Coronaviruses are an established cause of severe respiratory, enteric and systemic infections

Four genera:

1)Alphacoronavirus **= infect mammals**

[**human coronavirus NL63 (HCoV-NL63), HCoV-229E,**porcine transmissible gastroenteritis coronavirus (TGEV), PEDV, and porcine respiratory coronavirus (PRCV)]

↓

{ACE2 receptor}

2)Betacoronavirus **= infect mammals**

[**SARS-CoV, COVID-19**, **MERS-CoV,** bat coronavirus HKU4, **mouse hepatitis coronavirus (MHV),** bovine coronavirus (BCoV), and **HCoV-OC43, HCOV-HKU1**]

↓ ↓ ↓ ↓

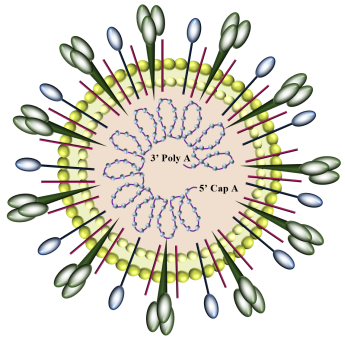
{ACE2 receptor} **{DPP4 receptor}** **{CEACAM1 receptor}**

3)Gammacoronavirus **= infect avian species**

[avian infectious bronchitis coronavirus (IBV)]

4)Deltacoronavirus **= infect both mammalian and avian species**

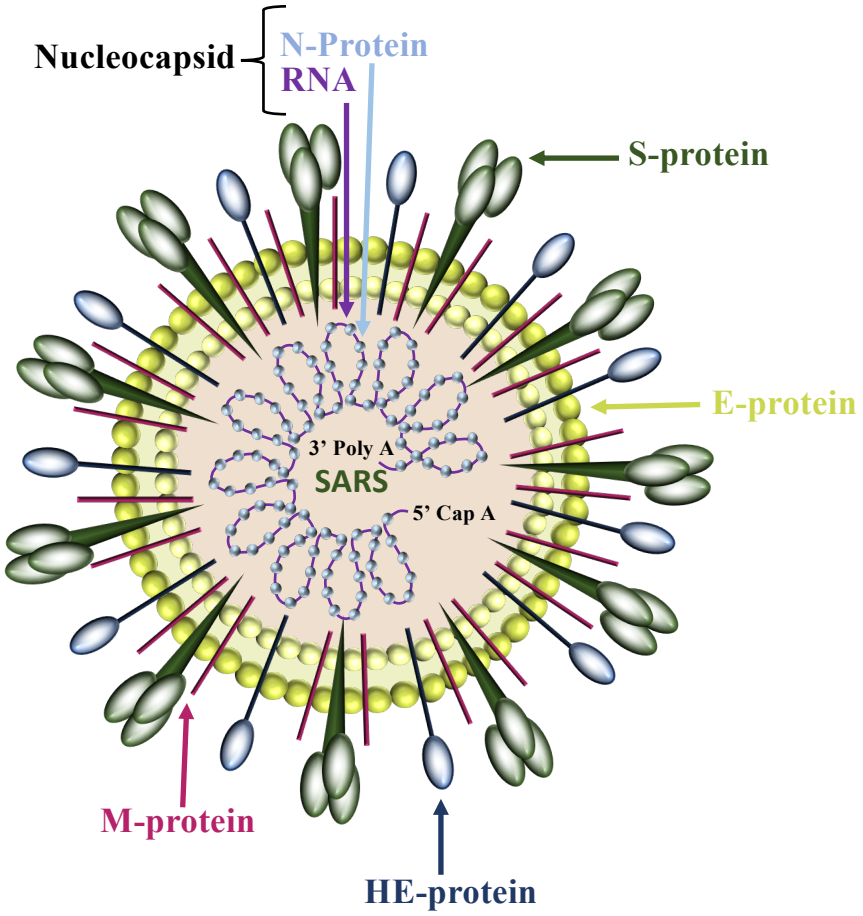
[porcine deltacoronavirus (PdCV)]



***The diversity of receptor usage is an outstanding feature of coronaviruses**

{e.g. Angiotensin-converting enzyme 2 gene (ACE2), carcinoembryonic antigen-related cell adhesion molecule 1 (CEAC[illegible]), dipeptidyl peptidase 4 (DPP4), sugar receptors}

PMID: 27578435 Annu Rev Virol. 2016 Sep 29;3(1):237-261.
PMID: 30646565 Viruses. 2019 Jan 14;11(1).



Coronavirus virus particles contain <u>four main</u> structural proteins encoded within the 3′ end of the viral genome.

