REVIEW | Virus-Host Cell Interactions and the Viral Life Cycle: Basic Science to Therapeutics

Inhibiting fusion with cellular membrane system: therapeutic options to prevent severe acute respiratory syndrome coronavirus-2 infection

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Inhibiting fusion with cellular membrane system: therapeutic options to prevent severe acute respiratory syndrome coronavirus-2 infection. Am J Physiol Cell Physiol 319: C500–C509, 2020. First published July 20, 2020; doi:10.1152/ajpcell.00260.2020.—Severe acute respiratory syndrome coronavirus (SARS-CoV), an enveloped virus with a positive-sense single-stranded RNA genome, facilitates the host cell entry through intricate interactions with proteins and lipids of the cell membrane. The detailed molecular mechanism involves binding to the host cell receptor and fusion at the plasma membrane or after being trafficked to late endosomes under favorable environmental conditions. A crucial event in the process is the proteolytic cleavage of the viral spike protein by the host’s endogenous proteases that releases the fusion peptide enabling fusion with the host cellular membrane system. The present review details the mechanism of viral fusion with the host and highlights the therapeutic options that prevent SARS-CoV-2 entry in humans.

ACE2; endocytosis; receptor binding motif; SARS-CoV-2; serine protease inhibitors

INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV) belonging to the order Nidovirales and family Coronaviridae has shown efficient transmission to humans causing global health crises (13, 70, 72). Three of the coronaviruses, SARS-CoV, MERS-CoV (Middle-East respiratory syndrome coronavirus), and SARS-CoV-2, have inflicted pandemic pathogenicity, while four others, HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E, are endemic strains exhibiting milder symptoms (57). As of today, there is no therapy or vaccine available to combat the coronavirus (CoV) infection. Among all the RNA viruses, coronaviruses have the largest genome that is packed inside a helical nucleocapsid and surrounded by an envelope (29). Associated with the viral envelope is the transmembrane spike (S) glycoprotein that facilitates viral entry to the host cell through receptor binding and membrane fusion (35) and serves as a central determinant of virulence and host selectivity (45).

A series of molecular interactions between SARS-CoV-2 spike protein and the host cellular membrane system is essential for the coronavirus infection. Proteolytic processing of the viral spike protein as well as the interaction of the fusion peptide with the host cell membrane constitutes a critical step for the viral entry and consequently serves as a crucial target for therapeutic intervention. The present review provides insights on how endogenous proteases and their inhibitors could regulate SARS-CoV-2 interaction with the host cell membrane and describes in detail the therapeutic options to impede viral infection to the human host.

RECEPTOR BINDING BY SARS-CoV-2 AND ITS PREVENTION BY SOLUBLE RECEPTOR DECOY

Viral entry to the host cell is mediated by the highly glycosylated transmembrane protein Spike (S) comprising of S1 and S2 subunit, S1 subunit being the domain that recognizes and binds to the host receptor (65) (Fig. 1). The receptor binding pattern is diverse among different coronaviruses. SARS-CoV-2 and HCoV-NL63, despite being members of different genera, bind to the human angiotensin-converting enzyme 2 (hACE2), a carboxypeptidase that hydrolyzes the active bradykinin metabolite [desArg-973] bradykinin (DABK) and attenuates the inflammatory response mediated through bradykinin receptor (B1R) (55). On the contrary, MERS-CoV, though a member of the same genera as that of SARS-CoV-2, recognizes a different receptor, dipeptidyl peptidase 4 (DPP4), for the host cell entry (6, 28, 29, 59). This apparent conundrum of receptor usage by coronaviruses has been resolved by crystal structures of S1 domains highlighting the presence of a specific receptor-binding motif (RBM) that interacts with virus binding motifs (VBM) of the host receptor determining the binding affinity and preference (28) (Fig. 1).
The receptor-binding domain (RBD) of MERS-CoV, for example, has a core structure similar to that of SARS-CoV, but their markedly different RBMs account for the different receptor specificity for host tropism (29).

