
लोकाः समस्ताः सुखिनोभवंतु

COVID-19

“Evolving Concepts in Pathophysiology”

“All professions are conspiracies against the laity”

George Bernard Shaw gave these words to the kindly old curmudgeon, Sir Patrick Cullen, in his 1906 play The Doctor's Dilemma.

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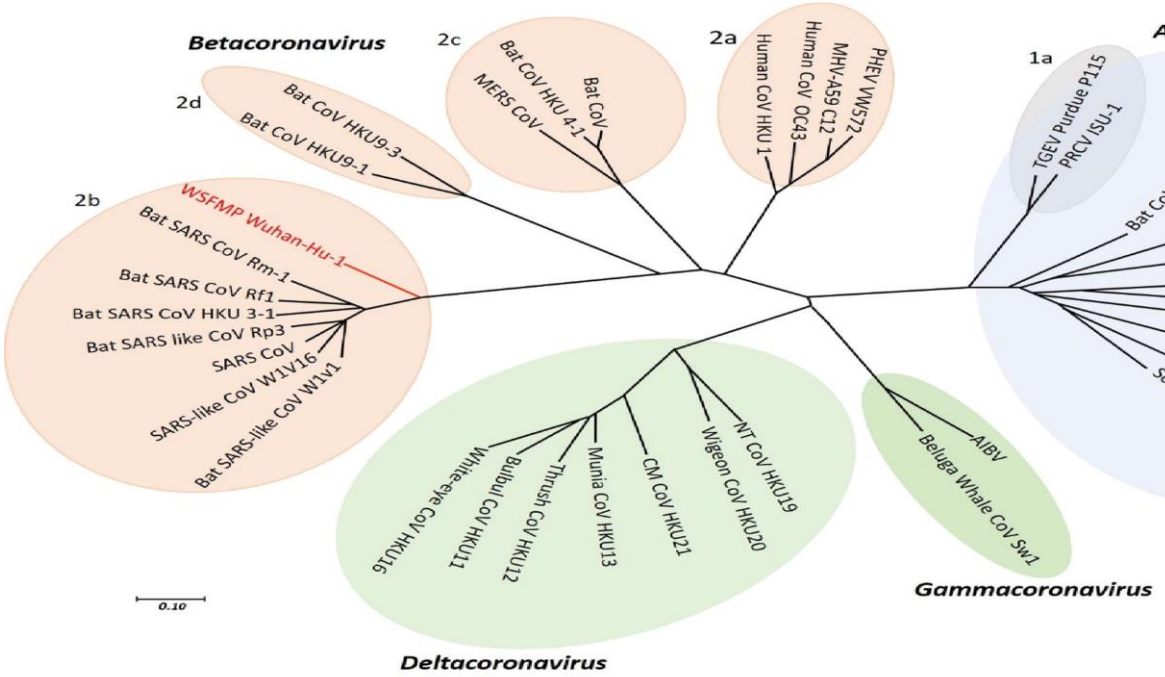
Clinical Professor of Medicine and Surgery University of Illinois

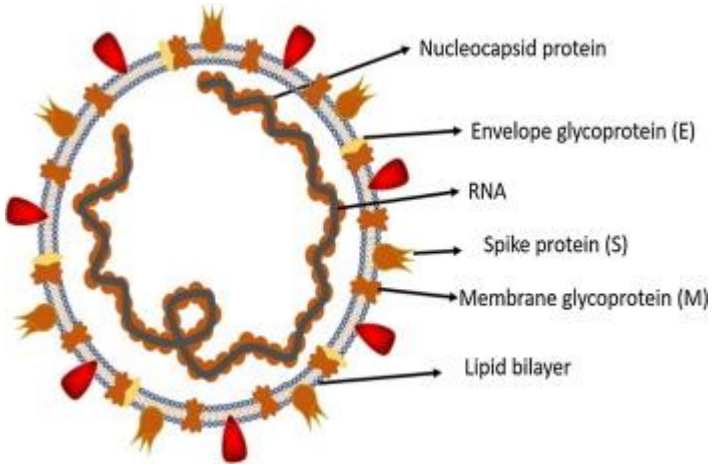
https://www.linkedin.com/groups/10412579

