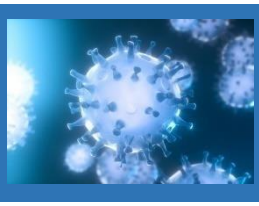

RESEARCH PUBLICATIONS

Publication Date: Feb 17, 2021

A potential interaction between the SARS-CoV-2 spike protein and nicotinic acetylcholine receptors

Abstract

Changeux *et al.* recently suggested that the SARS-CoV-2 spike protein may interact with nicotinic acetylcholine receptors (nAChRs), and that such interactions may be involved in pathology and infectivity. This hypothesis is based on the fact that the SARS-CoV-2 spike protein contains a sequence motif similar to known nAChR antagonists. Here, we use molecular simulations of validated atomically detailed structures of nAChRs, and of the spike, to investigate the possible binding of the Y674-R685 region of the spike to nAChRs. The binding of the Y674-R685 loop to three nAChRs, namely the human α4β2 and α7 subtypes and the muscle-like αβγδ receptor was examined, from Tetronarce californica. The results predict that Y674-R685 has affinity for nAChRs. The region of the spike responsible for binding contains a PRRA motif, a four-residue insertion not found in other SARS-like coronaviruses. The conformational behaviour of the bound Y674-R685 is highly dependent on the receptor subtype: it adopts extended conformations in the α4β2 and α7 complexes, but is more compact when bound to the muscle-like receptor. In the α4β2 and αβγδ complexes, the interaction of Y674-R685 with the receptors forces the loop C region to adopt an open conformation, similar to other known nAChR antagonists. In contrast, in the α7 complex, Y674-R685 penetrates deeply into the binding pocket where it forms interactions with the residues lining the aromatic box, namely with TrpB, TyrC1 and TyrC2. Estimates of binding energy suggest that Y674-R685 forms stable complexes with all three nAChR subtypes. Analyses of simulations of the glycosylated spike show that the Y674-R685 region is accessible for binding. It was suggested that a potential binding orientation of

the spike protein with nAChRs, in which they are in a non-parallel arrangement to one another.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(21)00146-6

Biomechanical characterization of SARS-CoV-2 spike RBD and human ACE2 protein-protein interaction

Abstract

The current COVID-19 pandemic has led to a devastating impact across the world. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (the virus causing COVID-19) is known to use the receptor-binding domain (RBD) at viral surface spike (S) protein to interact with the angiotensin-converting enzyme 2 (ACE2) receptor expressed on many human cell types. The RBD-ACE2 interaction is a crucial step to mediate the host cell entry of SARS-CoV-2. Recent studies indicate that the ACE2 interaction with the SARS-CoV-2 S protein has a higher affinity than its binding with the structurally identical S protein of SARS-CoV-1, the virus causing the 2002–2004 SARS outbreak. However, the biophysical mechanism behind such binding affinity difference is unclear. This study utilizes combined single-molecule force spectroscopy and steered molecular dynamics (SMD) simulation approaches to quantify the specific interactions between SARS-CoV-2 or SARS-CoV-1 RBD and ACE2. Depending on the loading rates, the unbinding forces between SARS-CoV-2 RBD and ACE2 range from 70 to 105 pN and are 30–40% higher than those of SARS-CoV-1 RBD and ACE2 under similar loading rates. SMD results indicate that SARS-CoV-2 RBD interacts with the N-linked glycan on Asn90 of ACE2. This interaction is mostly absent in the SARS-CoV-1 RBD-ACE2 complex. During the SMD simulations, the extra RBD-N-glycan interaction contributes to a greater force and prolonged interaction lifetime. The observation is confirmed by our experimental force spectroscopy study. After removing N-linked glycans on ACE2, its mechanical binding strength with SARS-CoV-2 RBD decreases to a similar level of the SARS-CoV-1 RBD-ACE2 interaction. Together, the study uncovers the mechanism behind the difference in ACE2 binding between SARS-CoV-2 and SARS-CoV-1 and could help develop new strategies to block SARS-CoV-2 entry.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(21)00141-7

Publication Date: Feb 16, 2021

COVID-19 and the human innate immune system

Abstract

The introduction of SARS-CoV-2 into the human population represents a tremendous medical and economical crisis. Innate immunity - as the first line of defense of our immune system - plays a central role in combating this novel virus. Here, a conceptual framework was provided for the interaction of the human innate immune system with SARS-CoV-2 to link the clinical observations with experimental findings that have been made during the first year of the pandemic. Review was evidenced that variability in innate immune system components among humans is a main contributor to the heterogeneous disease courses observed for COVID-19, the disease spectrum induced by SARS-CoV-2. A better understanding of the pathophysiological mechanisms observed for cells and soluble mediators involved in innate immunity is a prerequisite for the development of diagnostic markers and therapeutic strategies targeting COVID-19. However, this will also require additional studies addressing causality of events, which is so far lacking behind.

Reference

https://www.cell.com/cell/fulltext/S0092-8674(21)00218-X

Publication Date: Feb 15, 2021

Preterm care during the COVID-19 pandemic: A comparative risk analysis of neonatal deaths averted by kangaroo mother care versus mortality due to SARS-CoV-2 infection

Abstract

Background: COVID-19 is disrupting health services for mothers and newborns, particularly in low- and middle-income countries (LMIC). Preterm newborns are particularly vulnerable. We undertook analyses of the benefits of kangaroo mother care

(KMC) on survival among neonates weighing ≤2000 g compared with the risk of SARS-CoV-2 acquired from infected mothers/caregivers.

Methods: Two scenarios over 12 months were modelled. Scenario 1 compared the survival benefits of KMC with universal coverage (99%) and mortality risk due to COVID-19. Scenario 2 estimated incremental deaths from reduced coverage and complete disruption of KMC. Projections were based on the most recent data for 127 LMICs (~90% of global births), with results aggregated into five regions.

Findings: The worst-case scenario (100% transmission) could result in 1,950 neonatal deaths from COVID-19. Conversely, 125,680 neonatal lives could be saved with universal KMC coverage. Hence, the benefit of KMC is 65-fold higher than the mortality risk of COVID-19. If recent evidence of 10% transmission was applied, the ratio would be 630-fold. We estimated a 50% reduction in KMC coverage could result in 12,570 incremental deaths and full disruption could result in 25,140 incremental deaths, representing a 2·3–4·6% increase in neonatal mortality across the 127 countries.

Interpretation: The survival benefit of KMC far outweighs the small risk of death due to COVID-19. Preterm newborns are at risk, especially in LMICs where the consequences of disruptions are substantial. Policymakers and healthcare professionals need to protect services and ensure clearer messaging to keep mothers and newborns together, even if the mother is SARS-CoV-2-positive.

Reference

https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(21)00013-4/fulltext

Patient factors and temporal trends associated with COVID-19 in-hospital mortality in England: an observational study using administrative data

Abstract

Background: Analysis of the effect of COVID-19 on the complete hospital population in England has been lacking. The aim was to provide a comprehensive account of all hospitalised patients with COVID-19 in England during the early phase of the pandemic and to identify the factors that influenced mortality as the pandemic evolved.

Methods: This was a retrospective exploratory analysis using the Hospital Episode Statistics administrative dataset. All patients aged 18 years or older in England who

completed a hospital stay (were discharged alive or died) between March 1 and May 31, 2020, and had a diagnosis of COVID-19 on admission or during their stay were included. In-hospital death was the primary outcome of interest. Multilevel logistic regression was used to model the relationship between death and several covariates: age, sex, deprivation (Index of Multiple Deprivation), ethnicity, frailty (Hospital Frailty Risk Score), presence of comorbidities (Charlson Comorbidity Index items), and date of discharge (whether alive or deceased).

Findings: 91 541 adult patients with COVID-19 were discharged during the study period, among which 28 200 (30·8%) in-hospital deaths occurred. The final multilevel logistic regression model accounted for age, deprivation score, and date of discharge as continuous variables, and sex, ethnicity, and Charlson Comorbidity Index items as categorical variables. In this model, significant predictors of in-hospital death included older age (modelled using restricted cubic splines), male sex (1·457 [1·408–1·509]), greater deprivation (1·002 [1·001–1·003]), Asian (1·211 [1·128–1·299]) or mixed ethnicity (1·317 [1·080–1·605]; vs White ethnicity), and most of the assessed comorbidities, including moderate or severe liver disease (5·433 [4·618–6·392]). Later date of discharge was associated with a lower odds of death (0·977 [0·976–0·978]); adjusted in-hospital mortality improved significantly in a broadly linear fashion, from 52·2% in the first week of March to 16·8% in the last week of May.

Interpretation: Reductions in the adjusted probability of in-hospital mortality for COVID-19 patients over time might reflect the impact of changes in hospital strategy and clinical processes. The reasons for the observed improvements in mortality should be thoroughly investigated to inform the response to future outbreaks. The higher mortality rate reported for certain ethnic minority groups in community-based studies compared with our hospital-based analysis might partly reflect differential infection rates in those at greatest risk, propensity to become severely ill once infected, and health-seeking behaviours.

Reference

https://www.thelancet.com/journals/lanres/article/PIIS2213-2600(20)30579-8/fulltext

SARS-CoV-2 infects human pluripotent stem cell-derived cardiomyocytes, impairing electrical and mechanical function

Abstract

COVID-19 patients often develop severe cardiovascular complications, but it remains unclear if these are caused directly by viral infection or are secondary to a systemic response. Here the cardiac tropism of SARS-CoV-2 in human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) and smooth muscle cells (hPSC-SMCs) was examined. It was found that that SARS-CoV-2 selectively infects hPSC-CMs through the viral receptor ACE2, whereas in hPSC-SMCs there is minimal viral entry or replication. After entry into cardiomyocytes, SARS-CoV-2 is assembled in lysosome-like vesicles and egresses *via* bulk exocytosis. The viral transcripts become a large fraction of cellular mRNA while host gene expression shifts from oxidative to glycolytic metabolism and up-regulates chromatin modification and RNA splicing pathways. Most importantly, viral infection of hPSC-CMs progressively impairs both their electrophysiological and contractile function, and causes widespread cell death. These data support the hypothesis that COVID-19-related cardiac symptoms can result from a direct cardiotoxic effect of SARS-CoV-2.

