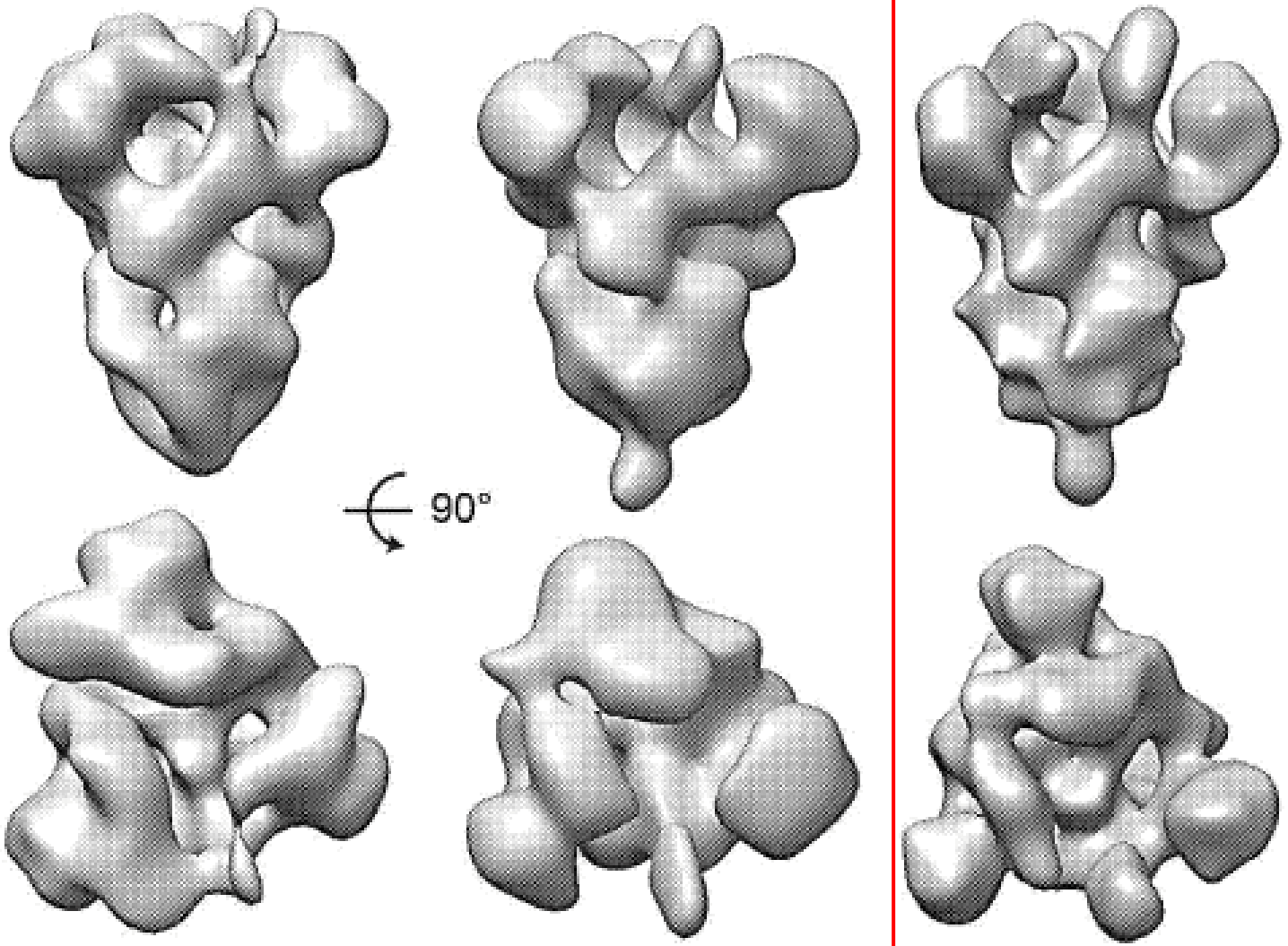

FIG. 14D

OC43 S-2P

FIG. 14E

WIV1 S-2P

FIG. 14F

PEDV S-2P

FIG. 14G

229E S-2P

Snake Venom Peptides

Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation

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Contributed by Ivet Bahar, June 29, 2020 (sent for review May 26, 2020; reviewed by Talal A. Chatila, Ruth Nussinov, and Celia A. Schiffer)