Comparative analysis of the crystal structures of the S1-CTD of pathogenic pandemic strain SARS-CoV-2 and low pathogenic endemic strain HCoV-NL63 complexed with hACE2 provides an overview of the pattern of receptor recognition by CoV spike proteins. The structure of RBD of SARS-CoV-2 and NL63-CoV are different: the SARS-CoV-2 possesses a single long continuous RBM (60) whereas, in NL63-CoV, RBM is discontinuous, comprising of three short loops (61) (Fig. 1). Notwithstanding their very different primary, secondary, and tertiary structures, the two RBMs recognize the common amino acid residues at overlapping ACE2 virus dealing motif (VDM) (60, 62) (Fig. 1). Contrary to the NL63-CoV-RBD, the crystal structure of SARS-CoV-2-CTD reveals the presence of RBD similar to that of SARS-CoV (60) showing 50% identity within their RBMs (57). However, the binding affinity of SARS-CoV-2 RBD with VDM of hACE2 is higher as SARS-CoV-2 RBM has more residues at the binding interface and displays enhanced Vander Waals interaction and hydrogen bonding compared with SARS-CoV (60). Monoclonal and polyclonal antibodies against SARS-CoV spike protein do not recognize their counterpart in SARS-CoV-2, highlighting their distinct epitope features (60). Nonetheless, all three viruses (hCoV-NL63, SARS-CoV, and SARS-CoV-2), bind to the overlapping regions on their common receptor hACE2, indicating the existence of an evolutionary conserved VDM hotspot that favors hCoV binding.

RBD of hCoV, from an evolutionary perspective, is mutation prone and hence not suitable for antiviral drug development. However, the conserved VDM hotspots on the host hACE2 receptor provide an opportunity for broad therapeutic intervention. Monteil et al. have demonstrated that the recombinant soluble hACE2 receptor that lacks the membrane anchor could significantly block SARS-CoV-2 infections in Vero 6 cells, engineered human blood vessel organoids, and human kidney organoids (37). The application has a dual advantage: in addition to its role as a decoy to prevent SARS-CoV-2 entry to the host cell, the recombinant soluble hACE2 receptor could prevent bradykinin dependent angioedema in lungs, a typical clinical feature of SARS-CoV-2 pathogenesis (55). A similar decoying strategy could prevent the future coronavirus infection using the hACE2 receptor as a port of entry; however, an in-depth assessment of soluble hACE2 receptor vis-à-vis toxicity and therapeutic benefits is necessary before progression for clinical trials.

**PROCESSING OF SARS CoV-2 SPIKE PROTEINS: REGULATION BY PROTEASE INHIBITORS**

The spike (S) glycoprotein, like other class I viral fusion proteins, mediates membrane fusion through its S2 subunit (Fig. 2). The receptor binding and fusion are sequential events; following binding of the S1 subunit to the cellular receptor, the S2 subunit undergoes conformational reorientation that facilitates the insertion of the fusion peptide to the host cell membrane (29, 63). To execute this conformational transformation, spike protein undergoes proteolytic processing by host serine proteases. Several serine proteases are present in airways.
epithelial cells (25) that includes mini plasmin at folded epithelial cells of bronchioles (39), tryptase club at bronchiolar epithelial club cells (club cells were formerly known as Clara cells; 26, 54), anionic trypsin in stromal cells of the peribronchiolar region (25) and transmembrane serine protease TM-PRSS2 (epitheliasin) and TMPRSS11D (human airway trypsin-like protease, HAT) in the cells of the upper and lower aero-digestive tract (4). In addition to endogenous proteases from airways epithelial cells, infiltrating immune cells under various pro-inflammatory, inflammatory, and pathological circumstances also secrete serine proteases capable of processing class I viral membrane fusion proteins (2). Viruses of different strains efficiently exploit the proteolytic processing capacity of these endogenous proteases to mediate host cell entry.

In the case of SARS-CoV-2, two proteolytic events are essential: one at the polybasic S1/S2 boundary that harbors several arginine residues (8, 20), and the other at the conserved KRSFIEDLDLNKV motif at the NH2 terminus of the internal fusion peptide (S2′ cleavage site) (21). The polybasic multiple arginine cleavage sites at S1/S2 boundary of the spike protein undergoes proteolytic cleavage by serine protease furin (8, 20), the other proteolytic processing at the S2′ cleavage site being mediated by TMPRSS2 (21) expressed in the upper respiratory tract and bronchial transient secretory cells (31). Both the proteolytic processing events are essential for the fusion of the spike protein with the host cell plasma membrane (20, 21) (Fig. 2).