1) Spike (S)-protein: trimeric protein that mediates attachment to the host receptor, can be cleaved by a host cell furin-like protease into two separate polypeptides noted S1 (binding domain) and S2 (stalk)

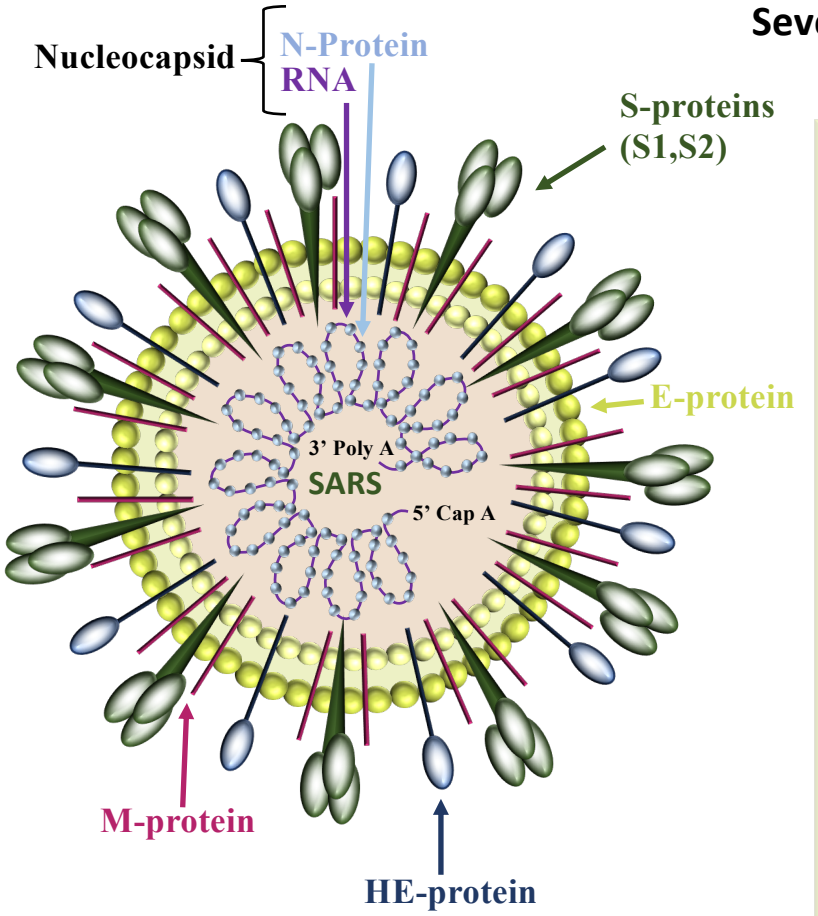
2) Membrane (M)-protein: most abundant structural protein in the virion.

3) Envelope (E)-protein: facilitates assembly and release of the virus, the ion channel activity in SARS-CoV E protein is required for pathogenesis

4) N-Protein: constitutes the only protein present in the nucleocapsid composed of 2 domains that bind RNA

5) Hemagglutinin-esterase (HE)-protein: present in a subset of β-coronaviruses, binds sialic acids on surface glycoproteins and contains acetyl-esterase activity

PMID: 25720466 Methods Mol Biol. 2015;1282:1-23.



Severe acute respiratory syndrome coronavirus 2 (SARS-Cov2): COVID-19

➔Sourced from Huanan Seafood Wholesale Market, which trades in fish and a variety of live animal species including poultry, bats, marmots, and snakes

➔Positive-strand RNA betacoronavirus

➔Highly affects older patients with multiple comorbidities

➔S1 directly binds to the peptidase domain of ACE2, exposing an S2 cleavage site critical for viral infection

➔S2 is subsequently cleaved by endosomal cysteine proteases cathepsin B and L (CatB/L) and/or serine protease TMPRSS2 prior to fusion where the latter may be preferred.

➔In vitro, camostat mesylate inhibits TMPRSS2 & ammonium chloride inhibits CatB/L

PMID: 32112977 Int J Surg. 2020 Feb 26;76:71-76.
PMID: 32142651 Cell. 2020 Mar 4. pii: S0092-8674(20)30229-4
PMID: 29217279 Virology. 2018 Apr;517:9-15..

