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Communications

Fever temperatures modulate intraprotein dynamics and enhance the binding affinity between monoclonal antibodies and the spike protein from SARS-CoV-2

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ABSTRACT

Fever is a typical symptom of most infectious diseases. While prolonged fever may be clinically undesirable, mild reversible fever (<39°C, 312 K) can potentiate the immune responses against pathogens. Here, using molecular dynamics and free energy calculations, we investigated the effect of febrile temperatures (38°C to 40°C, 311 K to 313 K) on the immune complexes formed by the SARS-CoV-2 spike protein with two neutralizing monoclonal antibodies. In analyzing the conformational dynamics of the interactions between the antibodies and the spike protein under different thermal conditions, we found that, at mild fever temperatures (311–312 K), the binding affinities of the two antibodies improve when compared to the physiological body temperature (37°C, 310 K). Furthermore, only at 312 K, antibodies exert distinct mechanical effects on the receptor binding domains of the spike protein that may hinder SARS-CoV-2 infectivity. Enhanced antibody binding affinity may thus be obtained using appropriate temperature conditions.

1. Introduction

SARS-CoV-2 is a new coronavirus responsible for the ongoing pandemic of coronavirus disease 2019 (COVID-19). To gain entry into human cells, SARS-CoV-2 uses the transmembrane viral S glycoprotein (Spike) that protrudes from the viral surface and constitutes the main target of current vaccines [1]. On COVID-19 infection, fever is a main symptom and a key feature of the immune response [2]. Fever that accompanies COVID-19 is generally treated with the aim of eliminating it [3]. Recently, a study showed that at 313 K (40°C), Spike attachment to ACE2 receptor is impaired [4]. It is unclear, however, what the role of fever may be on the formation of immune complexes. Here, we show that protein complexes formed between therapeutic antibodies and Spike proteins benefit from the effects of febrile temperatures. We choose two potent monoclonal antibodies (mAb), CV30 and S309, which are reported to neutralize SARS-CoV-2 [5,6]. We determine that at 312 K (39°C), the binding affinity of mAb towards Spike is highest when compared to 310 K (37°C, core body temperature), 311 K (38°C, mild fever) or 313 K (40°C, acute fever). Further, the detailed modes of the mechanisms of antibody neutralization under fever temperatures were uncovered: at 312 K, CV30 "pulls away" the receptor binding domain (RBD_1) off the Spike monomer it is bound to, from the RBD_2 and RBD_3 belonging to the other chains that do not contact the mAb. In so doing, CV30 exposes the $RBD_{2,3}$ to cell receptor binding or to further mAb attachment. In contrast, at 312 K and at 313 K, the S309 has the reverse role on Spike organization and in effect closes the trimer structure, rendering cell entry impossible. An overview of the dynamics between Spikes and these mAb at relevant temperatures is shown in Fig. 1.

We established the center of mass (COM) for each of the three RBDs and determined the extent of translations with respect to each other, at fixed intervals of 0 ns, 250 ns and 500 ns, using the positions of COM of each RBD at 310 K as reference (Supplementary Table 1). For the CV30: Spike system, at 312 K, the distances between RBD_1 (chain A) versus $RBD_{2,3}$ of chain B or chain C increase by 6.2 Å and 20.1 Å, respectively. For the S309: Spike complex, at 312 K, RBD_1 approaches RBD_2 by 10.6 Å, while at 313 K, the distance between same RBDs is reduced by 13.8 Å.