Reference

https://www.cell.com/stem-cell-reports/fulltext/S2213-6711(21)00088-6

Common genetic variation in humans impacts *in vitro* susceptibility to SARS-CoV-2 infection

Abstract

The host response to SARS-CoV-2, the etiologic agent of the COVID-19 pandemic, demonstrates significant interindividual variability. In addition to showing more disease in males, the elderly, and individuals with underlying comorbidities, SARS-CoV-2 can seemingly afflict healthy individuals with profound clinical complications. It was hypothesized that, in addition to viral load and host antibody repertoire, host genetic variants influence vulnerability to infection. Here we apply human induced pluripotent stem cell (hiPSC)-based models and CRISPR engineering to explore the host genetics

of SARS-CoV-2. It was demonstrated that a single-nucleotide polymorphism (rs4702), common in the population and located in the 3′ UTR of the protease FURIN, influences alveolar and neuron infection by SARS-CoV-2 *in vitro*. Thus, we provide a proof-of-principle finding that common genetic variation can have an impact on viral infection and thus contribute to clinical heterogeneity in COVID-19. Ongoing genetic studies will help to identify high-risk individuals, predict clinical complications, and facilitate the discovery of drugs.

Reference

https://www.cell.com/stem-cell-reports/fulltext/S2213-6711(21)00090-4

Publication Date: Feb 12, 2021

Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection

Abstract

SARS-CoV-2 has caused the global COVID-19 pandemic. Although passively delivered neutralizing antibodies against SARS-CoV-2 show promise in clinical trials, their mechanism of action in vivo is incompletely understood. Here, correlates were defined of protection of neutralizing human monoclonal antibodies (mAbs) in SARS-CoV-2-infected animals. Whereas Fc effector functions are dispensable when representative neutralizing mAbs are administered as prophylaxis, they are required for optimal protection as therapy. When given after infection, intact mAbs reduce SARS-CoV-2 burden and lung disease in mice and hamsters better than loss-of-function Fc variant mAbs. Fc engagement of neutralizing antibodies mitigates inflammation and improves respiratory mechanics, and transcriptional profiling suggests these phenotypes are associated with diminished innate immune signaling and preserved tissue repair. Immune cell depletions establish that neutralizing mAbs require monocytes and CD8+ T cells for optimal clinical and virological benefit. Thus, potently neutralizing mAbs utilize Fc effector functions during therapy to mitigate lung infection and disease.

Reference

https://www.cell.com/cell/fulltext/S0092-8674(21)00176-8

Structural insight reveals SARS-CoV-2 ORF7a as an immunomodulating factor for human CD14+ monocytes

Abstract

Dysregulated immune cell responses have been linked to the severity of coronavirus disease 2019 (COVID-19), but the specific viral factors of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were currently unknown. Herein, we reveal that the Immunoglobulin-like fold ectodomain of the viral protein SARS-CoV-2 ORF7a interacts with high efficiency to CD14+ monocytes in human peripheral blood, compared to pathogenic protein SARS-CoV ORF7a. The crystal structure of SARS-CoV-2 ORF7a at 2.2 Å resolution reveals three remarkable changes on the amphipathic side of the four-stranded β-sheet, implying a potential functional interface of the viral protein. Importantly, SARS-CoV-2 ORF7a coincubation with CD14+ monocytes ex vivo triggered a decrease in HLA-DR/DP/DQ expression levels and upregulated significant production of proinflammatory cytokines, including IL-6, IL-1β, IL-8, and TNF-α. Our work demonstrates that SARS-CoV-2 ORF7a is an immunomodulating factor for immune cell binding and triggers dramatic inflammatory responses, providing promising therapeutic drug targets for pandemic COVID-19.

Reference

https://www.cell.com/iscience/fulltext/S2589-0042(21)00155-3

Factors associated with SARS-CoV-2 infection and outbreaks in long-term care facilities in England: A national cross-sectional survey

Abstract

Background: Outbreaks of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have occurred in long-term care facilities (LTCFs) worldwide, but the reasons why some facilities are particularly vulnerable to outbreaks are poorly understood. We aimed to identify factors associated with SARS-CoV-2 infection and outbreaks among staff and residents in LTCFs.

Methods: A national cross-sectional survey of all LTCFs was done providing dementia care or care to adults aged 65 years or older in England between May 26 and June 19, 2020. The survey collected data from managers of eligible LTCFs on LTCF characteristics, staffing factors, the use of disease control measures, and the number of confirmed cases of infection among staff and residents in each LTCF. Survey responses were linked to individual-level SARS-CoV-2 RT-PCR test results obtained through the national testing programme in England between April 30 and June 13, 2020. The primary outcome was the weighted period prevalence of confirmed SARS-CoV-2 infections in residents and staff reported via the survey. Multivariable logistic regression models were fitted to identify factors associated with infection in staff and residents, an outbreak (defined as at least one case of SARS-CoV-2 infection in a resident or staff member), and a large outbreak (defined as LTCFs with more than a third of the total number of residents and staff combined testing positive, or with >20 residents and staff combined testing positive) using data from the survey and from the linked survey–test dataset.

Findings: 9081 Eligible wLTCFs were identified, of which 5126 (56·4%) participated in the survey, providing data on 160 033 residents and 248 594 staff members. The weighted period prevalence of infection was 10·5% (95% CI 9·9–11·1) in residents and 3·8% (3·4–4·2) in staff members. 2724 (53·1%) LTCFs reported outbreaks, and 469 (9·1%) LTCFs reported large outbreaks. The odds of SARS-CoV-2 infection in residents (adjusted odds ratio [aOR] 0·80 [95% CI 0·75–0·86], p<0·0001) and staff (0·70 [0·65–0·77], p<0·0001), and of large outbreaks (0·59 [0·38–0·93], p=0·024) were significantly lower in LTCFs that paid staff statutory sick pay compared with those that did not. Each one unit increase in the staff-to-bed ratio was associated with a reduced odds of infection in residents (0·82 [0·78–0·87], p<0·0001) and staff (0·63 [0·59–0·68], p<0·0001. The odds of infection in residents (1·30 [1·23–1·37], p<0·0001) and staff (1·20 [1·13–1·29], p<0·0001), and of outbreaks (2·56 [1·94–3·49], p<0·0001) were significantly higher in LTCFs in which staff often or always cared for both infected or uninfected residents compared with those that cohorted staff with either infected or uninfected residents. Significantly increased odds of infection in residents (1·01 [1·01–1·01], p<0·0001) and staff (1·00 [1·00–1·01], p=0·0005), and of outbreaks (1·08 [1·05–1·10], p<0·0001) were associated with each one unit increase in the number of new admissions to the LTCF relative to baseline (March 1, 2020). The odds of infection in

residents (1·19 [1·12–1·26], p<0·0001) and staff (1·19 [1·10–1·29], p<0·0001), and of large outbreaks (1·65 [1·07–2·54], p=0·024) were significantly higher in LTCFs that were for profit versus those that were not for profit. Frequent employment of agency nurses or carers was associated with a significantly increased odds of infection in residents (aOR 1·65 [1·56–1·74], p<0·0001) and staff (1·85 [1·72–1·98], p<0·0001), and of outbreaks (2·33 [1·72–3·16], p<0·0001) and large outbreaks (2·42 [1·67–3·51], p<0·0001) compared with no employment of agency nurses or carers. Compared with LTCFs that did not report difficulties in isolating residents, those that did had significantly higher odds of infection in residents (1·33 [1·28–1·38], p<0·0001) and staff (1·48 [1·41–1·56], p<0·0001), and of outbreaks (1·84 [1·48–2·30], p<0·0001) and large outbreaks (1·62 [1·24–2·11], p=0·0004).

Interpretation: Half of LTCFs had no cases of SARS-CoV-2 infection in the first wave of the pandemic. Reduced transmission from staff is associated with adequate sick pay, minimal use of agency staff, an increased staff-to-bed ratio, and staff cohorting with either infected or uninfected residents. Increased transmission from residents is associated with an increased number of new admissions to the facility and poor compliance with isolation procedures.