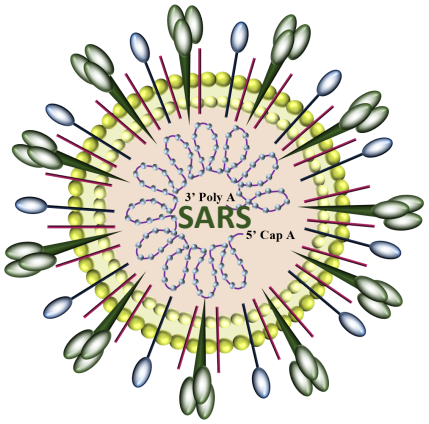
Identified ACE2 immunostaining in HUMANS is most prominent in lung and intestinal epithelia

Cell types:

Lung: type I and type II alveolar epithelial cells
Nasal: non-keratinizing squamous epithelium
GI: smooth muscle cells and endothelium of vessels from the stomach, small intestine, and colon
Skin: basal cell layer of the epidermis extending to the basal cell layer of hair
Kidney: glomerular and epithelial cells
All organs: arterial and venous endothelial cells
Immune cells of the spleen, thymus, lymph nodes, and bone marrow were consistently negative for ACE2

PMID: 15141377 J Pathol. 2004 Jun;203(2):631-7

Transmembrane protease, serine 2 (TMPRSS2, epitheliasin)



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Human transcript is observed in the following order:

prostate > colon > small intestine > pancreas > kidney > lung > liver

Epitheliasin transcripts in fetal tissues are observed only in kidney and lung.
Epitheliasin is induced by androgens

PMID: 11322890 Eur J Biochem. 2001 May;268(9):2687-99.

Expressed primarily in the apical surfaces of **mouse** renal tubular and airway epithelial cells.

PMID: 10683448 FEBS Lett. 2000 Feb 18;468(1):93-100.

A discernible phenotype in *Tmprss2*$^{-/-}$ mice is not identified, suggesting possible redundant functions in the absence of infection

PMID: 16428450 Mol Cell Biol. 2006 Feb;26(3):965-75.

ACE2 function is primarily identified in the renin/angiotensin system

In an ACE2 knockout model of lung injury, mice exhibited increased vascular permeability, lung edema and neutrophil influx in association with decreased lung function & this phenotype was rescued with a pharmacological inhibitor of the angiotensin II type 1 receptor (**AT_1R**)

PMID: 24776703

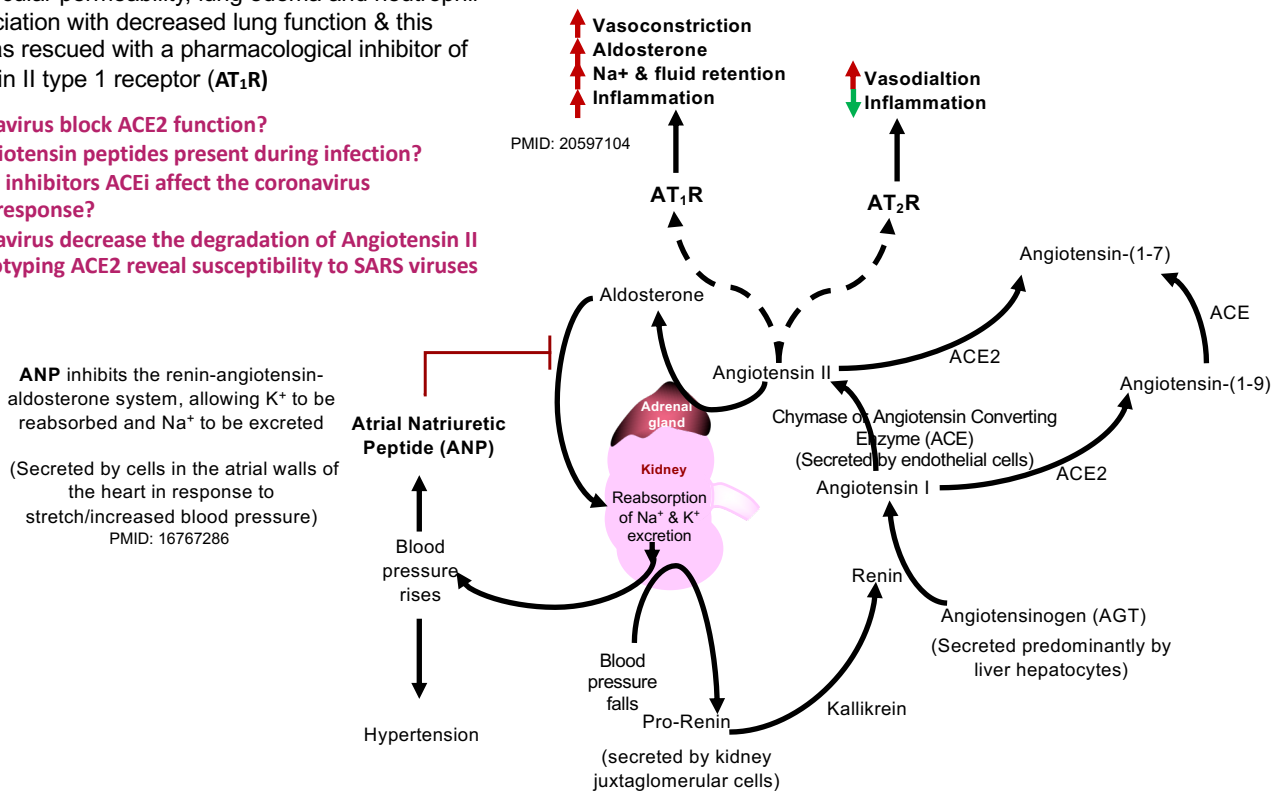
Q: Does coronavirus block ACE2 function?