Proteolytic cleavage of SARS-CoV-2 spike protein may also be accomplished at the late endosomes by lysosomal proteases cathepsin L following endocytosis of the virus (53, 66). However, the involvement of lysosomal proteases is restricted mostly in the kidney, gut, and blood vessels (53) as the enzyme has sparse distribution in the lungs and respiratory tract cells (43). The critical role of the host cell proteases in processing SARS-CoV-2 spike proteins highlight the therapeutic importance of the protease inhibitors for antiviral intervention. The emerging research indicates the viral route of entry to be dependent on the availability of the host proteases; accessibility to the plasma membrane proteases enables fusion via an early pathway whereas hCoV retains the flexibility to use the endosomal proteases like cathepsin L to mediate entry following fusion at the endosomal membrane through the late pathway (53). Type II transmembrane serine proteases (TTSPs), play a critical role in mediating viral entry through the plasma membrane pathway (early pathway) (35, 53). Camostat mesylate, a potent serine protease inhibitor of TTSP family protease TMPRSS2 that hinders the cellular entry of SARS-CoV-2 by blocking early entry pathway, is presently undergoing clinical trials.

Inhibition of furin is also a crucial therapeutic option for SARS-CoV-2 spike protein fusion with the host cell membrane (20). Protein-based furin inhibitors like α1-PDX, amidated ω-polyarginine based hexapeptide (DGR), and chloromethyl ketone (CMK)-based peptidomimetics have been reported in the literature but the paucity of the clinical data with these inhibitors limits the evaluation of their efficacy in humans (9). Van Rompaey et al. have described an innovative approach of furin inhibition by engineering furin recognition sequence at the bait region of alpha2 macroglobulin (α2M) (56). Given the efficacy of engineered α2M in inhibiting furin at nanomolar concentration, the localized application of the engineered α2M through intranasal delivery could be of clinical significance in the defense against SARS-CoV-2 infection.

The involvement of cathepsins, more particularly by cathepsin L for SARS-CoV infection has been reported in the liter-
turation (53, 35, 42). However, the expression of cathepsin L being sparse in the respiratory tract cells, the enzyme gets clinical prominence in the late stage of infection when the virus spreads beyond the lungs and infects other cells in the kidney, gut, and blood vessels (53). In this context, E64D (aloxistatin), a cysteine protease inhibitor that was unsuccessful in Phase III clinical trials for Duchene’s muscular dystrophy (40, 41), could be repositioned to impede the viral spread to the other organs. Specific cathepsin L inhibitor Z-FY(tBu)-DMK (22) and cysteine protease inhibitor K11777 (71) inhibit SARS-CoV infection and may have clinical significance in impeding SARS-CoV-2 entry to the host cell as well.

In addition to the recombinant protein-based and synthetic protease inhibitors, the human physiological system has its endogenous protease inhibitors that confer protection against viral infection. Histidine-rich human salivary peptides (histatin 3 and 5) reveal potent furin inhibitory activity (3) and may serve as a first line of defense against SARS-CoV-2 entry. Plasminogen activator inhibitor-1 (PAI-1), encoded by the SERPINE1 (serpin family E member 1) gene and expressed in several cell types in the lung (1) inhibits several airway proteases including TMRPSS2 (11) indicating its ability to impede SARS-CoV-2 spike protein processing. Submucosal tracheal serous cells (14), nonciliated epithelial cells in terminal bronchiole (38), and alveolar epithelial cells (47) possess secretory leukocyte protease inhibitor (SLPI), an important component of the innate mucosal immunity that inhibits proteolytic processing of the influenza virus (25) and human immunodeficiency virus (HIV-1) (24, 34). Intranasal administration of recombinant SLPI significantly reduces influenza virus infection in rat lung through its broad-spectrum serine protease inhibitory activity (25). Sulforaphane, a pan-histone deacetylase (HDAC) inhibitor, enhances the expression of SLPI (67) and ambroxol, a mucolytic and antioxidant agent, increases its secretion (25), providing therapeutic options for the augmentation of antiviral therapy. The involvement of serine proteases in processing spike protein along with the distribution of SLPI in the upper respiratory tract suggests at its putative role in defense against SARS-CoV-2 infection that needs experimental validation. The presence of these endogenous protease inhibitors in human saliva and respiratory tract defines the host’s defensive response against complex and diverse lung microbiome and their enhanced expression may provide a natural immunity against SARS-CoV-2 infection (Fig. 2). Moreover, these endogenous protease inhibitors confer protection against acute lung injury due to inflammatory cytokine storm, which is a typical pathological manifestation of SARS-CoV-2 infection. A population-based comparative study of PAI-1, α2M, and SLPI expression in normal volunteers and SAR-CoV-2 patients along with their activity to inhibit TMRPSS2 would throw more light on the role of endogenous protease inhibitors in preventing hCoV infection in the human population.