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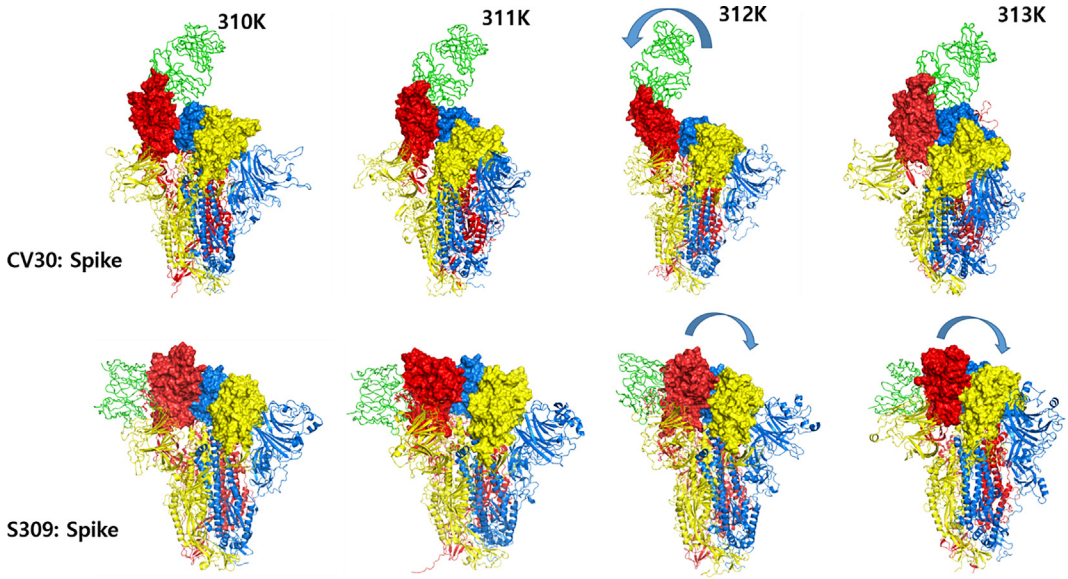

Fig. 1. Snapshots taken after 500 ns of the Spike proteins in complexes with CV30 or with S309, at the indicated temperatures. Chain A (red cartoon) contains the RBD_1 that is solely bound to respective antibodies (shown as green ribbons). Chains B and C are colored in yellow or blue cartoons, respectively; RBDs are shown as surfaces. Arrows indicate the translational movements of RBD_1 at 312 K or at 313 K. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Further, to gauge the extent of the changes in position of each RBD with respect to itself, across all temperatures, we determined the shifts along their rotation axes, using the RBD positions at 310 K as reference (Table 1).

At 312 K, each RBD in the CV30: Spike complex exhibits the largest translation away against own positions at 310 K, while the opposite situation is encountered for the RBDs from the S309: Spike immune protein complex at 312 K and 313 K. For either immune complex, the origins of these RBD movements within the Spike trimer are found in the presence of short β-strands (RBD_1 sequence residues 354–358, 452–455 and 492–494) that are connected by interstrands hydrogen bonds and help maintain a rigid binding interface with CV30 at 310, 311 and 313 K. Conversely, at 312 K, these secondary structures are lost and greater flexibility is measured that allows for the RBD_1 swing motions away from the other two RBDs (Supplementary Fig. 1). While the same phenomenon occurs with the immune complex formed with S309, the presence of β-strands (at 310 K and 311 K) or their absence (at 312 K and 313 K) involves residues that are not part of RBD_1 (residues 312–319). Additionally, for this complex, more extended β-strands are comparatively found at 310 K and 311 K, that help maintain the position of RBD_1 with respect to other RBDs (residues 592–594, 661–663), resulting in an enhanced flexibility only at 312 K and 313 K.

We quantified the extent of global structural changes across temperatures using the RMSD and Rg parameters (Supplementary Figure 2) as well as complex formation or dissociation between the mAb and the Spikes, using the Buried Surface Area (BSA) parameter (Table 2) and. For the CV30 bound to Spikes, BSA decreases by 18 % at 312 K, mirroring the movement away from the other RBDs, while it monotonically increases from 310 K to 312 K. In contrast, for the S309: Spike system, the increase in BSA is nonlinear with temperatures, peaking at 312 K, in line with RBD_1 movement towards the other RBDs.