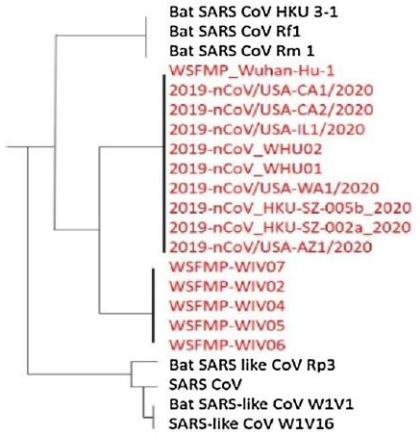
https://www.facebook.com/HAPPENforOneHealth

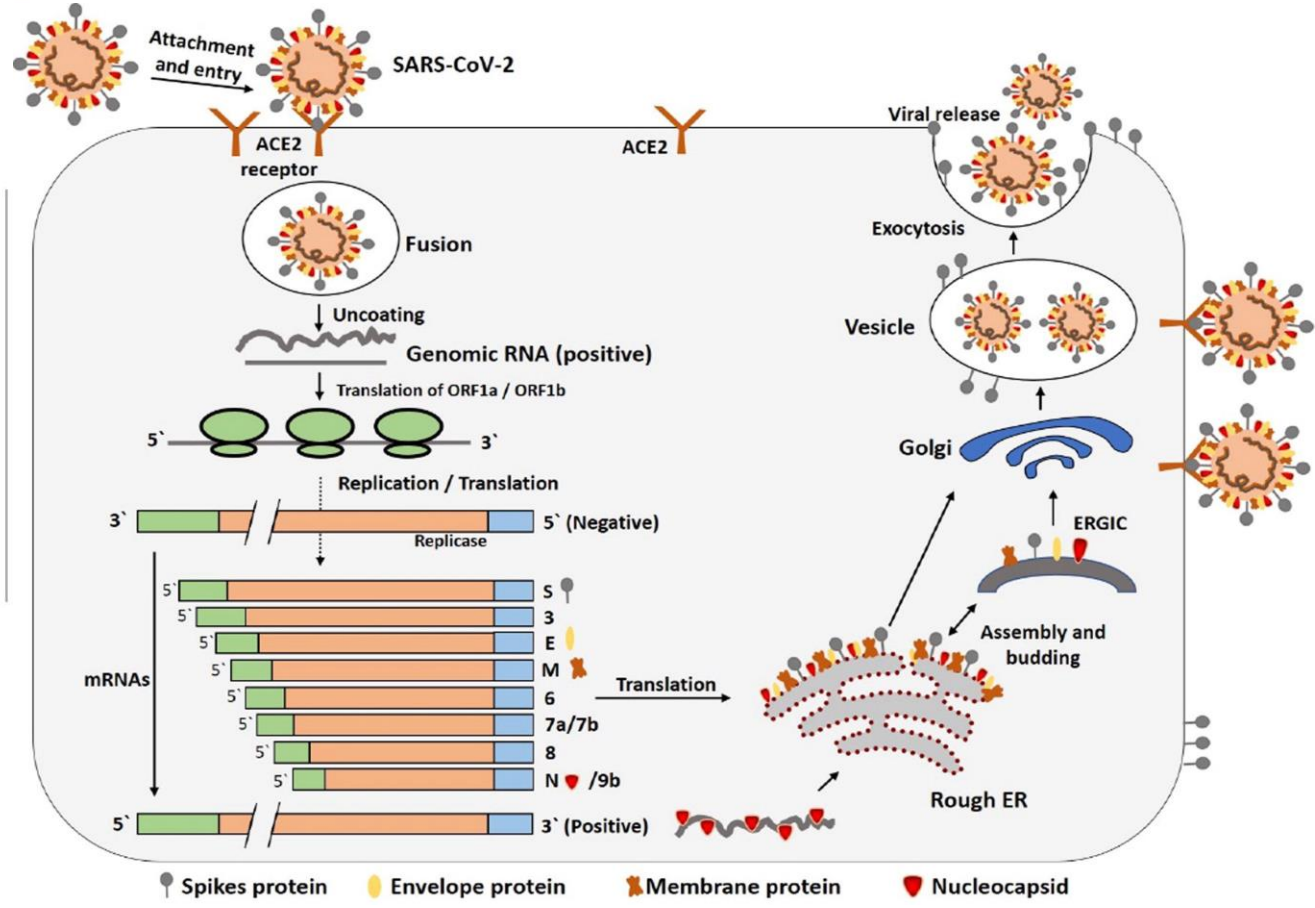
https://www.happenforonehealth.org

https://www.youtube.com/channel/UC5YM8FnS-O4JsjWZEPrqnCA

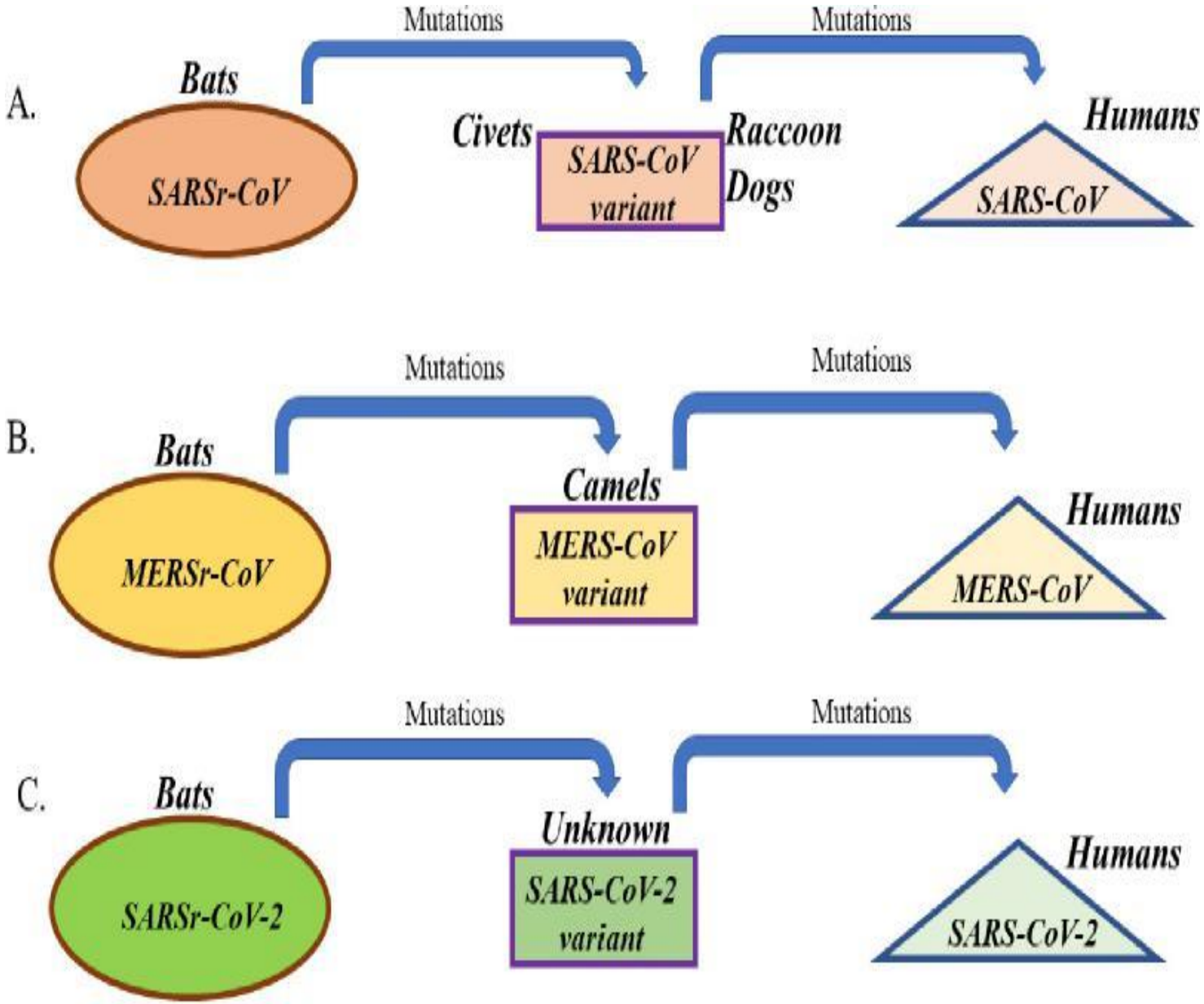




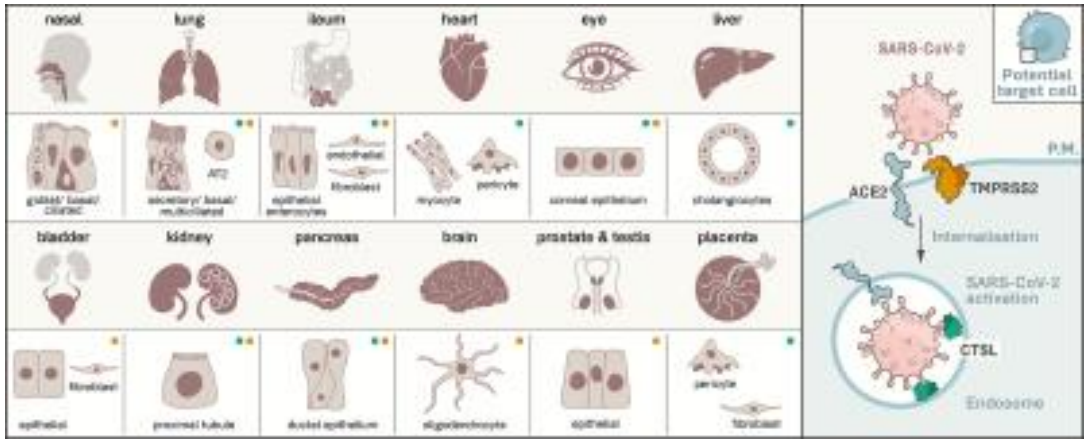




Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *Journal of Advanced Research*. 2020;24:91-98. doi:10.1016/j.jare.2020.03.005



WHY are Coronavirus infections not lethal for Bats ?



The Virus and affinity for cellular receptors: ACE 2; TMPRSS 2; DPP4; Neuropilin and Tissue Tropism as an explanation for organ dysfunction

Receptors for SARS-CoV-2 Present in Wide Variety of Human Cells. The Scientist Magazine®. Accessed July 15, 2020. https://www.the-scientist.com/news-opinion/receptors-for-sars-cov-2-present-in-wide-variety-of-human-cells-67496

Evolution of management paradigms

