THREE OPTIONS AGAINST SARS-CoV-2: PEDIATRIC SERA, MITHRIDATIZATION, DEWETTING ANTIBODIES

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Here we suggest three feasible options that could be useful in COVID-19 medical management.

1) COVID-19: CHILDREN SERA FOR PASSIVE ANTIBODY THERAPY?

Among the potential treatment options for COVID-19 [1], passive antibody therapy has been suggested. Because infusions of antibody-laden blood have been fruitful in prior outbreaks such as the SARS epidemic and the 1918 flu pandemic, it has been proposed to use human convalescent sera from individuals recovered from COVID-19 [2]. Supporting the idea of using plasma from previously affected patients, a preliminary study suggests that macaques do not develop a coronavirus infection the second time they are exposed [3]. Nevertheless, it is well known that children display less severe symptoms when infected by SARS-CoV-2 [4]: this means that it might be hypothesized that sera from pediatric population could be experimented as a promising option for containing COVID-19, both in prevention and treatment.

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2) MILD COVID-19 SYMPTOMS AND LOW VIRAL LOAD: A FORM OF MITHRIDATIZATION?

SARS-CoV-2 positive patients with few/no symptoms and modest levels of detectable viral RNA in the oropharynx have been described [1]. This claim, together with the observation that SARS-CoV-2 displays a well-known decay rate both in aerosols and various surfaces [2], suggests an intriguing possibility: when an otherwise healthy subject is exposed to a very low viral load, a partial immunization might occur that prevents severe COVID-19 forms. This mechanism, that we might term "mithridatization", has been already described in animal models of the 2009 pandemic influenza virus, where decreasing the challenge dose resulted in reduction in clinical signs and delay in virus production in the upper respiratory tract [3]. This would mean that a low-dose challenge with SARS-CoV-2 might be administered to decrease the COVID-19 symptomatology, in particular in fragile patients.

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3) DEWETTING ANTIBODIES AGAINST VIROPORINS MIGHT CONTRIBUTE TO DECREASE 2019-nCoV INFECTION SPREAD

Dewetting transition is transient phenomenon taking place inside the hydrophobic pores of ion channels that forbids water molecules to cross microscopic receptor cavities and leads to impairment of cellular performance. It has been recently suggested that artificially-provoked dewetting transition in ion channel hydrophobic pores might stand for a molecular candidate to erase viruses, bacteria and cancer cells, and to block autoimmune activities. A novel type of high-affinity monoclonal antibody has been suggested, that is equipped with lipophilic and/or hydrophobic fragments that prevent physiological water flow inside ion channels and targets specific trans-membrane receptor structures of influenza viruses. Here we suggest that these dewetting monoclonal antibodies targeting the 2019-nCoV viroporin channels, sprayed in the nasal cavities, might lead to virion impairment, thus preventing inter-human viral transmission.

In fluid mechanics, dewetting stands for the rupture of the thin, liquid continuous film on the solid–liquid or liquid–liquid interfaces, leading to formation of irregular patterns of droplets (Sharmaa, 1996; Sharmaa and Reiterb, 1996; Sackmann and Bruinsma, 2002; Anishkin and Sukharev, 2004; Rosen 2004; Tanaka et al., 2005; Karapanagiotis and Gerberich, 2005; Leroux et al., 2008; Young et al., 2010; Boreyko et al., 2011; Thompson 2012; Gonzalez-Rodriguez et al. 2012; Douezan and Brochard-Wyart, 2012; Rahe et al., 2012; Lapierre et al., 2013; Thiam et al., 2013; Lee et al., 2014; Vargas et al., 2014; Sándor et al., 2017; Alert and Casademunt 2018; Rego et al., 2019). This process, borrowed by physics, has been recently extended to describe also microscopic biological phenomena. In particular, dewetting transitions may occur inside the hydrophobic pores of cellular ion channels. In the narrowest, more hydrophobic parts of receptor holes, a metastable state of dewetting transition forbids water molecules to get inside the cavities, leading to a decrease in conductance channel closure, and impairment of cellular activity. Tozzi (2020) proposed that this peculiar process can be artificially produced to alter the physiological activity of noxious pathogens, such as viruses, bacteria and tumoral cells. In particular, the manufacture of monoclonal antibodies (against cellular receptors) has been suggested, equipped with lipophilic/hydrophobic caps. In the sequel, we will term these antibodies DEMA (DEwetting Monoclonal Antibodies). Once they link monoclonal targets, their artificial hydrophobic device blocks water flow inside receptors, contributing to malfunction of pathological organisms. The factors influencing dewetting in physical systems, in biological structures and also in nervous cells are described by Tozzi (2020).
In brief, a huge range of physical factors, that can be fine-tuned in experimental settings, may contribute to the attainment and preservation of dewetting regimes in countless systems equipped with solid–liquid or liquid–liquid interfaces. Summarizing, when water and ions are enclosed within the sub-nanometer, narrow cellular confines of a ion channel hydrophobic pore, they exhibit an odd behavior (Aryal et al., 2015): near a critical point, a stochastic liquid-vapor water phase transitions takes place (Anishkin and Sukharev, 2004). These transient vapor states are "dewetted", i.e., devoid of water molecules within all, or part of, the pore. The decreased amount of water molecules inside receptors leads to impaired conductance, energetic barriers to ion transit and closure of the channel, in a process termed "hydrophobic gating". It is noteworthy that the principles underlying the metastable dynamical state of hydrophobic gating require a very small tube radius and interactions with a strongly hydrophobic lining (Lapierre et al., 2013).
In the sequel, the above-mentioned physiological phenomenon of dewetting transition will permit us to build strategies to influence the dynamics of droplet formation inside biological channels of 2019-nCoV viroporin channels.