Next, we determined the extent of intra-monomer movements, using again BSA as a proxy for the formation of interfaces, as shown in Supplementary Table 2. While modest changes in BSA within the Spike monomers occur for the CV30: Spike complex (a 7 % increase at both 312 K and at 313 K with respect to 310 K), a 21 % peak increase at 312 K versus 310 K is present in the S309: Spike immune complex. Dissociation between the Spike monomers has two key roles for viral infectivity: to expose the fusion molecular machinery required for cell entry [7] and to promote RBD opening necessary for binding to cell entry receptor ACE2 [8]. Fever modulation of increased intra-monomer BSA may thus affect Spike intraprotein dynamics, with adverse consequences on viral entry into cells.

In order to further quantify the strength in the binding affinity between the mAb and the RBD_1, we estimate the changes in hydrogen bond formation across temperatures (Supplementary Figure 3 and Supplementary Table 4) and further calculated their free binding energy, as shown in Table 3.

In agreement with the results presented in Tables 1–3, we measured an inverse relation between increasing temperatures and the binding free energy of CV30 in complex with RBD_1, peaking at 311 K. In contrast, the highest affinity is present at 312 K for S309 bound to RBD_1, in line with the movements of this RBD within the Spike at various febrile temperatures. While the values measured at 311 K and at 312 K are not significantly higher than the reference values at 310 K, they do indicate that further improvements in binding affinity of mature mAb may be possible simply through appropriate temperature control, as experimentally determined with microcalorimetry for other viral complexes [9].

To control the ongoing pandemic, it is imperative to modulate the fundamental dynamics of the SARS-CoV-2 Spike, as its motions are key to the infection machinery [10]. Despite the increasing interest in the use of fever as an adjuvant to therapy [11], temperature modulation of immune complex formation, has just recently been investigated, at 313 K [4,9]. Observational studies reported worse outcomes among COVID-19 patients having a lower body temperature (<36°C, 309 K) on admission, suggesting an impaired immune response [12]. We here present the first results highlighting a mechanism wherein a range of febrile temperatures are shown to: (1) modulate domain movements in a viral protein and (2) increase mAb binding affinity against viral epitopes at particular fever values. In this study, antibodies bind directly to RBD, as with CV30 [5], or away from RBD, as in the case of S309 [6], revealing a possible allosteric effect of febrile temperatures in the formation of immune complexes. While fever is a beneficial and

Table 1
Translational shifts along the axes of rotations between the position of RBDs at 310 K and the positions of RBDs from the immune complexes under relevant febrile temperatures. The negative sign indicates translations away from the rotation axes.

Shift along axis of rotation (Å)	CV30: Spike			S309: Spike		
	311 K	312 K	313 K	311 K	312 K	313 K
RBD_1 (0 ns)	+2.1	−1.1	−1.5	+0.2	+0.1	+0.2
RBD_1 (500 ns)	+13.4	−35	−5.8	+3.5	+16.4	+0.9
RBD_2 (0 ns)	−1.2	−0.3	−0.4	+0.2	−0.2	−0.04
RBD_2 (500 ns)	+20	−40	−13	+3.9	+14	+14
RBD_3 (0 ns)	+1	+0.6	+1.1	+0.2	−0.1	−0.3
RBD_3 (500 ns)	+15	−58	+15	+5.5	+16	+10.7

Table 2
Changes in BSA between the mAb and the Spike proteins throughout the simulations at the indicated temperatures. In brackets, percentage increase or decrease against values at 0 ns.

BSA ($Å^2$)	310 K CV30: Spike	311 K CV30: Spike	312 K CV30: Spike	313 K CV30: Spike	310 K S309: Spike	311 K S309: Spike	312 K S309: Spike	313 K S309: Spike
0 ns	1285	1296	1533	1313	1287	1078	1068	1327
500 ns	1412 (+9%)	1480 (+14 %)	1260 (-18 %)	1679 (+27 %)	1534 (+19 %)	1510 (+40 %)	1660 (+55 %)	1936 (+45 %)

Table 3
Binding free energies between Spike and the two paratopes, at physiological and at febrile temperatures. In brackets, percentage increase or decrease with respect to the reference values at 310 K. Averaged values with standard deviations obtained every 50 ns throughtout the total 500 ns of simulations.