Q: Are the angiotensin peptides present during infection?

Q: Would AT_1R inhibitors ACEi affect the coronavirus inflammatory response?

Q: Does coronavirus decrease the degradation of Angiotensin II

Q: Would genotyping ACE2 reveal susceptibility to SARS viruses



*Both ACE and ACE2 bind integrins in an RGD-independent manner
*ACE2 increases cellular adhesion and affects integrin signaling
*The RGD motif present in the ectodomain of ACE2 is inaccessible
*sACE2 significantly reduces FAK phosphorylation levels.
PMID: 22523556 PLoS One. 2012;7(4):e34747.

An evolutionary RGD motif in the spike protein of SARS-CoV-2 may serve as a potential high risk factor for virus infection? PREPRINT PAPER: Notably, we found that S protein of SARS-CoV-2 produced an evolutionary mutation K403R, located at site 403, forming an RGD motif (Arg-Gly-AsP) in the S protein next to the binding site, which has neither been found in RaTG13 nor in SARS-CoV---**Q: Are integrins co-receptors for COVID-19? Is FAK required for viral entry?**

Q: Does sACE2 antagonize COVID-19?
Q: Would increasing ACE2 expression (e.g. ACEi) promote viral entry and/OR antagonize inflammation
Q: Would a COX2i dampen inflammation?

ACE2 purified from failing human heart was found to form a complex with integrin beta1 by immunoprecipitation
PMID: 15276642 Biochim Biophys Acta. 2004 Aug 4;1689(3):175-8.

Overexpression of ACE2 in neurons/brain antagonized COX2 and hypertension
PMID: 25489058 Hypertension. 2015 Mar; 65(3): 577–586.

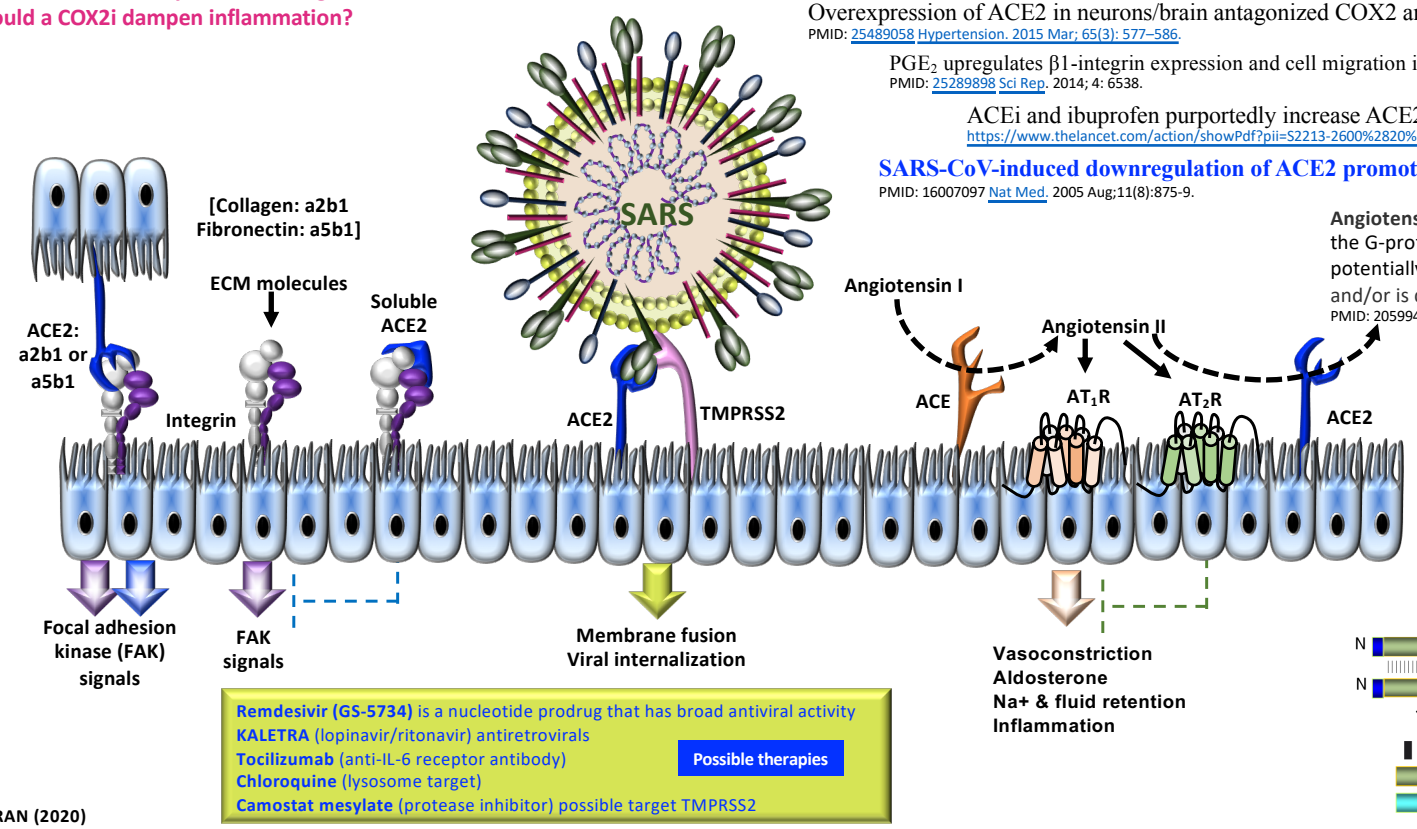
PGE_2 upregulates β1-integrin expression and cell migration in HCC cells
PMID: 25289898 Sci Rep. 2014; 4: 6538.