Reference

https://www.thelancet.com/journals/lanhl/article/PIIS2666-7568(20)30065-9/fulltext

SUPPLEMENT

Publication Date: Feb 12, 2021

Elucidating the mechanism of membrane destabilization by the preferred modes of insertion of the Sars-Cov2 fusion peptide

Cell penetration of the SARS-Cov2 virus after recognition by the ACE2 receptor requires the fusion of the viral envelope membrane with cellular membranes. The Spike protein (S) of the virus harbors a region identified as the “fusion peptide” (FP) which is liberated at its N-terminal site by a specific cleavage occurring in concert with the interaction of the receptor binding domain of the Spike. Studies have shown that the SARS-Cov2-FP binds Ca2+ ions and perturbs membranes in a calcium-dependent fashion. But the mechanisms of membrane insertion and destabilization remained unclear. We have identified the preferred modes of SARS-Cov2-FP insertion and the role of Ca2+ ions in mediating peptide-membrane interactions from extensive atomistic molecular dynamics (MD) simulations and trajectory analyses. Multiple Ca2+ binding modes in which the ion engaged with different pairs of acidic residues on the FP were observed. A systematic sampling of the interactions of these Ca2+-bound peptide models with lipid membranes showed that SARS-Cov2-FP penetrated the bilayer only in two modes involving different structural domains. In the first mode the hydrophobic residues F833/I834 from the middle region of the peptide are inserted. The second, and more prevalent mode of penetration involves residues L822/F823 in the freed N-terminus of the peptide. The latter insertion was related to the specific mode of Ca2+ association with the peptide in which one Ca2+ is bound to the D830/D839 pair and another to E819/D820. These findings explain the mechanistic role of the necessary cleavage (termed S2’) that frees the N-terminus of the peptide to effectuate the cell-entry of SARS-Cov2. Computational resources provided by the COVID-19 HPC Consortium are gratefully acknowledged.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31793-8

Essential dynamics that drive Sars-Cov-2 spike conformational changes

The ongoing COVID-19 pandemic caused by the SARS-CoV-2 virus has prompted the need for rapid development of effective vaccines to attenuate this global emergency. The Spike glycoprotein present on the surface of this virus is known to elicit immune response and has been a prime candidate for vaccine design. This trimeric protein plays a critical role in infection where its Receptor Binding Domain (RBD) moves upwards from the rest of protein making it free to bind with the host ACE2 receptor. This is followed by S1-S2 subdomain disengagement and cell entry. Structural understanding of such inter-domain motions is important for obtaining an effective molecular handle over this protein, and in turn, exploiting it towards improved immunogen development. We performed large-scale molecular dynamics simulations of the soluble form of the Spike in both 'down' and 'up' conformations of the RBD, and employed Principal Component Analysis (PCA) of the inter-residue correlated fluctuations to extract major collective modes that dominate the functional dynamics. We observe that there is significant influence of coupled dynamics connecting the RBD, the N-terminal Domain (NTD) and the S2 region that can drive RBD transitions. Moreover, both RBD and NTD sample disparate rotational space between the Up- and Down-RBD conformations, that can affect antibody interactions. Since the Spike is densely glycosylated, simulations were also performed for the dominant Spike glycoform to elucidate the effect of glycans on these essential dynamics. Our results further provide a mechanistic rationale for the allosteric modulations that distinguish the predominant D614G viral Spike form which has been shown to have enhanced infectivity and RBD-up probability.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31728-8

Modeling protein-lipid interactions during viral assembly of SARS-CoV-2

Specific protein-lipid interactions are key for cellular processes as well as for pathogens replication. Over the past months, SARS-CoV-2, the causative agent for COVID-19, infected more than thirty million people around the world, causing over a million deaths. The infectivity and transmission rate of the virus is unprecedented, and scientists have made important advances in record time to understand the virus mechanisms and develop therapies to combat it. A molecular level understanding of the interactions of

key viral proteins with the cellular host is needed to continue the development of therapies that block or control the infection. The M protein is among the four structural proteins of SARS-CoV-2 and is believed to play a crucial role in viral assembly, recruiting other viral proteins to the viral assembly site and modulating budding of the viral particle. We used all-atom molecular dynamics simulations to understand protein-protein and protein-lipid interactions of the M protein in complex membrane models. In this work, we characterize how interactions with specific lipids produce changes in the local membrane environment during viral assembly that in turn modulate protein-protein interactions. These atomistic simulations served to build a bottom-up coarse-grained model for the M protein, in an effort to study large-scale protein dynamics during viral assembly and membrane remodeling.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31444-2

Characterizing binding kinetics and thermodynamics of computer-designed nanobodies targeting SARS-CoV-2 RBD

The pandemics caused by SARS-CoV-2 has emerged on a global scale, and no vaccines or antivirals are available to prevent or treat COVID-19. We computer-designed three Nanobodies (Nb) (Nb-72, Nb-Hum, Nb-Ab) targeting neutralizing epitopes of the SARS-CoV-2 receptor-binding domain (RBD) to neutralize viral particles. The design was based on a previously reported SARS-CoV-1 neutralizing Nb, which prevented the neutralization of SARS-CoV-2, mainly due to its substantially higher dissociation constant. The crystallographic structure of Nb-SARS-CoV-1 was used as a template to model the structure of the Nb-SARS-CoV-2 complex. These systems were used as control of successful and unsuccessful binding partners. Given the firmly established importance of high affinity and rapid binding for therapeutic settings, the designed complexes must possess favorable binding kinetics and thermodynamics. To gain insight into molecular recognition, atomistic simulations in the aqueous environment were employed to reconstruct the free energy surface of the Nbs-RBD systems by 20 ns metadynamics using the Gromacs package interfaced with the Plumed plug-in, and the association rates were obtained through the Simulation of Diffusional Association (SDA) method, based in Brownian Dynamics. Both

methodologies reproduced the experimental observations for the control systems quantitatively. The free energy landscape for Nb-72 and Nb-Ab presented higher dissociation energy to the RBD than the control Nb-SARS-CoV-2, whereas for Nb-hum, similar binding energy was observed with regard to the control. However, the landscape for Nb-hum suggests an expected lower off-rate. The association rates indicate that the designed Nbs bind the target as fast as the Nb-SARS-CoV-1. The results suggest that the designed Nbs can strongly bind to the RBD and provide effective neutralization. Experimental assays are currently being carried out. Further characterization of interaction energetics in the interface will lead to rational affinity maturation of the Nbs.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31300-X

Automated computational technique to improve the quality of SARS-CoV-2 proteins

The COVID-19 pandemic caused by SARS-CoV-2 is a global health emergency. in order to develop an effective drug or a preventative vaccine, it is critical for research and development teams to have access to high-resolution and accurate viral protein structures. Current COVID-19 proteins available on the Protein Data Bank contain some moderate- to low-resolution structures. In order to improve the quality of the structures, a torsional optimization protocol was used that combines Protein Data Bank-based torsional optimization with real-space refinement against the electron density derived from crystallography or cryo-electron microscopy. The automated method converts moderate- to low-resolution protein structures at initial (e.g. backbone trace only) or late stages of refinement to structures with increased numbers of hydrogen bonds, improved crystallographic R-factors, and superior backbone geometry. Application of this automated method on COVID-19 proteins has produced high-quality structures that can further aid the counter-pandemic efforts.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31299-6

Combining computational modeling with library screening to adapt sars-cov-neutralizing antibody 80R to SARS-CoV-2

The worldwide COVID-19 pandemic has had enormous consequences in terms of lives lost, economic impact, and in affecting the quality of life in nearly every country around the globe. It has revealed the urgent need for generating therapeutics to mitigate the effects of novel viruses on a shorter timescale than that required to generate a vaccine. As one potential avenue to address this need, we report a new method for adapting antibodies to related virus types and subtypes. By combining computational modeling with targeted large-scale library generation and high-throughput screening, the SARS-CoV-neutralizing antibody80R was successfully mutated to bind to the homologous epitope on the spike protein (S) of SARS-CoV-2. The designed library generated 77 unique sequences that bound strongly to S of SARS-CoV-2, with an average of 6.5 mutations per sequence. Virus neutralization was demonstrated by plaque reduction using a VSV-SARS-CoV-2 pseudovirus. The combined approach mitigates the limitations of each when applied separately and provides a very powerful synergism able to meet the challenging demands of repurposing antibodies to related virus types and subtypes. We will report on the efficacy of this method, the timescale required, and suggest avenues for further improvements.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31298-4

Rationally designed chimeric antibodies for COVID-19 and future coronavirus variants

Most of the antibody treatments targeting the ACE2 receptor binding motif (RBM) on SARS-COV-2 spike glycoprotein are vulnerable to virus evasion through the occurrence of mutations in RBM. To circumvent this issue, a chimeric antibody composed of an IgG1 framework with “ACE2-units” grafted on complementarity-determining regions (CDRs) was developed to act as a decoy for virus binding and neutralization. ACE2-units were composed of spike-interacting regions of ACE2 that are then connected by Rosetta-designed linker peptides. Such a chimeric construct is designed to neutralize SARS-COV-2 by binding spike RBM and is expected to be tolerant to mutations, as long as ACE2 recognition is required for infection. The ACE2-units’ binding free energy to the

spike RBM were assessed by molecular dynamics simulation. In total, the free energy of 8 ACE2 units, with their size ranging from 69 to 259 amino acids, and whole ACE2 was assessed. The computation result surprisingly showed that some ACE2-units had similar or even stronger RBM binding than the whole ACE2. For example, two ACE2-units consisting of 17% and 43% the size of ACE2 maintained 78% and 123% binding free energy, respectively. A similar strategy using the whole ACE2 fused with the Fc region of IgG1 was proposed recently that claimed mutation resistance. The chimeric antibody offers the additional benefit of ACE2-units that not only have similar or even higher binding affinity, but can also be grafted on multiple CDRs due to their small size to increase the avidity. Additionally, the whole IgG1 construct should have a longer lifetime than the Fc-fusion protein.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31297-2

Predicting the ability of SARS-CoV-2 to utilize the ACE2 receptor for cell entry in north american rodents

SARS-CoV-2, the virus responsible for the ongoing COVID-19 pandemic, was first discovered in a human population in 2019, likely the result of cross-species transmission from bats to humans. While it is widely accepted that SARS-CoV-2 originated from an animal host, little is currently known about the role animal hosts play in the transmission of the disease. Due to the serious nature of the pandemic, there is an urgent need to identify potential animal host species of the virus. In order to gain entry into host cells, the receptor binding domain (RBD) on the spike protein of the virus must be able to dock to angiotensin converting enzyme 2 (ACE2), a membrane protein found in the cells of many organisms. Therefore, a calculation of the binding affinity of the RBD with the ACE2 receptor of an organism can allow us to predict the susceptibility of an organism toSARS-CoV-2. In this study, we developed a computational pipeline to predict the binding affinity of the RBD with the ACE2 receptor of animal species, with a focus on North American rodents. Sequences of the ACE2protein for more than100North American rodent species were obtained, and homology modeling was used to generate structures of the ACE2receptor for each sequence, using available human ACE2 structures as a template. Protein-protein

docking tools were then used to dock the RBD against the snapshots, generated using molecular dynamics simulations, of each ACE2homology model. The docking score for eachRBD-ACE2docked complex was used to compile a short list of North American rodents for empirical testing. Our data was expected to be vital in the identification of potentially susceptible animal species, in understanding the cross-species transmission of CoVs, animal surveillance, and the development of animal therapies and vaccines.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31296-0