THE FUSION OF VIRAL ENVELOPE WITH THE HOST MEMBRANE: TREATMENT OPTIONS

The prefusion structure of SARS-CoV and SARS-CoV-2 spike as revealed by cryo-electron microscopy displays S1 subunit on top of a trimeric S2 stalk that possesses a homotrimeric assembly of the heptad repeat 1 (HR1) region (63, 64) (Fig. 3A). Following proteolytic processing by host cell serine proteases at the S1/S2 and S2’ cleavage site, the S2 subunit undergoes conformational reorientation exposing the three highly conserved hydrophobic grooves on HR1 surface for binding of heptad repeat 2 (HR2) (Fig. 3A). The resultant six-helix bundle (6-HB) core structure exposes the fusion peptide as it brings the viral and cellular membranes in close apposition facilitating the fusion process. The fusion peptide undergoes a series of conformational changes from the prefusion metastable state to a postfusion stable state (27, 53, 63, 64) and the process requires a substantial calcium concentration as has been evidenced in case of SARS-CoV infection (35). The calcium dependency of the fusion process determines the site of viral entry, which is at either the plasma membrane or the specific late-endsosomal compartment.

The multilevel interaction of the fusion peptide with its structural components, host proteins, and signaling intermediates provides opportunities for several therapeutic interventions.

Antifusogenic Approach

Xia et al. proposed a peptide-based antifusogenic approach that inhibits the formation of a six-helix bundle fusion core thereby inhibiting the fusion process (63). The strategy was derived from DP-78 (Fuzeon), a synthetic peptide designed based on HR2 of HIV-1 envelope glycoprotein gp41. DP-78 inhibits HIV-1 infection by competitively binding to the HR1 region of gp41 subunit of HIV-1 (19). Xia et al. reported that peptide EK1, an optimized HR2 peptide derived from beta coronavirus HCoV-OC43, binds to the SARS-CoV-2 HR1 inhibiting the six-helix bundle (6-HB) fusion core formation and subsequent release of the fusion peptide (63) (Fig. 3A). Lipidated derivatives of EK1 inhibits SARS-CoV-2 spike (S) protein-mediated cell-cell fusion (63). More importantly, intranasal delivery of palmitate conjugated EK1 confers prophylactic and therapeutic benefits in the mouse model (63), highlighting a novel strategy of intervention against SARS-CoV-2 infection. However, the efficacy of these lipopeptides needs proper evaluation in clinical trials.