- The antiviral paradigm
- The Cytokine paradigm
- The Inflammasome paradigm and understanding attenuation of end organ damage
- Integrating all paradigms into a practical clinical approach

Laboratory Findings		Median (IQR)			
	Normal Range	All patients (n = 452)	Non-Severe (n = 166)	Severe (n = 286)	P value
Blood routine					
Leucocytes, × 10^9 per L	3.5–9.5	5.3 (3.9-7.5)	4.9 (3.7-6.1)	5.6 (4.3-8.4)	<0.001
Neutrophils, × 10^9 per L	1.8–6.3	3.9 (2.6-5.8)	3.2 (2.1-4.4)	4.3 (2.9-7.0)	<0.001
Neutrophil percentage, %	40.0-75.0	74.3 (64.3-83.9)	67.5 (57.8-75.8)	77.6 (68.9-86.5)	<0.001
Lymphocytes, × 10^9 per L;	1.1–3.2	0.9 (0.6-1.2)	1.0 (0.7-1.3)	0.8 (0.6-1.1)	<0.001
Lymphocyte percentage, %	20.0-50.0	17.5 (10.7-25.1)	21.4 (15.3-32.5)	14.1(8.8-21.4)	<0.001
Neutrophil-to-lymphocyte ratio		4.2 (2.5-7.7)	3.2 (1.8-4.9)	5.5 (3.3-10.0)	<0.001
Monocyte, × 10^9 per L	0.1–0.6	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.395
Monocyte percentage, %	3.0-10.0	7.1 (4.9-9.6)	8.4 (6.5-10.8)	6.6 (4.3-8.8)	<0.001
Eosinophils, × 10^9 per L	0.02-0.52	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	<0.001
Eosinophil percentage, %	0.4-8.0	0.0 (0.0-0.4)	0.2 (0.0-0.7)	0.0 (0.0-0.2)	<0.001
Basophils, × 10^9 per L	0.00-0.10	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.747
Basophil percentage, %	0.0-1.0	0.1 (0.1-0.2)	0.2 (0.0-0.3)	0.1 (0.0-0.2)	0.015
Infection-related biomarkers					
Procalcitonin, ng/mL	0.0–0.05	0.1 (0.0-0.2)	0.05 (0.03-0.09)	0.1 (0.0-0.2)	<0.001
Erythrocyte sedimentation rate,	0.0–15.0	31.5 (17.0-58.0)	28.0 (14.0-50.0)	34.0 (19.0-60.0)	0.123mm/h

Dysregulation of immune response in patients with COVID-19 in Wuhan, China

Prof. Dai-Shi Tian MD, PhD Oxford University Press for the Infectious Diseases Society of America.