TOWARDS DEWETTING MONOCLONAL ANTIBODIES

In this Section, we consider the possibility to build high-affinity monoclonal antibodies able to dewet the ion channels of target cells, thus leading to their impairment and, possibly, death. Antibodies with high affinity for receptors of pathological cells could be equipped with lipophilic structures, covalently attached, e.g., to their Fc region. These hydrophobic/lipophilic components must serve two purposes: a) to prevent water to penetrate inside the receptor ionic channels; b) to avoid immune Fc-mediated responses. In plain terms, we could state that, thanks to DEMA, a sort of cork provides a tight seal that prevents water to fill the receptor channels of pathogenic cells, causing collapse of unwanted organisms. DEMA could be used to counteract different types of pathogenic cells, such as viral, bacterial and tumoral ones. In the sequel, we will provide the example of Influenza A M2 proton channel, in an effort to develop a novel drug able to neutralize the virion. In Tozzi (2020), we provided an example, describing how to build D**EMA against influenza A virus M2 receptor** (Pielak and Chou, 2011; Rossman et al., 2010; Cho and Wrammert, 2016; Fiers et al., 2009; Cady et al., 2010; Homeyer et al., 2016).
The following paragraph is from Tozzi (2020), including the Figures. We suggest to use DEMA as a novel drug against all the strains of Influenza A (**Figure 1, top**). The very structure of M2 let us hypothesize that a monoclonal antibody that prevents water to enter the M2 channel might disrupt Influenza A pathogenetic activity. Indeed, water plays a foremost role in M2 functioning (**Figure 2A**). The channel, highly selective for protons, is activated by low pH and has a low conductance. Conduction mechanism involves: a) the exchange of protons between the His37 imidazole moiety, responsible for proton selectivity and pH modulation, and b) the water confined to the M2 bundle interior. Water molecules within the pore form hydrogen-bonded networks or "water wires", from the channel entrance to His37.

When a proton gradient occurs, conformational changes facilitate asymmetric diffusion through the channel. Indeed, protons diffusing through the channel need not be localized to a single His37 imidazole, but rather they may be delocalized over the entire His-box and associated water clusters. Furthermore, pore-lining carbonyl groups stabilize hydronium ions through second-shell interactions that involve the bridging of water molecules. A ring of methyl groups from Val27 tightens the N-terminal side of the pore to ~3.1 Å, narrowing the entrance and preventing water molecules from penetrating the channel (Pielak and Chou, 2011). Small motion or "channel breathing" may thus be required for water to enter the pore. It is widely accepted that water molecules are needed inside the channel cavity for supporting proton conduction. Water molecules, provided with a diameter of ~3 Å, start to exhibit liquid–vapor transitions and stochastic switches between wet and dry states within a hydrophobic pore of diameter less than ~14 Å. The most dynamic range for these transitions is between 9 and 12 Å: below this range, the pore will be largely dewetted (Aryal et al., 2015). The pore widens after Ser31 and becomes the widest at Gly34 position, that is equipped with an inner diameter of ~ 6 Å. The channel then narrows towards the C terminus, as the sidechains of His37 and Trp41 constrict the inner diameter to 1.7 and 1.4 Å, respectively.