Binding Free Energy (kCal/mol)	310 K	311 K	312 K	313 K
CV30: RBD_1	−18.6 ± 2.6	−24 ± 0.8 (+29 %)	−23 ± 1.2 (+23 %)	−19 ± 2.1(+2%)
S309: RBD_1	−71 ± 11	−59 ± 12 (-17 %)	−96 ± 10 (+35 %)	−88 ± 7(+23 %)

evolutionarily conserved immune reaction to acute infections [13,14], it is important to consider whether these febrile temperature values are important for COVID-19 therapy. In a hospital setting, four pertinent temperature-based clinical phenotypes have been identified indicating that normothermic patients with a maximum body temperature (MBT) of 37.1 ± 0.4°C and febrile, but rapid (1.4 ± 0.7 days) defervescent patients (MBT of 38.7 ± 0.5°C) had the highest survival rate [15]. In turn, the highest mortality was observed in the patients with gradual fever onset (MBT of 38.7 ± 0.4°C) and febrile, but slow (3.1 ± 1 days) defervescent group (39.3 ± 0.5°C) [15]. Furthermore, a majority of patients presented MBT above 38°C at ICU admission, with 44 % having between 38.1°C 39°C and 34 % with MBT above 39°C [16]. These results may indicate that, depending on duration of the fever onset, maintaining a fever temperature of around 39°C in for a brief period (e.g. up to 24 h), followed by chemically reducing MBT may potentiate the role of antibodies in recognizing viral epitopes and in reducing viral infectivity. Furthermore, such thermal therapy may assist patients at other levels of the immune response, as elevated febrile temperatures (40°C) were shown recently to prevent SARS-CoV-2 replication in respiratory epithelia without disruption of epithelium integrity [17]. It is important to stress that these therapeutical measures can only be administered in a hospital setting using whole body hyperthermia [18], so as to prevent significant side effects that can affect the cardiovascular or the nervous systems.

In conclusion, in infected patients, any advantage in the fight against viruses may be relevant, including through the use of fever as a means to control the dynamics of immune complexes. Further *in silico* and *in vitro* investigations are necessary to determine whether these observations can be extended to immune complexes belonging to other pathogens or to relevant cancers.

2. Methods

The complex structure of SARS-CoV-2 spike and each antibody was obtained from the Protein Data Bank (PDB ID: 6VSB for Spike alone, 6WPS for S309 and 6XE1 for CV30). As the complex structure of CV30 was defined only using the receptor binding domain (RBD) of the spike protein, the spike bound state was modelled using Modeller [19]. Simulations were performed using GROMACS 2020.2. Structures were pre-minimized using amber99sb force field with SPC/E explicit solvent model, equilibrated to each tested temperature (310–313 K) using NVT ensemble, followed by NPT ensemble equilibration. The production run was conducted for 500 ns (400 ns for Spike system) with 2 fs time step and snapshots were taken every 10 picoseconds. The leapfrog integration method, V-rescale thermostat and Parrinello-Rahman barostat were used. LINCS constraints were applied to hydrogen bonds and long range interactions were included using Particle Mesh Ewald method. The changes in root-mean-square deviation (RMSD) of the whole complex and radius of gyration of the system vs. time were calculated using GROMACS. The preservation of the binding event was examined from the changes in BSA of receptor binding motif (Asn437-Tyr508). The binding free energy was computed using MM-PBSA method, for each of the three simulated trajectories, every 50 ns (11 snapshots per run). Center of mass for each RBD and RBD translational movements with respect to each other were calculated using UCSF ChimeraX [20] and the Domain Rotation tool.

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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Author Contributions

RCS designed the study. RCS and YC secured funding. DGK performed the experiments. RCS wrote the manuscript. DGK, HSK, YC revised the final draft.

Classification

Biophysics and computational biology.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2022.10.045.

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