Exploring the role of glycans in the interaction of SARS-CoV-2 RBD and human receptor ACE2

COVID-19 is a highly infectious respiratory disease caused by the novel coronavirus SARS-CoV-2. It has become a global pandemic for which there is currently no effective treatment. During viral infection, the spike RBD of SARS-CoV-2 binds the human host cell receptor ACE2, enabling the virus to enter the host cell. Factors determining RBD-ACE2 binding may include interatomic contact formation and/or the effect of different glycan species that are on ACE2. Detailed understanding of these determinants is key for the development of novel therapeutic strategies. To this end, we perform extensive all-atom simulations of (i) the RBD-ACE2 complex without glycans, (ii) RBD-ACE2 with MAN9 glycans, and (iii) RBD-ACE2 with FA2 glycans. These simulations identify the key residues at the RBD-ACE2 interface that form contacts with higher probabilities, thus providing a quantitative evaluation that complements recent structural studies. in addition, we find that this RBD-ACE2 contact signature is not altered by the presence of MAN9 or FA2 glycans, suggesting that RBD-ACE2 contacts are inherently robust. Further, our simulations reveal how the glycan on Asn90 of ACE2 can play a distinct role in the binding/unbinding of RBD. Finally, an analysis of enthalpic contribution indicates that the binding energetics of RBD and ACE2 may depend on individual glycan types. Together, our results provide a more comprehensive view of the detailed interplay between virus and human receptor, which is much needed for the discovery of effective treatments that aim at modulating the physical-chemical properties of this virus.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31266-2

The extended intermediate of the SARS-CoV-2 spike protein uses extreme reach and flexibility to capture host cell membranes

The time for the EI was measured to capture a host cell membrane for a range of viral-host separations. Interestingly, the ability of the EI to capture target membranes is determined by its length and flexibility which endows it with a remarkably large capture volume. These quantitative results will help to assess antiviral strategies that target the S protein and the fusion process leading to viral entry.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32934-9

Rapid characterization of SARS CoV2 proteins with scattering methods

Structural characterization of SARS CoV2 proteins has provided directions for treatments of COVID 19. Fortunately, studies of the earlier SARS CoV1 provided a head start. To tackle truly new pathogens, rapid methods for structural characterization are needed. Solution scattering provides one avenue to provide structural insights rapidly. During the COVID 19 pandemic, solution scattering method was coordinated, developed and applied to interrogate the RNA dependent RNA polymerase from SARS CoV2. Insights were provided into the function and assembly of this complex. Furthermore, antibody/antigen interactions were characterized to help develop diagnostics and viral blocking treatments. The results will presented and application of scattering methods for tackling emerging pathogens.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31265-0

Topography, spike dynamics and nanomechanics of individual native SARS-CoV-2 virions

SARS-CoV-2, the virus responsible for the COVID-19 pandemic, displays a corona-shaped layer of spikes which play fundamental role in the infection process. Recent structural data suggest that the spikes possess orientational freedom and the

ribonucleoproteins segregate into basketlike structures. How these structural features regulate the dynamic and mechanical behavior of the native virion, however, remain unknown. By imaging and mechanically manipulating individual, native SARS-CoV-2 virions with atomic force microscopy, it was demonstrated that their surface displays a dynamic brush owing to the flexibility and rapid motion of the spikes. The virions are highly compliant and able to recover from drastic mechanical perturbations. Their global structure is remarkably temperature resistant, but the virion surface becomes progressively denuded of spikes upon thermal exposure. Thus, both the infectivity and thermal sensitivity of SARS-CoV-2 rely on the dynamics and the mechanics of the virus.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31268-6

Coronavirus pathogenicity is determined by stability of the spike protein open conformation

SARS-CoV-2, the virus inducing the COVID-19 illness, has claimed over 1 million lives since emerging in December 2019, while disrupting many more. It is the seventh coronavirus to emerge in human populations. However, what makes one coronavirus more deadly than another is not well understood. Furthermore, no tools exist to reliably assess pathogenic potential in coronavirus strains threatening future emergence from animal populations. Here, it was attempted to answer, why one coronavirus is more pathogenic than another and describe a tool for future predictive uses. Coronaviruses invade host cells using a spike glycoprotein. To bind to the host receptor and initiate fusion with the host membrane, the spike protein's receptor binding domain (RBD) must first transition from a closed to open conformation to avail itself for binding. It was proposed that the proportion of time spent in the open receptor-ready conformation, related to this conformation's stability, indicates the pathogenic potential of the coronavirus strain. To test this hypothesis, we compared the spike protein RBDs from three human ACE2 binding coronavirus strains with varying pathogenic potential: SARS-CoV-1, SARS-CoV-2, and hCoV-NL63, using molecular dynamics simulations. We employed both long equilibrium simulations and umbrella sampling to derive closed to open transition free energy profiles. The results suggest a strong correlation between the ability of the RBDs to transition to the open state and pathogenic potential. With

additional testing, our technique may prove useful in assessing animal coronaviruses' pathogenic potential, highlighting them as a high or low-risk strain before they can emerge into human populations.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31270-4

Mapping binding interfaces and allosteric changes in the SARS-COV-2 spike protein using hydrogen/deuterium exchange mass spectrometry

Coronaviruses (CoVs), including the severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) viruses, have and continue to pose a major threat to human health. In October of 2020 the COVID-19 pandemic, caused by the coronavirus SARS-CoV-2, surpassed one million global deaths. CoVs are enveloped positive strand RNA viruses that display surface spike proteins which recognize host receptors. The spike proteins then undergo conformational changes that allow for attachment to the host membrane and eventually facilitate membrane fusion and viral entry. Due to their exposure on the virus surface and their essential role in coronavirus infection, most antibody development strategies and many therapeutic development strategies have focused on the spike protein.

While much focus has been given to the structure of spike, successful design of therapeutics requires understanding the conformational dynamics and alternative conformations not accessible using traditional structural methods. We have successfully applied Hydrogen/Deuterium Exchange Mass Spectrometry (HDX/MS) on this large (>400 kDa) glycosylated trimeric complex to investigate these important conformational changes; allowing us to identify the binding interfaces as well as the induced allosteric changes upon binding to the human receptor ACE2, neutralizing patient antibodies, and synthetic binders. We also compare the conformational flexibility of coronavirus homologues and naturally occurring SARS-CoV-2 spike variants allowing us to connect changes in the conformational ensemble to functional, phenotypic differences in these variants. Understanding the native conformational ensemble, and interactions between receptors or antibodies with CoV spike proteins will not only improve our understanding of CoV biology and the host immune response to CoV but also aid in the design of therapeutics and vaccines for current and future CoVs.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31887-7

Conserved elements in the 3'-UTR of SARS-CoV-2: Involvement in genomic dimerization and interactions with cellular micrornas

SARS-CoV-2, the coronavirus responsible for the COVID-19 pandemic, belongs to the Coronaviridae family of viruses. Members of this family contain highly conserved structural elements within their 5’ and 3’ untranslated regions (UTRs), which are believed to play roles in genome replication and circularization. One structure of interest in the 3’-UTR is a highly conserved 41-nucleotide stem-loop II-like motif (s2m), which is found not only within coronaviruses, but also within Astroviridae, Calciviridae, and Picornaviridae families. While the specific function remains unresolved, it has been suggested to play roles in viral replication, hijacking of host protein synthesis, and RNA interference pathways. Here, the formation of a homodimeric kissing complex was demonstrated by the SARS-CoV-2 s2m element, which is converted to a stable, extended duplex by the viral nucleocapsid (N) protein. Subsequently, a potential role of host cellular microRNA-1307-3p hijacking by the s2m element was established, a function which is altered by s2m dimerization. The purported roles of this microRNA include translational regulation of various interleukins and interleukin receptors, including those found elevated in severe COVID-19 patients. Furthermore, a second region was identified in the 3’- UTR of SARS-CoV-2 that binds another host miRNA involved in interleukin expression. The data indicates that the SARS-CoV-2 virus may act as a “microRNA sponge”, sequestering host cellular microRNAs and preventing them from performing their canonical functions. The molecular mechanisms put forth in this study could act as a guide for future therapeutic drug development studies in order to alleviate the severe symptoms associated with COVID-19.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32889-7

Characterization of SARS-CoV-2 conserved elements' structures and their RNA-RNA interactions

On December 31, 2019 the first case of a novel coronavirus infection, designated as COVID-19 was reported in Wuhan, China. Since that time, the virus responsible for this outbreak, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread to over 200 countries with more than 33 million confirmed cases and over 1 million deaths. SARS-CoV-2 is a positive stranded RNA belonging to the coronavirus family. Although most of its 3'-untranslated region (UTR) is variable, it contains a 41-nucleotide sequence, the S2m element, that is highly conserved among coronaviruses and three other viral families. The S2m is predicted to fold into a stem-loop structure, which we noted contains the palindromic sequence "GUAC" in its terminal loop. Thus, similarly to the dimer initiation sites of HIV-1 and HCV, we hypothesized that the SARS-CoV-2 S2m motif might be implicated in the genomic RNA dimerization through a similar mechanism involving a kissing dimer intermediate that is converted to an extended duplex by the nucleocapsid N protein. Such dimerization might be relevant for the coronaviruses' recombination events. Moreover, since emerging mutations in the S2m motif have been reported in SARS-CoV-2, we conducted a bioinformatics analysis to determine the most common mutations. These mutants were tested to show the effect of the mutations on the S2m dimerization, conversion to the extended duplex, and its interactions with host microRNAs and other small molecules.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32890-3