The presence of a hydrophobic (or lipophilic) part located on the constant chain of DEMA, could, according to our theoretical previsions, stop water flow inside the M2 channels (**Figure 2B**), leading to viral malfunction and, possibly, removal from the infected human body.

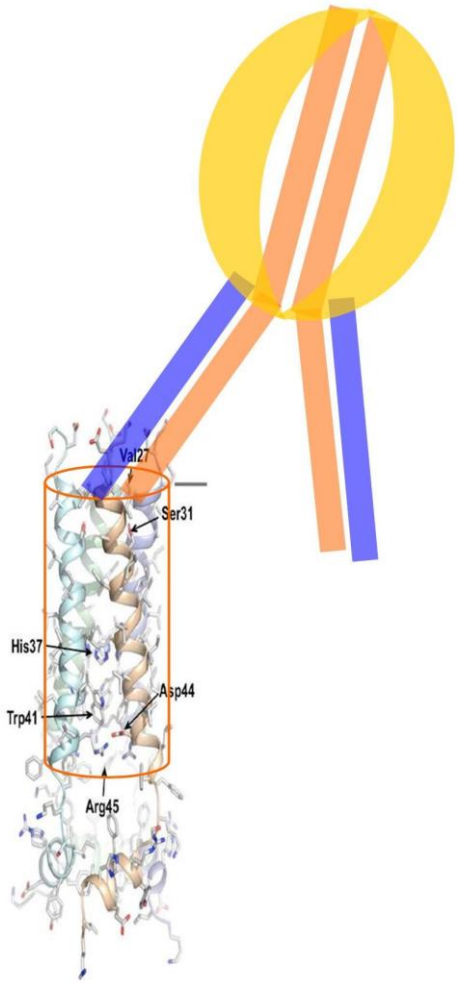

Figure 1. **Bottom**: high resolution structures of the AM2 channel domain. Solution structure of residues 18–60 in 1,2-Dihexanoyl-sn-Glycero-3-Phosphocholine micelles at pH 7.5 (Modified from Pielak and Chou, 2011). **Top**: Dewetting antibody (DEMA) against the Val27 area. The Fc region of this artificial antibody is surrounded by an hydrophobic structure (yellow shape). Channel and antibody are drawn to scale: the transmembrane section (red cylinder) is about 30 Å long. From: Tozzi (2020).

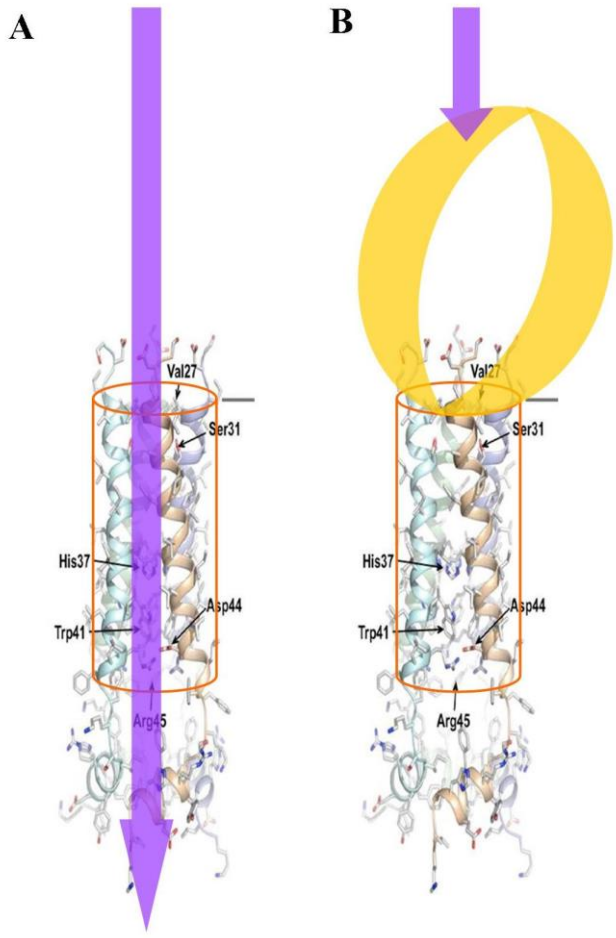

Figure 2. Water flow inside M2 channel. **2A**: In physiological conditions, water is allowed to flow inside the M2 channel, giving rise to proton gradients crucial for pathogenic activity of Influenza A virus. **2B**: When a DEMA targets the upper part of the M2 receptor, its hydrophobic component prevents water to go through the M2 receptor channel (**right side**), leading to impairment of virionic metabolic pathways. From: Tozzi (2020).