ACEi and ibuprofen purportedly increase ACE2
https://www.thelancet.com/action/showPdf?pii=S2213-2600%2820%2930116-8

SARS-CoV-induced downregulation of ACE2 promotes lung injury
PMID: 16007097 Nat Med. 2005 Aug;11(8):875-9.

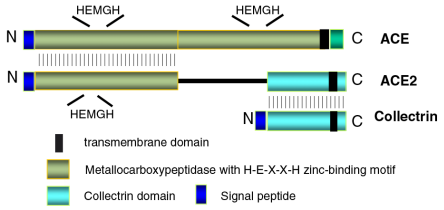
Angiotensin 1–7 (Ang 1–7; DRVYIHPF), binds the G-protein-coupled receptor Mas to potentially induce vasoprotective responses and/or is degraded to inactive peptides.
PMID: 20599443 Pharmacol Ther. 2010 Oct;128(1):119-28.

Q: Is Ang1-7 a diagnositic marker



Remdesivir (GS-5734) is a nucleotide prodrug that has broad antiviral activity
KALETRA (lopinavir/ritonavir) antiretrovirals
Tocilizumab (anti-IL-6 receptor antibody)
Chloroquine (lysosome target)
Camostat mesylate (protease inhibitor) possible target TMPRSS2
Possible therapies

Collectrin, a truncated form of ACE2, has no intrinsic angiotensinase activity
PMID: 17825789 Biochem Biophys Res Commun. 2007 Nov 9;363(1):1-5.



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Middle East respiratory syndrome coronavirus (MERS-CoV)

*MERS-CoV uses the N-terminal part of its spike (S)—the so called S1 protein to bind to two host cell surface molecules, dipeptidyl peptidase-4 (DPP4) and α2,3-sialic acids.
*Post attachment, MERS-CoV uses the C-terminal part of its S protein—known as S2—to interact with host proteases, such as furin, TMPRSS2, and cathepsins
*TMPRSS2 and DPP4 are held in one complex at the cell surface by a scaffolding protein, the tetraspanin CD9, leading to a rapid and efficient entry of MERS-CoV into the susceptible cells
PMID: 30893947 Viruses. 2019 Mar 19;11(3). pii: E280.
*DPP-4 is a cell surface aminopeptidase that was originally characterized as a T cell differentiation antigen (CD26)
*DPP-4 is expressed ubiquitously and found in many cell types, including the endothelial cells in multiple vascular beds, rendering the enzyme highly accessible to the peptide substrates circulating through the gut, liver, lung, and kidney------ the kidney expresses the highest levels of DPP-4 per organ weight
*The loss of DPP-4 cell surface expression has shown to be associated with decreased phosphorylation of integrin β1 at the S785 residue
PMID: 26877767 Fibrogenesis Tissue Repair. 2016 Feb 13;9:1.