Condensed Liquid Phase 3D Structure of SARS-CoV-2 s2m Guided by NMR Spectroscopy

The Covid-19 pandemic, caused by the SARs-CoV-2 virus, has become a research priority due to lack of preventative and therapeutic options. Development depends partly upon accurate three-dimensional structure and knowledge of interactions. We report on the elucidation of the condensed liquid phase 3D structure of a highly conserved region in the SARS-CoV-2 3' untranslated region (UTR), known as the s2m element. The s2m element has high homology with the SARS-CoV virus, as well as with viruses in four other families, making this region a potential target for preventative and therapeutic

measures. An X-ray structure of the SARS-CoV s2m element, which caused the 2003 outbreak, is available as PDB ID 1XJR, and has been the structure of choice for structural studies of SARS-CoV-2. Despite having high sequence homology differing by two nucleotides, the secondary structure of SARS-CoV s2m element does not correspond to recently published NMR secondary structure data for SARs-CoV-2. Specifically, we find that adaptation of the SARs-CoV s2m sequence to match the SARs-CoV-2 s2m region starting with the 1XJRcrystal structure does not yield a SARS-CoV-2 3D structure that corresponds to published proton NMR resonances after 1.5 microseconds of molecular dynamics simulation time. We have designed a SARS-CoV-2 s2m model consistent with experimental proton NMR resonances, resulting in a different 3D structure than the SARS-CoV s2m element that retains the appropriate base-pairs as indicated by the available NMR data after 1.5 microseconds. This work provides a 3D model of the condensed liquid phase of SARS-CoV-2 consistent with NMR data for future research and discovery.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32891-5

Modeling the structure of the frameshift-stimulatory pseudoknot in SARS-CoV-2 reveals multiple possible conformers

The coronavirus causing the COVID-19 pandemic, SARS-CoV2, uses the process of −1 programmed ribosomal frame-shifting (−1 PRF) to control production of gene products like the viral polymerase. In −1 PRF, a pseudoknot in the viral genome stimulates the shift in reading frame. Because the frameshifted gene products are essential to viral replication, the frameshift-stimulatory pseudoknot is a strategic target for attenuating the virus. However, the atomic structure of the pseudoknot has not yet been solved experimentally. The structure of the pseudoknot computationally was modeled, using multiple blind prediction tools followed by μs-long all-atom molecular dynamics simulations. The plausibility of the equilibrated models was assessed by comparison to nuclease-protection assays and single-molecule force spectroscopy measurements of the SARS-CoV pseudoknot. Several possible conformations with distinct fold topologies were found, including models exhibiting single-strand threading through junctions between stems 1 and 2 or stems 1 and 3 in the pseudoknot. Isolated monomeric

pseudoknots as well as monomers paired through a dimerization domain in loop 2 was modeled, finding similar fold topologies in both paired and unpaired pseudoknots. These structural models should help interpret future experiments and support efforts to discover ligands inhibiting −1 PRF in SARS-CoV-2.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32893-9

Structural dynamics of SARS-CoV-2 frameshift signal studied by single-molecule force spectroscopy reveal topologically distinct conformers

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the COVID-19 pandemic uses −1 programmed ribosomal frameshifting (−1 PRF) to control expression of key viral proteins. −1 PRF involves a shift in the reading frame of the ribosome at a specific location in the RNA message, stimulated by a pseudoknot structure in the mRNA located 5-7 nt downstream of the 'slippery' sequence where the reading-frame shift occurs. To understand how this pseudoknot responds to the mechanical tension applied by ribosomes during translation, which is thought to play a key role in stimulating frameshifting, its structural dynamics were probed under force using optical tweezers. Unfolding curves revealed that the frameshift signal formed multiple different structures: at least two pseudoknotted conformers with distinct unfolding forces and pathways, as well as alternative stem-loop structures. Using anti-sense oligomers to bind specific parts of the RNA and inhibit their folding, the pattern of intermediates involved in forming the different states, were identified. Remarkably, it was found that the two pseudoknotted conformers were consistent with models having different fold topologies, one with the 5′ end threaded through a helix junction to form a ring-knot—not seen in any other frameshifting element—and the other without threading. These results solve the folding mechanism of the frameshift signal in SARS-CoV2 and highlight the conformational heterogeneity of this RNA, with important implications for structure-based drug-discovery efforts.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32894-0

Distinct differences in the interactions of receptor binding domains of SARS-CoV-2 and SARS-CoV with human ACE2

Coronaviruses have created multiple infectious diseases in the last two decades with dreadful global impact, including most recently COVID-19 caused by the SARS-CoV-2 virus. The SARS-CoV-2 virus binds to the human angiotensin-converting enzyme (hACE2) using the spike glycoprotein. While ACE2 acts as a receptor for both SARS-CoV-2 and SARS-CoV, it binds much more strongly (~4 times) to the spike glycoprotein of SARS-CoV-2. To investigate this difference in binding affinity, were modeled two RBD-hACE2 complex systems for the two viruses by adding appropriate glycans at the N- and O-glycosylation sites as suggested by mass spectroscopy experiments. We performed multiple microsecond-length molecular dynamics simulations of the RBD-hACE2 complex for viruses. The analysis demonstrates that the glycan shielding in this complex behaves differently between SARS-CoV-2 and SARS-CoV. Additionally, we also show that each of the glycans has a specific interaction pattern that is unique to each virus. Finally, we compute rigorously the free energy change for mutating residues on the RBD-ACE2 interface identified from the equilibrium molecular dynamics simulation. Collectively, it was cogently explained why hACE2 interacts more favorably with the RBD of SARS-CoV-2 compared to that from SARS-CoV.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32818-6

SARS-CoV-2 simulations go exascale to capture spike opening and reveal cryptic pockets across the proteome

The SARS-CoV-2/COVID-19 pandemic continues to threaten global health and socioeconomic stability. Experiments have revealed snapshots of many viral components but not the moving parts of these molecular machines. To capture these essential processes, over a million citizen scientists have banded together through the Folding@home distributed computing project to create the world's first exascale computer and simulate an unprecedented 0.1 seconds of the viral proteome. The dataset contains a wealth of information on the workings of the virus, from its ability to infect host cells, evade and suppress the immune response, replicate, and package viral RNA. Here, a few major highlights were presented. In full atomic detail, we capture

a dramatic transition of the spike complex was captured from a closed state (immune evading) to an open state (infection initiating). Differences in open populations between Spike homologues and quantify antigen exposures were reported, which have strong implications for vaccine developments. The simulations of the viral proteome uncovered over 50 'cryptic' pockets and provide a quantitative atlas for the design of antivirals and related therapeutics. Lastly, our datasets, final models, and depiction of cryptic pockets can be accessed freely online as a resource.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32816-2

The SARS-CoV-2 spike variant D614G favors an open conformational state

The COVID-19 global pandemic is an international health emergency. It is caused by the SARS-CoV-2 virus, which binds with its trimeric Spike protein to the ACE2 receptor in the human lung. Early in 2020, researchers observed the emergence of a single amino acid variant at residue 614 of the Spike trimer, in which the aspartic acid of the original "D-form" was replaced by a glycine in the emergent "G-form." The G-form rapidly became the most dominant form, displaying heightened infectivity and transmissibility. To gain understanding of the molecular mechanisms underlying how a single amino acid shift could cause such a drastic change, we performed the first extensive all-atom simulations of the Spike trimer in explicit solvent in both the D-form and the G-form. For each form, we simulated the "all down" conformational state, which is not infection-capable, and the "one up" state, which is infection capable due to the "blossoming" outward of one of the three trimers. It was shown that a shift in the inter-protomer contacts likely leads to a shift in energetics that causes the G-form to have a heightened population of Spikes in the one up state. While there is no significant difference between the exposure of the ACE2 binding site when comparing the D- and G-forms, there is a difference when comparing the all down and one up states, so a heightened population in the one up state corresponds to higher infectivity. Overall, this work presents molecular-level understanding of the differences between the D- and G-forms that is of crucial importance for vaccine design and thus combating the COVID-19 pandemic.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32811-3

Anti-frameshifting ligand active against SARS Coronavirus-2 is resistant to natural mutations of the frameshift-stimulatory pseudoknot

Currently the world is undergoing major heath and economic crisis due to the global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is structurally and functionally similar to SARS-CoV. Like all coronaviruses, it uses −1 programmed ribosomal frameshifting (−1 PRF), whereby host cell ribosomes are directed shift reading frame at a specific location in the viral genome, to control expression of key viral proteins. Among the gene products arising from −1 PRF is the RNA-dependent RNA polymerase required for replicating genomic and sub-genomic RNA from the virus. Modulating −1 PRF by binding ligands to RNA pseudoknot that stimulates −1 PRF may therefore have therapeutic potential by attenuating the virus. Several mutations in the pseudoknot have occurred during the pandemic, but how they affect −1 PRF efficiency and ligand activity is unknown. We studied a panel of six mutations observed in samples taken from COVID-19 patient, located in key regions of the pseudoknot. We found that most mutations did not change −1 PRF levels, even when base-pairing was disrupted. However, one led to a striking 3-fold decrease, suggesting SARS-CoV-2 may be less sensitive to −1 PRF modulation than expected. Examining the effects of a small-molecule −1 PRF inhibitor active against SARS-CoV-2, it was seen to have a similar effect on all of the 6 mutants, regardless of their basal −1 PRF efficiency, indicating that anti-frameshifting activity can be resistant to natural pseudoknot mutations. These results have important implications for therapeutic strategies targeting SARS-CoV-2 through modulation of −1 PRF.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32745-4

Differential dynamic behavior of prefusion spike glycoproteins of SARS coronaviruses 1 and 2