Multisystem Inflammatory Syndrome in Children (MIS-C) associated with COVID-19 is a newly recognized condition in children with recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. These children and adult patients with severe hyperinflammation present with a constellation of symptoms that strongly resemble toxic shock syndrome, an escalation of the cytotoxic adaptive immune response triggered upon the binding of pathogenic superantigens to T cell receptors (TCRs) and/or major histocompatibility complex class II (MHCII) molecules. Here, using structure-based computational models, we demonstrate that the SARS-CoV-2 spike (S) glycoprotein exhibits a high-affinity motif for binding TCRs, and may form a ternary complex with MHCII. The binding epitope on S harbors a sequence motif unique to SARS-CoV-2 (not present in other SARS-related coronaviruses), which is highly similar in both sequence and structure to the bacterial superantigen staphylococcal enterotoxin B. This interaction between the virus and human T cells could be strengthened by a rare mutation (D839Y/N/E) from a European strain of SARS-CoV-2. Furthermore, the interfacial region includes selected residues from an intercellular adhesion molecule (ICAM)-like motif shared between the SARS viruses from the 2003 and 2019 pandemics. A neurotoxin-like sequence motif on the receptor-binding domain also exhibits a high tendency to bind TCRs. Analysis of the TCR repertoire in adult COVID-19 patients demonstrates that those with severe hyperinflammatory disease exhibit TCR skewing consistent with superantigen activation. These data suggest that SARS-CoV-2 S may act as a superantigen to trigger the development of MIS-C as well as cytokine storm in adult COVID-19 patients, with important implications for the development of therapeutic approaches.

shock syndrome (TSS) (8, 9) (Table 1), rather than Kawasaki disease (KD), due to marked demographic, clinical, and laboratory differences (6). Indeed, a recent uncontrolled retrospective case study concluded that MIS-C is distinct from KD and KD shock syndrome (10). The similarities to TSS and the association of MIS-C with COVID-19 led us to hypothesize that SARS-CoV-2 may possess superantigenic fragments that induce an inflammatory cascade and may also contribute to the hyperinflammation and cytokine storm observed in severe adult COVID-19 patients (3, 4). The question we raised is, does SARS-CoV-2 spike (S) possess superantigenic fragments that could elicit such reactions upon binding proteins involved in the host cell cytotoxic adaptive immune response? Such a reaction was not observed in the SARS-CoV pandemic of 2003 (SARS1 hereafter). What is unique to SARS-CoV-2, and how might recent mutations in SARS-CoV-2 S have promoted such an increased virulence?

TSS can be caused by two types of superantigens (SAgs): bacterial or viral. Bacterial SAgs have been broadly studied. They include proteins secreted by *Staphylococcus aureus* and *Streptococcus pyogenes* that stimulate massive production of

Significance

A hyperinflammatory syndrome reminiscent of toxic shock syndrome (TSS) is observed in severe COVID-19 patients, including children with Multisystem Inflammatory Syndrome in Children (MIS-C). TSS is typically caused by pathogenic superantigens stimulating excessive activation of the adaptive immune system. We

Analysis of NGS immunosequencing data from COVID-19 patients

Blood collection from 38 patients (42 samples) with mild/moderate COVID-19, and 8 patients (24 samples) with severe/hyperinflammatory COVID-19 was performed under institutional review board approval number 2020-039. The patients and controls, and their immune repertoires, were part of a previously published cohort (16). For details of NGS data acquisition, please refer to our earlier work (16). Only productive TRB rearrangements were used and all repertoires were normalized to 20,000 reads. For the analyses, we used R version 3.5.1 for plotting of TRBV and TRBJ gene usage as previously described (17, 18). Differences in principal component analysis were studied by Pillai–Bartlett test of MANOVA. To study TRBJ gene diversity, J genes were extracted if

they were part of rearrangements containing TRBV rearrangements expanded in patients with hyperinflammatory COVID-19. Frequencies of J gene families were summarized per repertoire and plotted separately for each rearrangement. See Fig S7.