Within COVID-19, many different and unique residues (R408, Q409, T445, V417, L461, D467, S469, L491, N492, D493, Y 494, T497, T150, Y504) are predicted to interact with CD26
Emerg Microbes Infect. 2020 Dec;9(1):601-604. PMID: 32178593

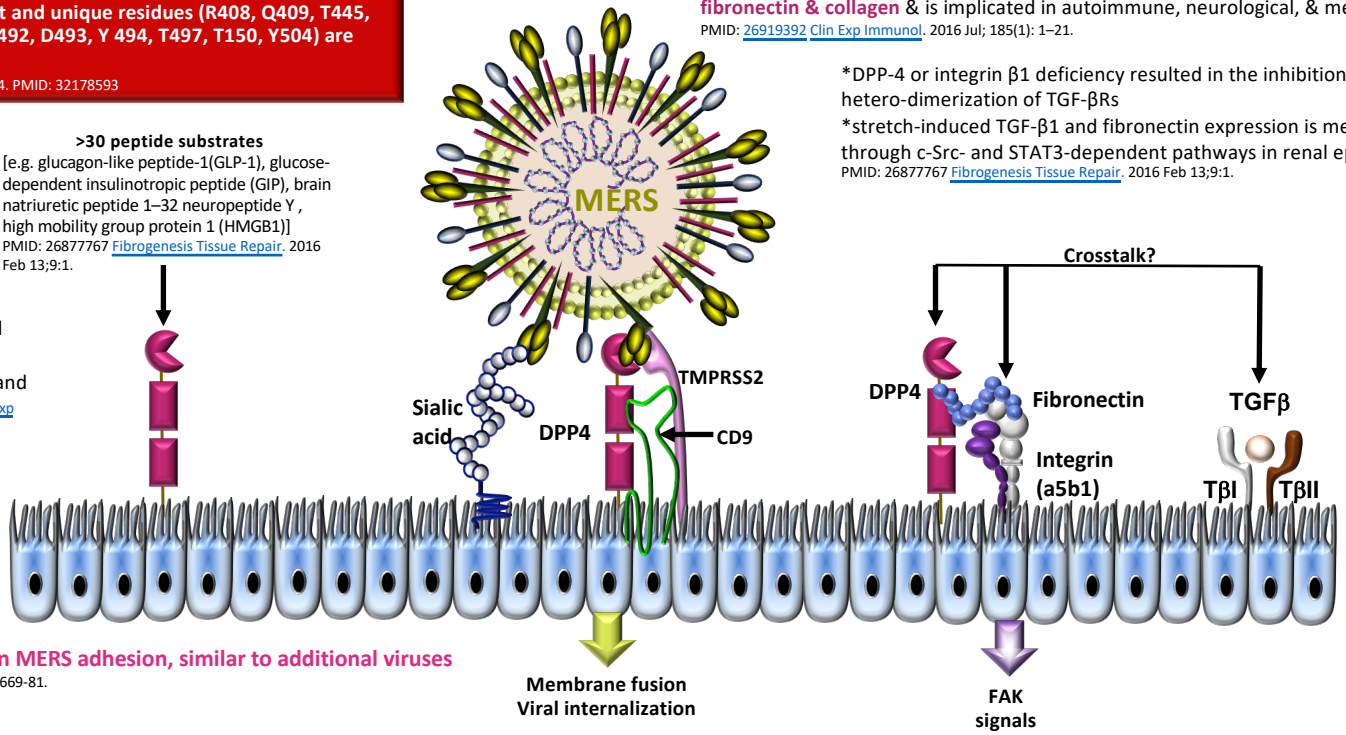
***CD26/DPP4 has shown the ability to act as a non-integrin receptor, being able to bind fibronectin & collagen** & is implicated in autoimmune, neurological, & metabolic diseases
PMID: 26919392 Clin Exp Immunol. 2016 Jul; 185(1): 1–21.

*DPP-4 or integrin β1 deficiency resulted in the inhibition of TGF-β2-stimulated hetero-dimerization of TGF-βRs
*stretch-induced TGF-β1 and fibronectin expression is mediated by β1-integrin through c-Src- and STAT3-dependent pathways in renal epithelial cells
PMID: 26877767 Fibrogenesis Tissue Repair. 2016 Feb 13;9:1.

>30 peptide substrates
[e.g. glucagon-like peptide-1(GLP-1), glucose-dependent insulinotropic peptide (GIP), brain natriuretic peptide 1–32 neuropeptide Y , high mobility group protein 1 (HMGB1)]
PMID: 26877767 Fibrogenesis Tissue Repair. 2016 Feb 13;9:1.

*Sitagliptin, a DPP4 inhibitor, enhanced angiotensin II–induced increase of perfusion pressure in isolated kidneys from both lean and obese ZSF1 rats PMID: 20374254 Clin Exp Pharmacol Physiol. 2010 Jul;37(7):689-91.

Q: Does MERS inhibit DPP4 expression and/or function?



Q: Would integrins play a role in MERS adhesion, similar to additional viruses
PMID: 26321473 Arch Virol. 2015 Nov;160(11):2669-81.