Within the last two decades, severe acute respiratory syndrome (SARS) coronaviruses 1 and 2 (SARS-CoV-1 and SARS-CoV-2) have caused two major outbreaks. For

reasons yet to be fully understood, the COVID-19 outbreak caused by SARS-CoV-2 has been significantly more widespread than the 2003 SARS epidemic caused by SARS-CoV-1, despite striking similarities of the two viruses. Coronavirus spike protein mediates a crucial step in the infection, i.e., the host cell recognition and viral entry, which starts with binding to host cell angiotensin converting enzyme 2 (ACE2) protein in both viruses. Recent structural and functional studies have shed light on the differential binding behavior of the SARS-CoV-1 and SARS-CoV-2 spike proteins. In particular, cryogenic electron microscopy studies show that ACE2 binding is preceded by a large-scale conformational change in spike protein to expose the receptor binding domain (RBD) to its binding partner. Unfortunately, these studies do not provide detailed information on the dynamics of this activation process. Here, an extensive set of unbiased and biased microsecond-timescale all-atom molecular dynamics (MD) simulations of SARS-CoV-1 and SARS-CoV-2 spike protein ectodomains in explicit solvent was used to determine the differential behavior of spike protein activation in the two viruses. The results indicate that the active form of SARS-CoV-2 spike protein is considerably more stable than that of the SARS-CoV-1 spike protein. The deactivation is in part due to interactions between the N-terminal domain (NTD) and the RBD of SARS-CoV-1 spike protein that are absent in SARS-CoV-2. Understanding how coronavirus spike proteins undergo conformational changes prior to binding to host cell ACE2 receptors is key to the development of COVID-19 vaccines and therapeutics, which requires a dynamic rather than a static picture to provide a reliable structure-based drug design framework.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32666-7

SARS-CoV-2 glycosylated spike activation mechanism - simulations of the full unbiased pathway

The SARS-CoV-2 virus is responsible for the COVID-19 pandemic and has taken over one million lives do date. Infection occurs through glycosylated spike trimers, which protrude the surface of viral particles and bind to human ACE2 receptors. For the spikes to bind ACE2, the receptor binding domains (RBD)s must transition from a closed to open state. To access the biological timescales of this transition, we used the weighted

ensemble (WE) enhanced sampling method to generate unbiased, all-atom molecular dynamics simulations of the glycosylated spike undergoing RBD activation. Rather than adding an external biasing force, the WE method relies on running many short simulations in parallel along chosen reaction coordinate(s). The trajectories that rarely sample high energy regions are replicated, while the trajectories that frequently sample low energy regions are merged, focusing computational resources on sampling rare events. The trajectories also carry probabilities or weights, which are continuously updated, and there is no statistical bias added to the system. Hence, both thermodynamic and kinetic properties from the WE simulations were directly obtained, which are not possible to accurately predict from most other enhanced sampling methods. From these simulations, it was uncovered the primary glycans and residues responsible for facilitating the activation mechanism, identified the internal barriers for initiating RBD opening, and obtained extensive sampling of spike conformations. These conformations can be used to train machine learning algorithms, test the effect of perturbations (mutations, small molecules, *etc*.) and for *in silico* design of SARS-CoV-2 Spike targeting therapeutics.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32665-5

Real-time conformational dynamics of SARS-CoV-2 spikes on virus particles

SARS-CoV-2 spike (S) mediates entry into cells and is critical for vaccine development against COVID-19. Structural studies have revealed distinct conformations of S, but real-time information that connects these structures, is lacking. Here single-molecule Förster Resonance Energy Transfer (smFRET) imaging was applied to observe conformational dynamics of S on virus particles. Virus-associated S dynamically samples at least four distinct conformational states. In response to hACE2, S opens sequentially into the hACE2-bound S conformation through at least one on-path intermediate. Conformational preferences of convalescent plasma and antibodies suggest mechanisms of neutralization involving either competition with hACE2 for binding to RBD or allosteric interference with conformational changes required for entry. The findings inform on mechanisms of S recognition and conformations for immunogen design.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32664-3

Vitamin D and Its derivatives as promising drugs against COVID-19 - A computational study

Abstract

COVID-19 pandemic caused by SARS-CoV-2 presents a great threat to public health. Epidemiologic correlation of SARS-CoV-2 infection with vitamin D deficiency patients with critical symptoms was observed worldwide. Vitamin D has a central role in regulating body calcium homeostasis and musculoskeletal system. Vitamin D derivative 1,25(OH)2D3 also has important pleiotropic effects affecting almost all body functions and organs including neuroendocrine and immune systems. SARS-CoV-2 entry involves interaction between receptor binding domain (RBD) of spike protein in SARS-CoV-2 and human angiotensin-converting enzyme 2 (hACE2) receptor. Molecules with potential to inhibit the interaction of SARS-CoV-2 RBD and hACE2 could prevent SARS-CoV-2 cellular entry. In this study, it was aimed to determine the potential of vitamin D and its derivatives to inhibit hACE2 and SARS-CoV-2 RBD interaction and its underlying structural basis using combined molecular docking and molecular dynamics (MD) simulations. Available electron microscopy structure of hACE2 and SARS-CoV-2-RBD and the known binding sites between hACE2 and SARS-CoV-2-RBD provide structural basis for this study. Results showed that vitamin D3 and its derivatives are all favorable to bind either ACE2 or SARS-CoV-2-RBD at ACE2-RBD binding site. These results indicated that vitamin D3 and its derivatives have the potential to inhibit or block SARS-CoV-2-RBD binding hACE2. Electrostatic analysis results of 1,25(OH)2D3 with hACE2 and SARS-CoV-2-RBD showed that charged residues in the binding sites between ACE2 and SARS-CoV-2-RBD steadily hold 1,25(OH)2D3 polar groups through electrostatic interaction. Our MD simulations results showed that 1,25(OH)2D3 interaction with ACE2 and SARS-CoV-2-RBD resulted in their conformation and dynamical motion changes, particularly for its binding site(s), which further support the potential of vitamin D3 and its derivatives inhibition of SARS-CoV-2 binding hACE2 for entry. The results could propose vitamin D and its derivatives as promising drugs against COVID-19.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32303-1

Identification of FDA approved antiviral drugs for COVID-19 treatment using unbiased virtual screening

Abstract

COVID-19 has led to a worldwide pandemic and treatments are limited and only used in severe cases. This study aims to identify FDA-approved antiviral drugs for the inhibition of host proteins of ACE2 and TMPRSS2 and key SARS-CoV-2 proteins of Mpro, NSP15, RBD of S protein, and RdRp domain of NSP12 for potential COVID-19 treatment through unbiased virtual screening. To reduce the bias of using a single molecular docking program for virtual screening, we used three docking programs, AutoDock Vina, AutoDock4, and RosettaLigand, and adopted unbiased rank-by-rank scoring method to identify top FDA-approved antiviral drug candidates for each receptor protein, which could be repurposed for potential COVID-19 treatment. A series of positive and negative controls of ligand-receptor binding were used to validate the unbiased virtual screening methods and set binding free energy threshold values as positive ligand-receptor binding for each docking program. With the validated unbiased virtual screening method, top 20 FDA-approved antiviral drugs for each of the studied host and SARS-CoV-2 proteins were identified. The FDA-approved antiviral drugs that could inhibit multiple studied receptors are also identified. The top drug candidates targeting multiple receptors are FDA-approved anticancer drug, HIV-1 antiretroviral drug, and hepatitis C (HCV) antiviral drugs. Interactions of the top drug candidate with target receptors are investigated. Results from this study presented the potential of repurposing FDA-approved drugs to target the host proteins and key SARS-CoV-2 proteins to inhibit SARS-CoV-2 from binding to host proteins and stop viral replications. The identified FDA-approved drugs with the reposition potential for COVID-19 treatments could inspire clinical trials, further accelerating the translation efforts to treat COVID-19. Clinical data from UAB showed that one of the identified drugs is correlated with a lower mortality rate among COVID19+ patients.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32301-8

Prediction and analysis of multiple sites and inhibitors of SARS-CoV-2 proteins

In the current COVID-19 pandemic, it is critical to understand, as swiftly as possible, how the viral proteins function and how their function might be modulated. The machine learning method Partial Order Optimum Likelihood (POOL) is used to predict binding sites in protein structures from SARS-CoV-2, the virus that causes COVID-19. Using the 3D structure of each protein as input, POOL uses computed electrostatic and chemical properties to predict the amino acids that are biochemically active, including residues in catalytic sites, allosteric sites, and other secondary sites. Docking studies are then performed to predict ligands that bind to each of these predicted sites. For instance, for the x-ray crystal structures of the main protease, POOL predicts two sites: the known catalytic site containing the catalytic dyad His41 and Cys145 and a second nearby site on an adjacent face of the protein surface. The x-ray crystal structure of the SARS-CoV-2 2'-O-ribose RNA methyltransferase (NSP16) protein has been reported in complex with its activating partner NSP10 and with two bound ligands, S-adenosylmethionine (SAM) and β-D-fructopyranose (BDF). POOL predicts three binding sites, including the catalytic SAM-binding site, the BDF binding site on the opposite side, and a third site adjacent to the catalytic / SAM-binding site. Predicted binding ligands (including selected compounds from the ZINC and Enamine databases, Chemical Abstract Service database compounds, and COVID-specific libraries from Enamine and Life Chemicals) are reported for several SARS-CoV-2 proteins. Kinetics assays to test for catalytic activity of the main protease and of 2'-O-ribose RNA methyltransferase in the presence of predicted binding ligands with high scores are underway. Theoretical and experimental methods are aimed at identifying molecules having inhibitory effects on the function of viral proteins.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32298-0

Identifying hotspots in binding of SARS-CoV-2 spike glycoprotein and human ACE2

Coronaviruses are a large family of viruses that can cause respiratory infections with varying severity from common cold to severe diseases such as novel coronavirus disease (COVID-19). It has been declared as a global pandemic by the World Health