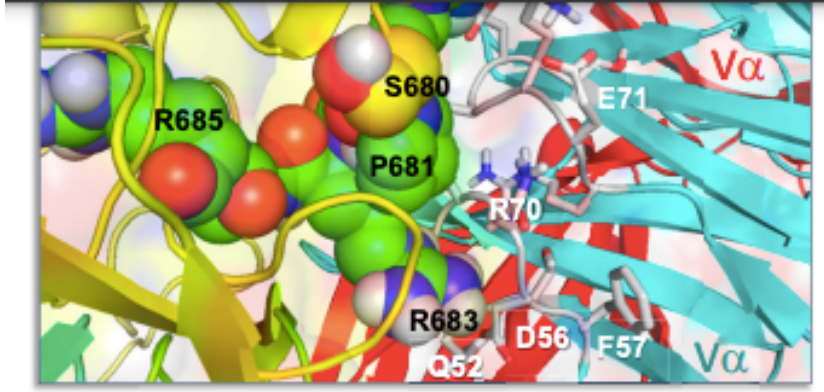

Fig. 1. Binding of TCR to SARS-CoV-2 spike trimer near the "PRRA" insert. (*A*) Overall and (*B*) close-up views of the complex and interfacial interactions. In *A*, the spike monomers are colored white, ice blue/gray, and spectrally from blue (N-terminal domain) to red, all displayed in surface representation. The N and C termini and RBD of the spectrally colored monomer, which also binds the TCR, are labeled; for better visualization, the S trimer is oriented such that its RBDs are at the bottom. TCR α- and β-chains are in red and cyan ribbons. In *B*, the segment S_{680}PPRAR$_{685}$ including the PRRA insert and the highly conserved cleavage site R685 is shown in van der Waals representation (black labels); nearby CDR residues of the TCR Vβ domain are labeled in blue/white. See additional information in *SI Appendix*, Fig. S1.

Further Examination of the Motif near PRRA Reveals Close Structural Similarity to the SEB Superantigen as well as Sequence Similarities to Neurotoxins and a Viral SAg. The insertion PRRA together with seven sequentially preceding residues and succeeding R685 (conserved among β-CoVs) form a motif, Y_{674}QTQTNSPRRAR$_{685}$, homologous to those of neurotoxins from *Ophiophagus* (cobra) and *Bungarus genera*, as well as the neurotoxin-like regions from three RABV strains (20) (Fig. 2*D*). We further noticed that the same segment bears close similarity to the HIV-1 glycoprotein gp120 SAg motif F164 to V174. This close sequence similarity to both bacterial and viral SAgs, in support of the potential superantigenic character of the stretch Y674 to R685 of SARS-CoV-2 S, directed us to further analyze its local sequence and structure.

Our analysis led to an interesting sequence similarity between the fragment T678 to Q690 of SARS-CoV-2 S and the SEB superantigenic peptide T_{150}NKKKATVQELD$_{161}$ (Fig. 3*A*). This aligned against various bacterial or viral SAgs (Figs. 2*C* and 3 *A*–*C*) with or without the participation of the adjoining amino acids. However, combined broader sequence and structure analysis in Fig. 3 *A* (*Right*) and *B* and *C*, reveals an even more compelling feature: This putative SAg core is structurally consolidated by spatial proximity to a conserved acidic segment, $E_{661}CD_{663}$, which forms a highly stable salt bridge with the polybasic segment PRRAR of SARS-CoV-2 S, much in the same way as the salt bridge observed in SEB (but not in SARS1 S), complemented by an asparagine shared between SARS-CoV-2 S and SEB (but not SARS1 S), and the SAg character may be conferred by this type of structural scaffolding.

We note that the SEB superantigen peptide Y_{150}NKKKATVQELD$_{161}$ has been reported to bind CD28 (21), a TCR that provides costimulatory signals required for T cell activation and survival. CD28 and TCRV domains share the same (immunoglobulin, Ig) fold (Fig. 3*E*), and the binding mechanism shown in Fig. 1*B* could adapt, with minor rearrangements, to interactions with other Ig-fold molecules including neutralizing antibodies. Because of the homologous superantigenic segment of SEB binding CD28, we also tested the potential binding of SARS-CoV-2 spike residues E661 to R685 onto CD28. Our simulations indicated that the same segment can equally bind to CD28, further supporting the strong propensity of the fragment to stimulate T cell activation.

An ICAM-1–like Motif Shared between SARS1 and SARS-CoV-2 Spikes Interacts with TCRVα to Further Stabilize the S-TCR Complex. The existence of potential superantigenic, toxic, or intercellular adhesion molecule (ICAM)-like fragments in SARS1 was thoroughly examined by Li et al. (23) following the 2003 pandemic. This led to the identification of the nine sequence stretches including three *Botulinum* neurotoxin type D or G precursors and two motifs that are highly similar to ICAM-1. Comparative analysis with SARS-CoV-2 S sequence revealed that seven of these motifs are conserved (with >68% sequence identity) between SARS1 and SARS-CoV-2 spikes (*SI Appendix*, Fig. S3). Among them, the ICAM-1 (CD54)-like motif Y_{279}NENGTITDAVDCALDPLSETKC$_{301}$ also participates in the association between the SARS-CoV-2 S and αβTCR as shown in Fig. 4.

ICAM-1 involvement is critical to mediating immune and inflammatory responses. The observed interaction of the ICAM-1–like motif of SARS-CoV-2 S with TCRVα, in tandem with the interaction of the above discussed putative SAg motif (around