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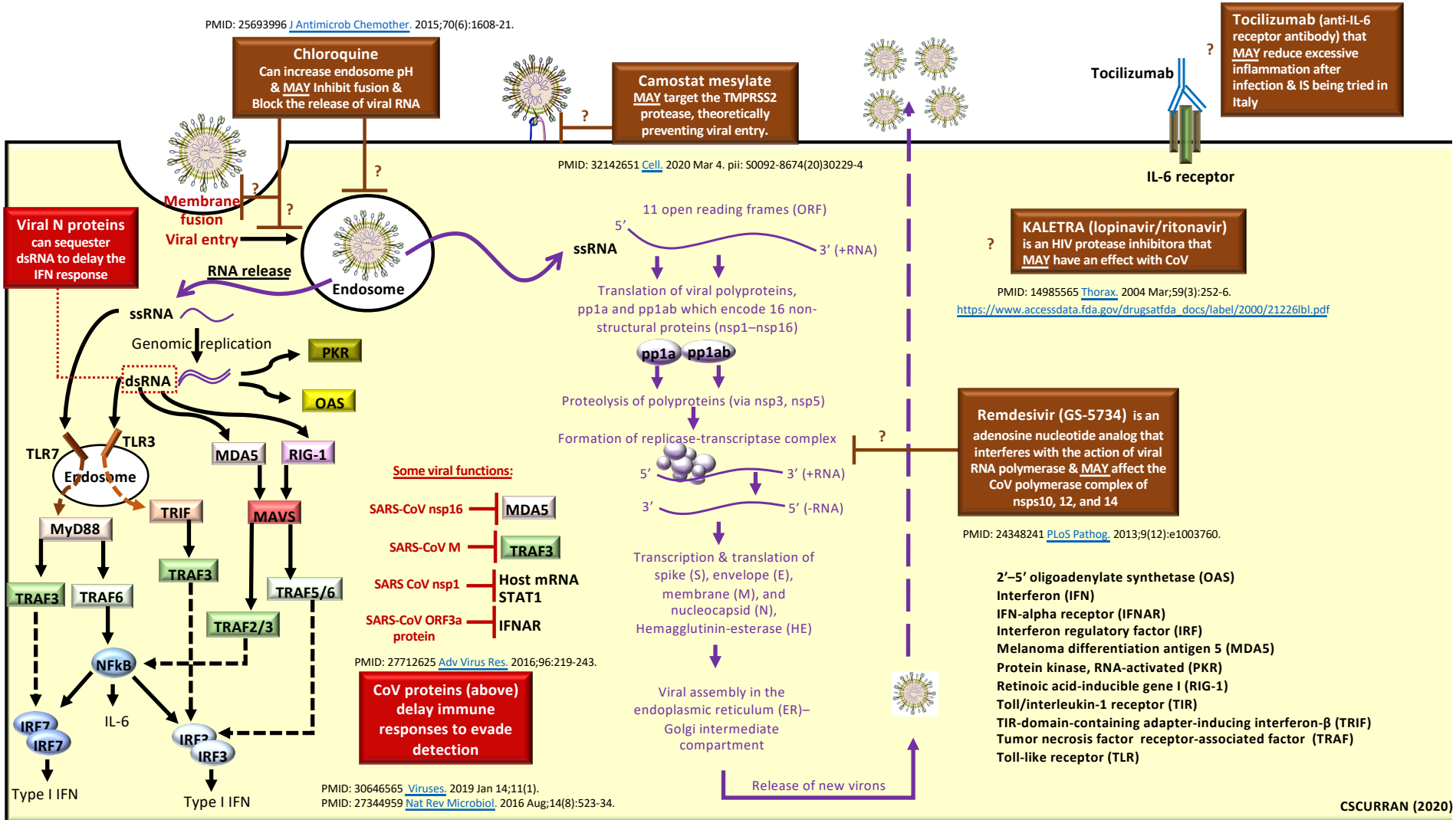
PMID: 25693996 J Antimicrob Chemother. 2015;70(6):1608-21.
Chloroquine
Can increase endosome pH & MAY Inhibit fusion & Block the release of viral RNA
Camostat mesylate
MAY target the TMPRSS2 protease, theoretically preventing viral entry.
PMID: 32142651 Cell. 2020 Mar 4. pii: S0092-8674(20)30229-4
Tocilizumab (anti-IL-6 receptor antibody) that MAY reduce excessive inflammation after infection & IS being tried in Italy
Tocilizumab
IL-6 receptor
Membrane fusion
Viral entry
RNA release
Endosome
Viral N proteins can sequester dsRNA to delay the IFN response
ssRNA
Genomic replication
dsRNA
PKR
OAS
TLR7
TLR3
Endosome
MDA5
RIG-1
TRIF
MAVS
MyD88
TRAF3
TRAF6
TRAF3
TRAF5/6
TRAF2/3
NFkB
IL-6
IRF7
IRF7
IRF3
IRF3
Type I IFN
Type I IFN
11 open reading frames (ORF)
5′
3′ (+RNA)
ssRNA
Translation of viral polyproteins, pp1a and pp1ab which encode 16 non-structural proteins (nsp1–nsp16)
pp1a
pp1ab
Proteolysis of polyproteins (via nsp3, nsp5)
Formation of replicase-transcriptase complex
5′
3′ (+RNA)
3′
5′ (-RNA)
Transcription & translation of spike (S), envelope (E), membrane (M), and nucleocapsid (N), Hemagglutinin-esterase (HE)
Viral assembly in the endoplasmic reticulum (ER)–Golgi intermediate compartment
Release of new virons
KALETRA (lopinavir/ritonavir) is an HIV protease inhibitora that MAY have an effect with CoV
PMID: 14985565 Thorax. 2004 Mar;59(3):252-6.
https://www.accessdata.fda.gov/drugsatfda_docs/label/2000/21226lbl.pdf
Remdesivir (GS-5734) is an adenosine nucleotide analog that interferes with the action of viral RNA polymerase & MAY affect the CoV polymerase complex of nsps10, 12, and 14
PMID: 24348241 PLoS Pathog. 2013;9(12):e1003760.
Some viral functions:
SARS-CoV nsp16 | MDA5
SARS-CoV M | TRAF3
SARS CoV nsp1 | Host mRNA STAT1
SARS-CoV ORF3a protein | IFNAR
PMID: 27712625 Adv Virus Res. 2016;96:219-243.
CoV proteins (above) delay immune responses to evade detection
PMID: 30646565 Viruses. 2019 Jan 14;11(1).
PMID: 27344959 Nat Rev Microbiol. 2016 Aug;14(8):523-34.
2′–5′ oligoadenylate synthetase (OAS)
Interferon (IFN)
IFN-alpha receptor (IFNAR)
Interferon regulatory factor (IRF)
Melanoma differentiation antigen 5 (MDA5)
Protein kinase, RNA-activated (PKR)
Retinoic acid-inducible gene I (RIG-1)
Toll/interleukin-1 receptor (TIR)
TIR-domain-containing adapter-inducing interferon-β (TRIF)
Tumor necrosis factor receptor-associated factor (TRAF)
Toll-like receptor (TLR)
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The human immune response to COVID-19