Organization on March 11, 2020 and still continues to this date. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2 uses its spike glycoprotein (Sgp) to bind human angiotensin converting enzyme 2 (hACE2) receptor, and mediates membrane fusion and virus entry. The recognition of Sgp to human ACE2 and its high affinity for it has been of great importance since this provides the first step in viral entry to human cells. Therefore, it is important to identify key residues (hotspots) in this process. In this study, computational Ala Scanning has been performed for Sgp and hACE2. The residues identified with significance in binding and other residues in close proximity with those were studied further through molecular mechanics-based protein binding free energy change prediction methods. The comparison of our findings with all previous studies revealed interesting results that would need to be further investigated.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32278-5

Rapid clinical diagnostic viral detection with saliva by a novel single step nested mango-NASBA assay

The recent COVID-19 pandemic has clearly highlighted the necessity for rapid and fieldable diagnostic coronavirus tests. However, few such methods meet all requirements of rapidity, sensitivity, and affordability, which hiders the prevention of uncontrolled widespread transmission of SARS-Cov-2. Here, a new rapid clinical diagnostic test was reported for viral RNA detection using a recently developed fluorogenic RNA aptamer, Mango. This assay is based on an isothermal amplification of ribonucleic acid termed NASBA (nucleic acid sequence-based amplification). The assay merely consists of three enzymes and two sets of primers. This assay was optimized to increase its sensitivity, such that authentic COVID-19 viral fragments can be detected within <30 min in a single tube. Importantly, RNA extraction is not required for this assay, and the viral fragments are directly detected from saliva. The annealing step of primers can be also omitted, the target RNAs are amplified at low constant temperature. Taken together, the features of this Mango-NASBA assay satisfy all requirements for a rapid and fieldable clinical diagnostic coronavirus test. It was anticipated that the implementation of such coronavirus test platforms will help control spread of viruses.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32252-9

Amplification-free detection of viruses in minutes using single-particle imaging and machine learning

The increasing frequency and magnitude of viral outbreaks in recent decades, epitomized by the current COVID-19 pandemic, has resulted in an urgent need for rapid and sensitive viral diagnostic methods. To address this need, a novel method wasdeveloped to rapidly detect and identify intact virus particles using wide-field fluorescence imaging, advanced image analysis and deep learning. The method utilizes cation-mediated labelling of enveloped viruses, a labelling method not specific to any virus. Doubly labelled viruses on the surface of a glass slide was subsequently immobilised, collect diffraction-limited images containing thousands of labelled particles, and finally use image analysis and deep learning to identify different viruses in biological and clinical samples. The software initially segments the images and only keeps the signals present in both the green and the red channel. Those signals are then saved as individual images and fed into a custom convolutional neural network for classification. The network exploits the differences between the labelling efficiency for different viruses, as well as their size and shape differences. The assay achieves labelling, imaging and virus identification in less than five minutes; further, the whole procedure does not require any lysis, purification or amplification steps. The trained neural network was able to differentiate SARS-CoV-2 from negative clinical samples, as well as from other common respiratory pathogens (such as influenza and seasonal human coronaviruses). The sensitivity and specificity for lab-adapted strains exceeded 90% per signal, and the overall validation accuracy for Flu A clinical samples was 86.5% per signal. Single-particle imaging combined with deep learning therefore provides a promising alternative to traditional viral diagnostic methods and has the potential for significant impact.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32248-7

Dynamics and binding strength of the spike protein of Sars-Cov-2 probed by high-speed atomic force microscopy

The ongoing pandemic of the coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). One of the first steps in the infection process is the binding of the spike (S) protein of the viral membrane to the angiotensin-converting enzyme 2 (ACE2) receptor present on human cell. The S-protein forms a homotrimer that is post-translationally cleaved into 2 subunits (S1 and S2). S1 presents the N-terminal domain (NTD), the receptor binding domain (RBD) and two C-terminal domains. Different cryoEM structures of the SARS-CoV-2 S-trimer have been reported, including that of the S ectodomain stabilized in the prefusion state with the double proline mutation. Structural information revealed that the RBD adopts at least two conformations: ‘up’ to allow receptor (ACE2) binding and ‘down’ preventing ACE2 binding. However, little is known about the dynamics of these conformational changes. in addition, while the unbinding forces of the RBD and S1 subunit of the S-protein to ACE2 have been reported, the binding strength of the trimeric S-protein/ACE2 complex is unknown. Here, high-speed atomic force microscopy (HS-AFM) was used to visualize the conformational dynamics of wild type (Swt) and pre-fusion mutant (Spp) S-trimer. Single molecule force spectroscopy (SMFS) was applied to determine the binding strength of the S-trimer/ACE2 interaction (Swt and Spp). The results show dynamic conformational changes, suggesting remarkable flexibility and displaying simultaneous opening of at least two RBD domains. Preliminary SMFS data suggest similar binding strength on Swt and Spp and that the S-trimer may provide multiple binding regulation. The results will improve the rational development of blocking agents and neutralizing antibodies.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)31035-3

The effect of mutations on binding interactions between the SARS-CoV-2 receptor binding domain and neutralizing antibodies

First discovered in 2019, SARS-CoV-2 is a beta-genus coronavirus responsible for COVID-19 and the current pandemic. Despite sharing similarities to SARS-CoV-1, responsible for an epidemic in 2003, the 2019 variant is far more infectious and deadly.

The severity of COVID-19 necessitates an understanding of how it could evolve to escape potential treatments as well as ways to strengthen treatments against it. While there has been work devoted to understanding the impact of possible mutations in the spike S protein and its ability to bind to angiotensin-converting enzyme 2, there has not been such an effort to study interactions with antibodies. Here, a computational pipeline was used that was previously designed in our lab and applied it to the SARS-CoV-2 S protein receptor binding domain (RBD) bound to a neutralizing antibody (Ab). Molecular dynamics simulations were used to generate trajectory snapshots. These snapshots were used as inputs for FoldX, a fast semi-empirical method for estimating folding and binding free energies. These free energy calculations were then averaged to get final estimates. Sites within both Ab and the RBD were mutated that were within 10 Å of the RBD-Ab binding interface. We found a large number of potential antibody escape mutations in the RBD (i.e., those predicted to destabilize RBD-Ab interactions), some of which agree with other studies. It was also found a smaller number of potential antibody strengthening mutations in Ab (i.e., those predicted to stabilize RBD-Ab interactions) that could be used to improve the therapeutic value of Ab. These results provide a basis for further studies on the effects of mutations in the RBD and antibodies and provide a starting point for building a list of potential escape mutations for antibodies.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32145-7

Investigational treatments for COVID-19 may increase ventricular arrhythmia risk through drug interactions

Several drugs proposed for the treatment of COVID-19 have reported cases of cardiac adverse events such as ventricular arrhythmias. To properly weigh risks against potential benefits in a timely manner, mathematical modeling of drug disposition and drug action can be useful for predicting patient response. Here the potential effects were explored on cardiac electrophysiology of 4 COVID-19 proposed treatments: lopinavir, ritonavir, chloroquine, and azithromycin, including combination therapy involving these drugs. To address this, simulations of pharmacokinetics (PK) were combined with mechanistic mathematical modeling of human ventricular myocytes to predict adverse events caused by these treatments. A mechanistic model were utilized

was utilized to construct heterogenous populations of 4 patient groups (healthy male, healthy female, diseased male, and diseased female) each with 1000 members, and studied the varied responses of drugs and combinations on each population. To determine appropriate drug concentrations for recommended COVID-19 regimen, we implemented PK models for each drug and incorporated these values into the mechanistic model. We found that: (1) drug combinations can lead to greater cellular action potential (AP) prolongation, analogous to QT prolongation, compared with drugs given in isolation; (2) simulations of chloroquine with azithromycin caused a significantly greater increase in AP duration (ΔAPD≈190 ms) compared to lopinavir with ritonavir (ΔAPD≈6 ms); (3) drug effects on different patient populations revealed that females with pre-existing heart disease are more susceptible to drug-induced arrhythmias as 85 members formed arrhythmias, and less than 20 in each of the other three; and (4) logistic regression analysis performed on the population showed that higher levels of the sodium-calcium exchanger may predispose certain females with heart failure to drug-induced arrhythmias. Overall, these results illustrate how PK and mechanistic modeling can be combined to precisely predict cardiac arrhythmia susceptibility of COVID-19 therapies.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)32992-1

Quantitative fluorescence microscopy on SARS-CoV-2

Severe acute respiratory coronavirus-2 (SARS-CoV-2) is the causative agent of Coronavirus-19 Disease (COVID-19) and remains a severe public health threat. SARS-CoV-2 particles contain four structural proteins - i.e., membrane (M), nucleocapsid (N), spike (S) and envelope (E). Early in virus assembly, the M, S and E proteins are inserted into the rough endoplasmic reticulum (ER). Upon their transport to the ER-Golgi intermediate compartment (ERGIC), there is interaction with N - which initiates particle biogenesis. While fluorescence microscopy offers a non-invasive method to quantitatively study the structural proteins expressed inside living cells, quantitative fluorescence microscopy studies of SARS-CoV-2 assembly have been thus far lacking in the literature. Here, we present work examining the SARS-CoV-2 structural proteins in living cells using fluorescence techniques. We begin by introducing a fluorescence

toolkit for our studies which consists of fluorescent protein chimeras of M, S and N. We additionally are developing a virus-like particle (VLP) system and methods of VLP detection to further investigate particle biogenesis. Lastly, we present initial results using quantitative fluorescence microscopy techniques, such as superresolution imaging, fluorescence fluctuation spectroscopy and two-photon scanning, and report preliminary measurements of SARS-CoV-2 structural protein localization, mobility and complex stoichiometry.