CORONAVIRUS CHANNELS

Our account of dewetting transition paves the way for innovative strategies. To make an example, dewetting enables researchers to build synthetic asymmetric model membranes with lipid composition/architecture that mimics the outer membrane of human pathogens, such as Pseudomonas aeruginosa (Maktabi et al., 2019).

Here we take into account the 2019 novel coronavirus 2019-nCoV, recently announced by the World Health Organization. Bioinformatics analysis on a virus genome is available and has been compared with other related coronavirus genomes: overall, the genome of 2019-nCoV has 89% nucleotide identity with bat SARS-like-CoVZXC21 and 82% with that of human SARS-CoV (Chan et al., 2020). Recent studies on coronaviruses have described the presence of a viroporin, i.e., a ion-channel protein (**Figure 3**). The latter is generated by the CoV E protein when it forms homotypic interactions which allows it to oligomerise (Schoeman and Fielding, 2019). Viroporins are viral-encoded membrane pore-forming proteins that can modulate cellular ion channels; they have been suggested to regulate and function in multiple stages of the viral life cycle, from viral entry to assembly and release, and even pathogenesis (Schoeman and Fielding, 2019).

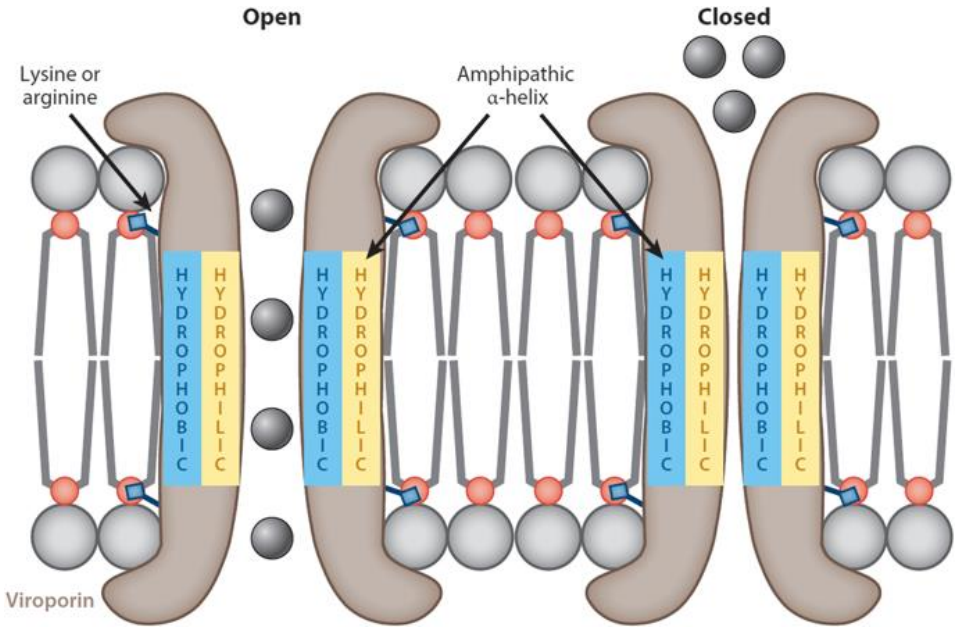

Figure 3. Coronaviruses' viroporin structure and motifs. For further details about this Figure, see Schoeman and Fielding (2019).

SUGGESTIONS

Dewetting transition, characterized by an unusual behavior of water supramolecular assembly, stands for an increasingly important feature that has been already used to assess countless morphological and/or functional biological structures, such as protein cavities, extracellular matrix and glycocalix, lipid droplets, lipid bilayers, cell adhesion, macroapertures opening in endothelial cells. In the narrowest, more hydrophobic part of cellular channels, a metastable state of dewetting transition of water molecules takes place, leading to decrease of conductance and closure of the pore.

Dewetting mechanisms can be used to achieve novel therapeutic weapons against a wide range of diseases. Further, the possibility to artificially modulate dewetting processes could lead to the development of new drugs (with mechanisms different from DEMA) that might be relevant in development, regeneration, self-immunity, infection and cancer.
Here we suggest to build a dewetting monoclonal antibody against the viroporins of 2019-nCoV (or against other viral channels characterized by dewetting transition). Once achieved a drug, it could be sprayed into the nose. Indeed, administred through the nose, such monoclonal dewetting antibody might contribute to inactivate the virions in the human airways, before 2019-nCoVs are able to link to the host's cellular membranes.

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