Generation of Neutralizing Antibodies

The S protein of SARS-CoV-2 elicits host immune response and generate virus-neutralizing antibodies (18, 44, 58). Zhang et al. identified a major antigenic determinant (Leu 803 to Ala 828) at the fusion peptide region at the S2 domain of SARS-CoV (69). A corresponding linear B cell epitope (S21P2) that encompasses the S2’ cleavage site and fusion peptide region is recognized by neutralizing antibodies from the sera of SARS-CoV-2 convalescent patients (44) (Fig. 3B). Several clinical trials are presently in progress using SARS-CoV-2 patients’ convalescent sera (51). In parallel, a consortium of the industry that includes Takeda, CSL Behring, Biotest, Bio Products Laboratory, Octapharma, and Microsoft has initiated developing hyperimmune globulin (H-Ig) purified from the pooled plasma of patients recovered from SARS-CoV-2 infection (49). In the same line, SAβ BioTherapeutics employed a proprietary approach involving an artificial chromosome in cattle to produce human antibodies (H-Ig) against SARS-CoV-2 spike protein for targeted immunotherapy (49). The polyclonal antibody cocktail (SAβ185) is presently in the preclinical stage and progressing for a rapid transition to clinical studies (Table 1).
Fig. 3. Treatment options to hinder the fusion of the viral envelope with the host cell membrane. **A**: inhibition of transition from prefusion to the postfusion conformation of spike protein by lipidated EK1/OC43-HR2P by hindering six helical bundle formation (6HB) (64). **B**: neutralizing antibodies in the convalescent patient’s sera recognize linear B cell epitope spanning the S2 cleavage site and fusion peptide of SARS-CoV-2 spike protein. **C**: inhibitors of plasma membrane route and endocytic route of SARS-CoV-2 entry. A red arrow marks the fusion peptide of the virus penetrating the host cell membrane system. ACE2, angiotensin-converting enzyme 2 receptor; CD, connector domain; CH, central helix; CT, cytoplasmic tail; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; IFP, internal fusion peptide; OC43-HR2P, HR2 domain of HCoV-OC43; PAI-1, plasminogen activator inhibitor 1; S1, S1 subunit; S2, S2 subunit; SLPI, secretory leukocyte protease inhibitor; TM, transmembrane; VM, viral membrane.
Inhibition of Endocytosis

Since SARS-CoV could opt for either plasma membrane (early entry) pathway or endocytic route (late entry) (53), a combinatorial approach of the inhibition of plasma membrane proteases and blockage of endosome maturation may provide a better therapeutic outcome. Genetic ablation of plasma membrane protease TMPRSS2 in C57BL/6 mice reduces SARS-

### Table 1. Drug candidates and treatment strategies to hinder SARS-CoV-2 entry to the human host

| Drug Candidate | Host Interacting Protein | SARS-CoV-2 Interacting Protein | Treatment Strategy | Status |
|----------------|--------------------------|-------------------------------|-------------------|--------|
| Recombinant soluble hACE2 receptor | [desArg-973] Bradykinin | Spike (S1 subunit: RBM) | Impediment of viral binding to host ACE2 receptor Inhibiting Bradykinin system and blunting SARS-CoV-2-induced ARDS | Pilot investigator initiated clinical trial (Apeiron) |
| Camostat mesilate | TMPRSS2 | Spike (S2 subunit: S2’ cleavage site) | Inhibition of TMPRSS2 | Marketed for treatment of pancreatitis. An investigator-initiated randomized, placebo-controlled, phase II a trial (Aarhus University Hospital, Denmark) |
| Decanoyl-RVKR-CMK engineered α2 macroglobulin (furin cleavage site at bait region) Aloxistatin (E64D) Z-FY (t-Bu)-DMK K11777 | Furin | Spike (polybasic S1’ S2’ cleavage boundary) | Furin inhibition to impede proteolytic processing of spike protein | Preclinical |
| Dekanoyl-RVKR-CMK engineered α2 macroglobulin (furin cleavage site at bait region) Aloxistatin (E64D) Z-FY (t-Bu)-DMK K11777 | Cathepsin L | Proteolytic processing of spike protein | Cysteine protease inhibitor: cathepsin inhibition to impede proteolytic processing of spike protein | Preclinical |
| EK1 (HR2-based peptides) Neutralizing antibodies from COVID-19 convalescent patients SAB 185 (polyclonal antibody cocktail against SARS-CoV-2 spike protein) | NA | HR1 of S2 subunit of spike protein | Inhibition of conformation changes of S2 subunit of spike impeding release of fusion peptide | Preclinical |
| Neutralizing antibodies from COVID-19 convalescent patients SAB 185 (polyclonal antibody cocktail against SARS-CoV-2 spike protein) | NA | Conserved fusion peptide region (Leu 803 to Ala 828) of SARS-CoV-2 spike protein | Inhibition of fusion of viral membrane with host cell membrane and impeding viral entry to host | Phase II clinical studies (Erasmus Medical Center, Rotterdam, Netherlands; Assistance Publique Hopitaux de Paris, France); Preclinical |
| Apilimod | Phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) | NA | Inhibits vesicle trafficking and viral endocytosis | Preclinical |
| SAR 405 | Class III PI3K (PI3KC3) | NA | Inhibits autophagosome formation | Primary indication for anticancer therapy; possibility of repositioning to prevent SARS-CoV-2 endocytosis |
| Imatinib | Abelson kinase | NA | Inhibition of Abelson kinase and regulation of cytoskeletal organization | Marketed for acute lymphoblastic leukemia, chronic myeloid leukemia, gastrointestinal stromal tumors, and myeloproliferative diseases; Possibility of repositioning to prevent SARS-CoV-2 endocytosis |
| Amiodarone | Endosomal/lysosomal calcium channels | NA | Endosomal/lysosomal calcium channel inhibitor | Marketed as antiarrhythmic medication; Possibility of repositioning to prevent SARS-CoV-2 endocytosis |
| Hydroxychloroquine | Endosome maturation | NA | Prevents endosomal acidification; autophagy inhibitor | Marketed as antimalarial drug and for autoimmune disorders (systemic lupus erythematosus). Clinical trials for treatment of hospitalized patients suffering from SARS-CoV-2 infection (NIH ORCHID study Phase IIa) (Novartis phase III) |
| Bacille Calmette-Guérin (BCG) vaccine | Rab7, RILP, Hops complex | NSP7, ORF3a | Mechanism unknown | Phase III BCG-CORONA trial, University Medical Center Utrecht in collaboration with Radboud University, Netherlands. Phase III BRACE trial Murdoch Children’s Research Institute, Australia |