*Most patients also developed lymphopenia and pneumonia with characteristic pulmonary ground glass opacity changes on chest CT.
***Severe case cytokines (41 patients):** IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNFα
***99 patients**: increased total neutrophils (38%), reduced total lymphocytes (35%), increased serum IL-6 (52%) and increased c-reactive protein (84%) were observed.
*SARS-Co-V directly infects macrophages and T cells, a key feature in SARS-CoV-mediated pathogenesis, in COVID-19?. Only minimal percentages of monocytes/macrophages in the lung expressed ACE2.
*SARS-CoV induces delayed type I IFN and loss of viral control in an early phase of infection, in COVID-19?
*Type I IFN for treatment in mouse models of either SARS-CoV or MERSCoV infection, the timing of administration is key to yield protective response.
*SARS-CoV infection induces seroconversion as early as day 4 after onset of disease and was found in most patients by 14 days.
*Long lasting specific IgG and neutralizing antibody are reported as long as 2 years after infection
*Sera from 5 patients of confirmed COVID-19 show some cross-reactivity with SARS-CoV, but not other coronavirus.
*The virus specific T cells from the severe group tended to be a central memory phenotype with a significantly higher frequency of polyfunctional CD4+ T cells (IFNγ, TNFα, and IL-2) and CD8+ T cells (IFNγ, TNFα and degranulated state), as compared with the mild-moderate group.
*Strong T cell responses correlated significantly with higher neutralizing antibody while more serum Th2 cytokines (IL-4, IL-5, IL-10) were detected in the fatal group
PMID: 32105090 Asian Pac J Allergy Immunol. 2020 Feb 27.

Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19
https://www.nature.com/articles/s41591-020-0819-2
*Increased antibody-secreting cells (ASCs-$CD3^{-}CD19^{+}CD27^{hi}CD38^{hi}$), follicular helper T cells (T_{FH} cells-$CD4^{+}CXCR5^{+}ICOS^{+}PD\text{-}1^{+}$), activated $CD38^{+}HLA\text{-}DR^{+}$ $CD4^{+}$ T cells and $CD38^{+}HLA\text{-}DR^{+}CD8^{+}$ T cells and immunoglobulin M (IgM) and IgG antibodies that bound the COVID-19 were detected in blood before symptomatic recovery.
*These immunological changes persisted for at least 7 d following full resolution of symptoms.
[detection of SARS-CoV-2 in sputum, nasopharyngeal aspirates and feces but not urine, rectal swab or whole blood]

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