	Normal Range	Mean (SD)			
		All patients (n =44)	Non-Severe (n = 17)	Severe (n = 27)	P value
Lymphocyte Subsets					
T cells+B cells+NK cells /ul	1100.0-3200.0	852.9 (412.0)	1020.1 (396.5)	743.6 (384.4)	0.032
T cells+B cells+NK cells %	95.0-105.0	98.9 (1.0)	99.2 (0.6)	98.6 (1.2)	0.103
B cells (CD3-CD19+) /ul	90.0-560.0	179.7 (143.1)	196.1 (144.9)	169.0 (140.9)	0.559
B cells (CD3-CD19+) %	5.0-18.0	20.5 (10.9)	18.5 (8.1)	21.8 (12.2)	0.353
T cells (CD3+CD19-) /ul	955.0-2860.0	541.5 (292.7)	663.8 (291.3)	461.6 (264.7)	0.027
T cells (CD3+CD19-) %	50.0-84.0	61.3 (10.1)	63.4 (8.5)	60.0 (10.8)	0.283
NK cells (CD3-/CD16+CD56+) /ul	150.0-1100.0	131.7 (83.1)	160.2 (90.8)	113.0 (71.8)	0.072
NK cells (CD3-/CD16+CD56+) %	7.0-40.0	17.0 (10.1)	17.2 (10.1)	16.9 (10.1)	0.926
Lymphocyte function					
IFN-γ+ CD4+ T cells /Th %	14.54-36.96	21.2 (12.2)	22.6 (10.2)	20.2 (13.3)	0.557
IFN-γ+ CD8+ T cells /Ts %	34.93-87.95	48.6 (13.7)	46.9 (11.6)	49.7 (14.8)	0.541
IFN-γ+ NK cells /NK %	61.2-92.65	68.0 (14.7)	66.7 (19.3)	68.8 (10.5)	0.677
T cells Subsets					
Th cells (CD3+CD4+) /ul	550.0-1440.0	338.6 (196.3)	420.5 (207.8)	285.1 (168.0)	0.027
Th cells (CD3+CD4+) %	27.0-51.0	38.3 (8.1)	39.8 (7.5)	37.2 (8.4)	0.314
Ts cells (CD3+CD8+) /ul	320.0-1250.0	173.4 (115.2)	201.9 (107.1)	154.7 (116.5)	0.197

Dysregulation of immune response in patients with COVID-19 in Wuhan, China

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Serum ferritin, ng/mL	15.0–150.0	662.4 (380.9-1311.9)	523.7 (299.1-840.4)	800.4 (452.9-1451.6)	<0.001
C-reactive protein, mg/L	0.0–1.0	44.1 (15.5-93.5)	33.2 (8.2-59.7)	57.9 (20.9-103.2)	<0.001
Inflammatory cytokines					
Tumor necrosis factor-α, pg/mL	0.0–8.1	8.6 (6.9-10.9)	8.4 (6.9-10.4)	8.7 (7.1-11.6)	0.037
Interleukin-1β, pg/mL	0.0–5.0	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	0.962
Interleukin-2R, U/mL	223.0–710.0	714.5 (514.5-1040.3)	663.5 (473.3-862.8)	757.0 (528.5-1136.3)	0.001
Interleukin-6, pg/mL	0.0–7.0	21.0 (6.1-47.2)	13.3 (3.9-41.1)	25.2 (9.5-54.5)	<0.001
Interleukin-8, pg/mL	0.0–62.0	16.7 (10.2-27.0)	13.7 (8.9-21.0)	18.4 (11.3-28.4)	<0.001
Interleukin-10, pg/mL	0.0–9.1	5.4 (5.0-9.7)	5.0 (5.0-7.0)	6.6 (5.0-11.3)	<0.001
Immunoglobulins					
Immunoglobulin A	0.82-4.53	2.21 (1.65-2.79)	2.14 (1.66-2.71)	2.26 (1.57-2.89)	0.285
Immunoglobulin G	7.51-15.60	11.75 (9.70-13.60)	11.85 (10.13-13.40)	11.7 (9.53-13.8)	0.551
Immunoglobulin M	0.46-3.04	0.95 (0.70-1.31)	1.02 (0.77-1.37)	0.90 (0.69-1.28)	0.033
Complement proteins					
C3	0.65-1.39	0.88 (0.77-1.00)	0.88 (0.77-1.00)	0.89 (0.77-1.00)	0.942
C4	0.16-0.38	0.26 (0.20-0.31)	0.26 (0.20-0.31)	0.26 (0.20-0.31)	0.851