ARDS, acute respiratory distress syndrome; CMK, chloromethyl ketone; hACE2, human angiotensin-converting enzyme 2; HR1; heptad repeat 1; HR2, heptad repeat 2; NA, not applicable; NSP7, nonstructural protein 7; PI3K, phosphatidylinositol 3-kinase; RBM, receptor-binding motif; RILP, Rab interacting lysosomal protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2.
CoV pathology (23) but the viral spread could still be detected in the alveoli providing clinical relevance of the endosomal route of infection.

One of the key regulators of the endocytic pathway is phosphoinositides, the lipid signaling molecules derived by the phosphorylation of the third, fourth, and fifth positions of the inositol head group of phosphatidylinositol (PI) by respective phosphoinositide kinases and phosphatases (30, 32, 36). Apilimod, the potent inhibitor of phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) that catalyzes the conversion of PI3P to PI (3, 5) P2 and regulates vesicle dynamics and trafficking, hinders SARS-CoV S pseudovirions entry in 293/hACE2 cells (42). Similarly, small molecule inhibitors, like SAR-405 from Sanofi, that inhibit Class III PI3K (PI3KC3) (46) and are presently being assessed in clinical studies as anticancer autophagy inhibitors (12), could be repositioned to hinder the endosomal route of SARS-CoV-2 infection (Table 1; Fig. 3C).

Cytoskeletal proteins of the host cell is another component of the endocytic pathway exploited by SARS-CoV for the host cell entry. SARS-CoV spike protein interacts with the intracellular filamentous cytoskeletal protein vimentin (68) and imatinib, the inhibitor of Abelson kinase that regulates cytoskeletal rearrangement, impedes SARS and MERS CoV fusion at the endosomal membrane (7) (Table 1; Fig. 3C). The data indicates the involvement of the cytoskeletal proteins in regulating hCoV fusion at endosomes. Consequently, Abelson kinase inhibitors, which regulate cytoskeletal organization, may have similar impeding efficacy on SARS-CoV-2 entry to the host cell.