Reference

https://www.cell.com/biophysj/fulltext/S0006-3495(20)33130-1

CORRESPONDANCE

Publication Date: Feb 15, 2021

Estimates of anti-SARS-CoV-2 antibody seroprevalence in Iran

Iran was among the first countries outside China to report a large outbreak of COVID-19, but the transmission dynamics across the country have largely remained unknown due to the scarcity of serological, epidemiological, and genomic data. One of the main barriers has been the fact that Iran's Ministry of Health and Medical Education (MoHME) stopped releasing province-level data on the number of confirmed COVID-19 cases from March 22, 2020, onward. Furthermore, provincial data on the number of confirmed COVID-19-related deaths were never released. Instead, MoHME reports the percentage change in the number of cases with respect to previous days as an indicator of the state of the epidemic in each province and colour-codes them from blue (low incidence) to yellow (medium incidence), orange (high incidence), and red (very high incidence).

Despite the significant implications of understanding the Iranian epidemic for the country and the Eastern Mediterranean region as a whole, research investigations have largely been hindered due to the lack of epidemiological data on the number of cases and deaths, age-stratified and sex-stratified data, both at the national and province level, and seroepidemiological analysis. The study by Hossein Poustchi and colleagues, sponsored by MoHME and carried out by the then Deputy Minister of Research and Technology of the Ministry of Health Reza Malekzadeh and his team, to measure SARS-CoV-2 antibody seroprevalence in the general population across 18 cities of Iran was the first systematic investigation into the geographical spread of COVID-19 across the country nearly a year after the first two cases were reported in Qom on Feb 19, 2020. Their analysis showed greatly varied levels of exposure in different cities, with some reaching very high levels (>50% in Qom and Rasht) by late April to early June.

Reference

https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(21)00053-0/fulltext

PERSPECTIVE

Publication Date: Feb 12, 2021

Neurotropic effects of SARS-CoV-2 modeled by the human brain organoids

COVID-19, caused by SARS-CoV-2, is a socioeconomic burden, which exhibits respiratory illness along with unexpected neurological complications. Concerns have been raised about whether the observed neurological symptoms are due to direct effects on CNS or associated with the virus's systemic effect. Recent SARS-CoV-2 infection studies using human brain organoids revealed that SARS-CoV-2 targets human neurons. Human brain organoids are stem cell-derived reductionist experimental systems that have highlighted the neurotropic effects of SARS-CoV-2. Here, the neurotoxic effects were summarized of SARS-CoV-2 using brain organoids and comprehensively discuss how brain organoids could further improve our understanding when they are fine-tuned. For more details, read the link given below.

Reference

https://www.cell.com/stem-cell-reports/fulltext/S2213-6711(21)00087-4

Tracking the UK SARS-CoV-2 outbreak

Following a year of relatively uneventful evolution, the emergence and global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants signals an urgent need for better genetic tracking. The United Kingdom (UK) has emerged as a leader in this domain. A £20 million investment in March 2020 established the COVID-19 Genomics UK (COG-UK) Consortium, which has produced >200,000 SARS-CoV-2 genomes, more than twice the number produced by any other country. Such a large volume of data provides an unprecedented opportunity to trace which human activities drive epidemic growth during a rapidly changing pandemic, but also introduces numerous bioinformatic challenges. du Plessis *et al.* describe a new hybrid phylogenetic approach that integrates genetic data with epidemiological and travel data to uncover the roots of the UK's severe spring epidemic. Notably, they find that the UK epidemic resulted from more than 1000 transmission lineages seeded by travelers from Europe. The study shows how last winter's control efforts were consistently one step behind the

virus, allowing SARS-CoV-2 to permeate national borders. Their analysis of ~26,000 UK sequences from January to June 2020, the largest study of its kind, reveals that the UK epidemic was primarily brought into the country by travelers from European neighbors: first Italy, then Spain and France. Peak viral flow into the UK occurred in March as the virus expanded across Western Europe, but surveillance lags led to restrictions still focusing on travelers arriving from Asia. By capturing large numbers of small transmission lineages that would not be detected at lower levels of virological surveillance, as well as >1600 singleton viruses with no observed progeny, the authors uncovered an unprecedented volume of cross-border virus traffic. Genetic patterns mirrored human movement patterns, as the number of viruses entering the UK rose and then fell after international travel plummeted in March. The UK is not the only country whose early focus on Asia as the pandemic epicenter allowed viruses to enter from European sources. Genetic data also traced the origins of epidemics in Brazil, Boston, and New York City back to Europe. Travel restrictions can be highly effective when stringently implemented, but these studies collectively highlight how easily SARS-CoV-2 infection can arise during even small lapses in border control, including the repatriation of Americans from Asia at the beginning of the pandemic. For more details, read the link given below.

Reference

https://science.sciencemag.org/content/371/6530/680

Single-domain antibodies make a difference

The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, mediates attachment of the virion to the host cell, binding to the receptor, and fusion of the viral and host cell membranes. This releases the viral genomic RNA into the host cell cytoplasm, which is the start of virus replication. Antibodies that interfere with SARS-CoV-2 spike function—in particular, antibodies that prevent the interaction between the receptor binding domain (RBD) of spike and human angiotensin-converting enzyme 2 (ACE2), the canonical receptor on the host cell surface—can neutralize the virus in vitro and are associated with protection from infection *in vivo*. Koenig group describe four SARS-CoV-2–neutralizing single-domain antibodies, or VHHs, and combinations thereof that can disable spike function. This

extends the growing list of reports on SARS-CoV-2 spike–specific single-domain antibodies that have been proposed as potential therapeutics for COVID-19 patients.

VHHs (present in camelid species) are the variable domains of heavy chain–only antibodies, which lack a light chain and the first constant domain of the heavy chain that occur in conventional antibodies. Koenig *et al.* immunized a llama and an alpaca with recombinant spike RBD antigen and inactivated SARS-CoV-2 virions to select high-affinity VHHs directed against SARS-CoV-2 spike. Structural analysis of the VHHs in complex with RBD or prefusionstabilized spike revealed their epitopes, explained their capacity to hinder ACE2 binding, and showed that two of the VHHs captured spike in its so-called up-conformation in which the RBD is flipped upward, which is essential for the virus to engage with ACE2. The closed state is the predominant form of spike on the virion. ACE2 binding combined with proteolytic activation of spike triggers membrane fusion. The authors propose that some of their VHHs—including biparatopic ones that bind two different, nonoverlapping epitopes on spike—can destabilize the prefusion conformation of the spike protein and thereby inactivate the virus fusion machinery. For more details, read the link given below.

Reference

https://science.sciencemag.org/content/371/6530/681

COMMENT

Publication Date: Feb 17, 2021

Clarifying the evidence on SARS-CoV-2 antigen rapid tests in public health responses to COVID-19

Abstract

The use of rapid lateral flow antigen testing (LFT) for SARS-CoV-2 has been questioned with uncorroborated reports of poor LFT sensitivity. The debate surrounding the use of the Innova Lateral Flow SARS-CoV-2 Antigen Test in the UK risks confusing policy makers internationally and potentially stalling deployment of LFTs in other countries. As scientists and health professionals evaluating some of the world's largest pilots of LFT, we wish to challenge those interpretations and clarify the evidence on how such testing might be used to detect SARS-CoV-2 in minutes and improve COVID-19 control measures.

Testing for SARS-CoV-2 is central to COVID-19 management and has relied on quantitative reverse transcriptase polymerase chain reaction (PCR) technology. PCR seeks the genetic code of the virus from nose or throat swabs and amplifies it over 30–40 cycles, doubling each cycle, enabling even miniscule, potentially single, copies to be detected. PCR is thus a powerful clinical test, specifically when a patient is, or was recently, infected with SARS-CoV-2. Fragments of RNA can linger for weeks after infectious virus has been cleared, often in people without symptoms or known exposures.

Reference

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)00425-6/fulltext

REPORT

Publication Date: Feb 17, 2021

Intranasal fusion inhibitory lipopeptide prevents direct-contact SARS-CoV-2 transmission in ferrets

Containment of the COVID-19 pandemic requires reducing viral transmission. SARS-CoV-2 infection is initiated by membrane fusion between the viral and host cell membranes, mediated by the viral spike protein. We have designed lipopeptide fusion inhibitors that block this critical first step of infection, and based on in vitro efficacy and in vivo biodistribution selected a dimeric form for evaluation in an animal model. Daily intranasal administration to ferrets completely prevented SARS-CoV-2 direct-contact transmission during 24-hour co-housing with infected animals, under stringent conditions that resulted in infection of 100% of untreated animals. These lipopeptides are highly stable and thus may readily translate into safe and effective intranasal prophylaxis to reduce transmission of SARS-CoV-2.

Reference

https://science.sciencemag.org/content/early/2021/02/16/science.abf4896

Publication Date: Feb 11, 2021

Revealing tissue-specific SARS-CoV-2 infection and host responses using human stem cell-derived lung and cerebral organoids

COVID-19 is a transmissible respiratory disease caused by a novel coronavirus, SARS-CoV-2, and has become a global health emergency. There is an urgent need for robust and practical in vitro model systems to investigate viral pathogenesis. Here, we generated human induced pluripotent stem cell (iPSC)-derived lung organoids (LORGs), cerebral organoids (CORGs), neural progenitor cells (NPCs), neurons, and astrocytes. LORGs containing epithelial cells, alveolar types 1 and 2, highly express ACE2 and TMPRSS2 and are permissive to SARS-CoV-2 infection. SARS-CoV-2 infection induces interferons, cytokines, and chemokines and activates critical inflammasome pathway genes. Spike protein inhibitor, EK1 peptide, and TMPRSS2

inhibitors (camostat/nafamostat) block viral entry in LORGs. Conversely, CORGs, NPCs, astrocytes, and neurons express low levels of ACE2 and TMPRSS2 and correspondingly are not highly permissive to SARS-CoV-2 infection. Infection in neuronal cells activates TLR3/7, OAS2, complement system, and apoptotic genes. These findings will aid in understanding COVID-19 pathogenesis and facilitate drug discovery.

Reference

https://www.cell.com/stem-cell-reports/fulltext/S2213-6711(21)00085-0