Viral entry either at the plasma membrane or through the endocytic route at the endosome-lysosome compartment (35) depends on the concentration of calcium present in the cellular environment. The supposition finds support in the inhibition of SARS-CoV entry by amiodarone, an arrhythmogenic drug that blocks endosomal/lysosomal calcium channels (50). The involvement of the late endosomal compartment for SARS-CoV entry put forward the rationale for the usage of lysosomotropic agents like chloroquine, hydroxychloroquine (10), and dimeric chloroquine (Lys-05) (33) that could prevent endosome maturation and subsequent host cell entry. Broad-spectrum antiviral effect of chloroquine and its derivatives, reflected in its efficacy to blunt lethal HCoV-O43 infections in newborn mice, makes it a drug of choice for the prevention against SARS-CoV-2 pandemic (48). Encouraging outcome of a pilot study showing minimum side effects has resulted in consideration of chloroquine/hydroxychloroquine as a therapy against SARS-CoV-2 pandemic (15, 16); however, an elaborate clinical trial regarding the establishment of optimal clinical dose and a careful evaluation of efficacy versus potential side effects is needed before advocating for its use as a therapy against SARS-CoV-2 infection.

The involvement of Rab7a, Rab 7B, VPS39, and other subunits of Hops complex at late endosome in wild-type MHV (mouse hepatitis virus) and feline coronavirus infection has been documented in the literature (5). A protein-protein interaction study using SARS-CoV-2 proteins as bait demonstrated the interaction of Hops complex with viral proteins encoded by ORF3a (17). The interactome analysis also reported an association of Rab7a with NSP7 nonstructural protein of SARS-CoV-2 viruses that complexes with NSP8 to form a part of viral RNA polymerase complex (17). The data indicates the possible involvement of SARS-CoV-2 with Rab7a-Hops complex underlying the importance of therapeutic intervention at the late endosomal level.

Presently none of the Rab7 inhibitors are in the clinical trial; nonetheless, mycobacterium bovis BCG (bacille Calmette-Guérin) and mycobacterium tuberculosis infection disrupt Rab7-RILP (Rab interacting lysosomal protein) interaction, preventing endosome maturation (52). Though at present there is no evidence that BCG or latent mycobacterium tuberculosis infection could prevent SARS-CoV-2 infection, clinical trials are presently underway that could address the postulation adequately (54a, 54b).

CONCLUSION

SARS-CoV-2, with its easy transmissibility and having an entire gamut of symptoms from “asymptomatic” to “severe that cause high mortality,” has reached pandemic proportions. Vaccines, considered as the holy grail to contain disease spread, are still months away, and with the virus having a high mutation rate to escape immunity, the strategy of vaccination would be required to be updated and administered in a regular basis before human population develops immunity to the novel coronavirus. The worldwide vaccination program is time consuming; to alleviate the human suffering in the present situation as well as to prevent a future threat to global health by the zoonotic transmission of new generation SARS coronaviruses, a broad-based antiviral therapy is of urgent necessity.

In this communication, I discussed the impediment of the viral entry as prophylaxis as well as a therapy against SARS-CoV-2 infection. The paradigm of prevention of SARS-CoV-2 infection involves decoying the virus by ensnaring it to a soluble receptor, inhibition of proteolytic cleavage of the spike protein, hindering fusion peptide insertion to the host membrane as well as impeding endosome maturation. A combinatorial therapy that hinders the viral entry through the plasma membrane pathway as well as through the endosomal route using new drug candidates and repositioning existing drugs (Table 1, Fig. 3C) could efficiently inhibit SARS-CoV-2 pandemic.

The present report also postulates a possibility of the hindrance of viral entry by endogenous protease inhibitors in body fluids like saliva, in the upper respiratory tract as well as in bronchial mucus. The enhancement of the secretion of endogenous protease inhibitors in the saliva and the upper respiratory tract may bolster the natural defense lessening the viral load and reducing the severity of SARS-CoV-2 pathogenesis. A dual approach of stimulating a natural defense by enhancing endogenous protease inhibitor secretion in the saliva and in the upper respiratory tract along with a combinatorial therapy that prevents early and late entry of the virus to the human host could be efficacious in preventing the SARS-CoV-2 infection and its devastating consequences on human health.

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AUTHOR CONTRIBUTIONS
P.M. prepared figures; P.M. drafted manuscript; P.M. edited and revised manuscript; P.M. approved final version of manuscript.

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