Dysregulation of immune response in patients with COVID-19 in Wuhan, China

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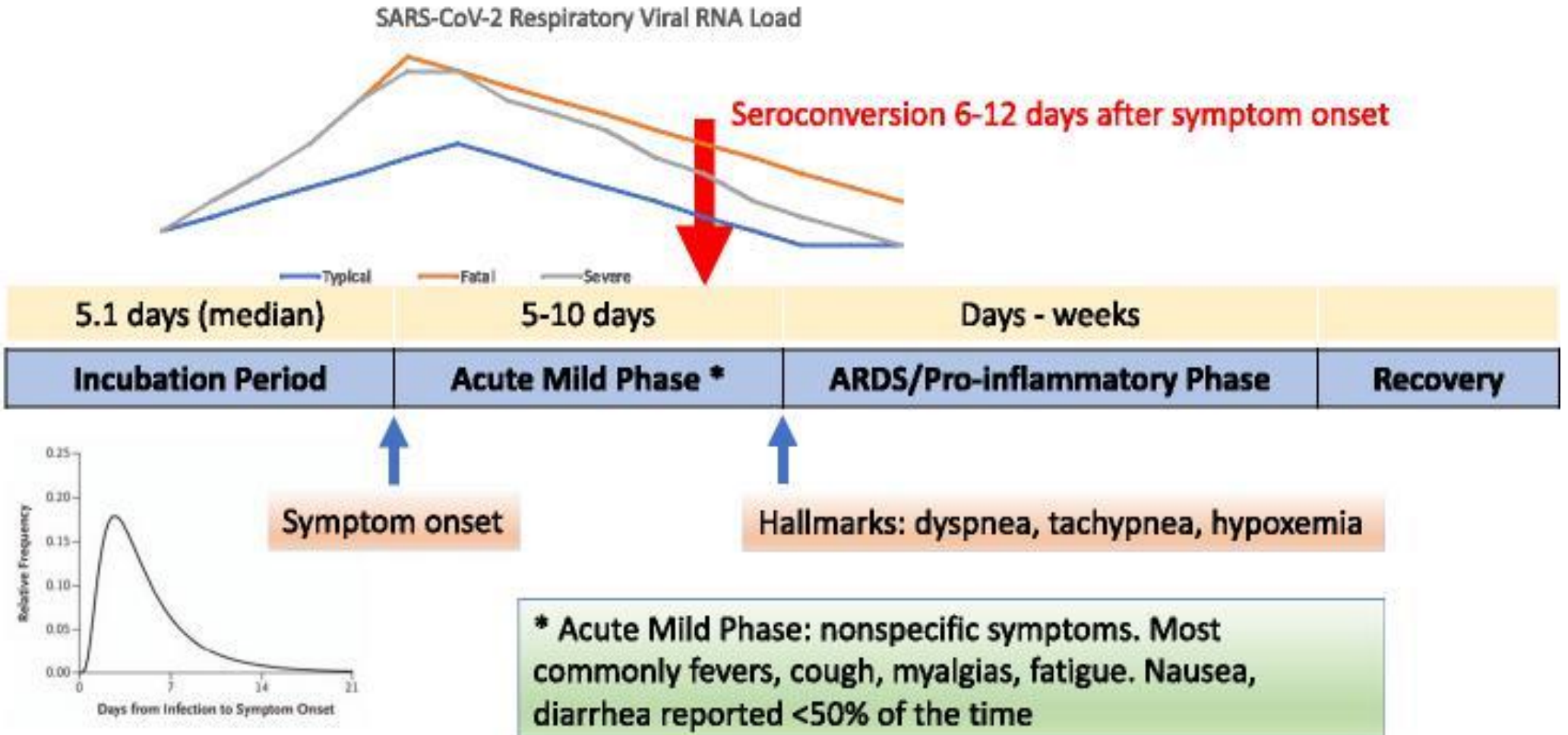
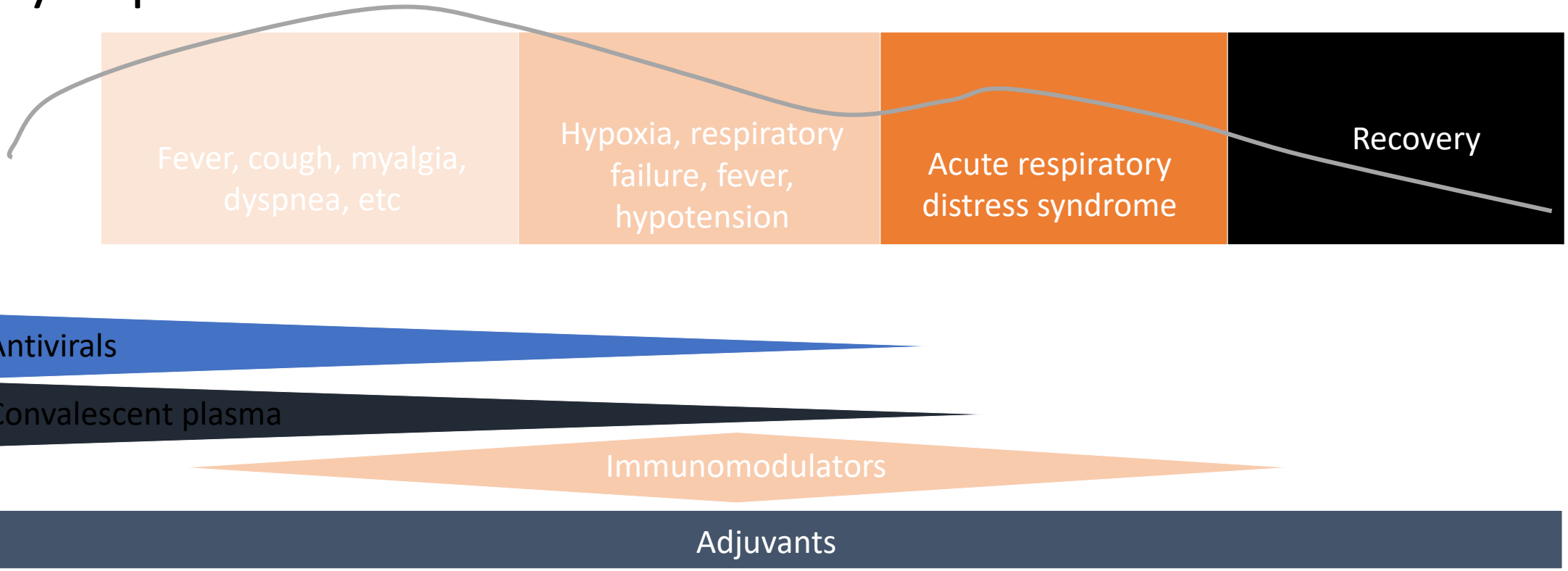
COVID-19 Illness Course



Pan Lancet ID 2020 https://doi.org/10.1016/S1473-3099(20)30113-4
Zou NEJM 2020 DOI: 10.1056/NEJMc2001737
Zhou Lancet 2020 https://doi.org/10.1016/S0140-6736(20)30566-3
Li NEJM 2020 DOI: 10.1056/NEJMoa2001316
Wang JAMA 2020 doi:10.1001/jama.2020.1585
Siddiqi JHLT 2020 doi:10.1016/j.healun.2020.03.012
Wolfel Nature doi:10.1038/s41586-020-2196-x

Meyerowitz EA, Vannier AGL, Friesen MGN, et al. Rethinking the role of hydroxychloroquine in the treatment of COVID-19. The FASEB Journal. 2020;34(5):6027-6037. doi:10.1096/fj.202000919

Timing of Treatment in Relation to Onset of Symptoms

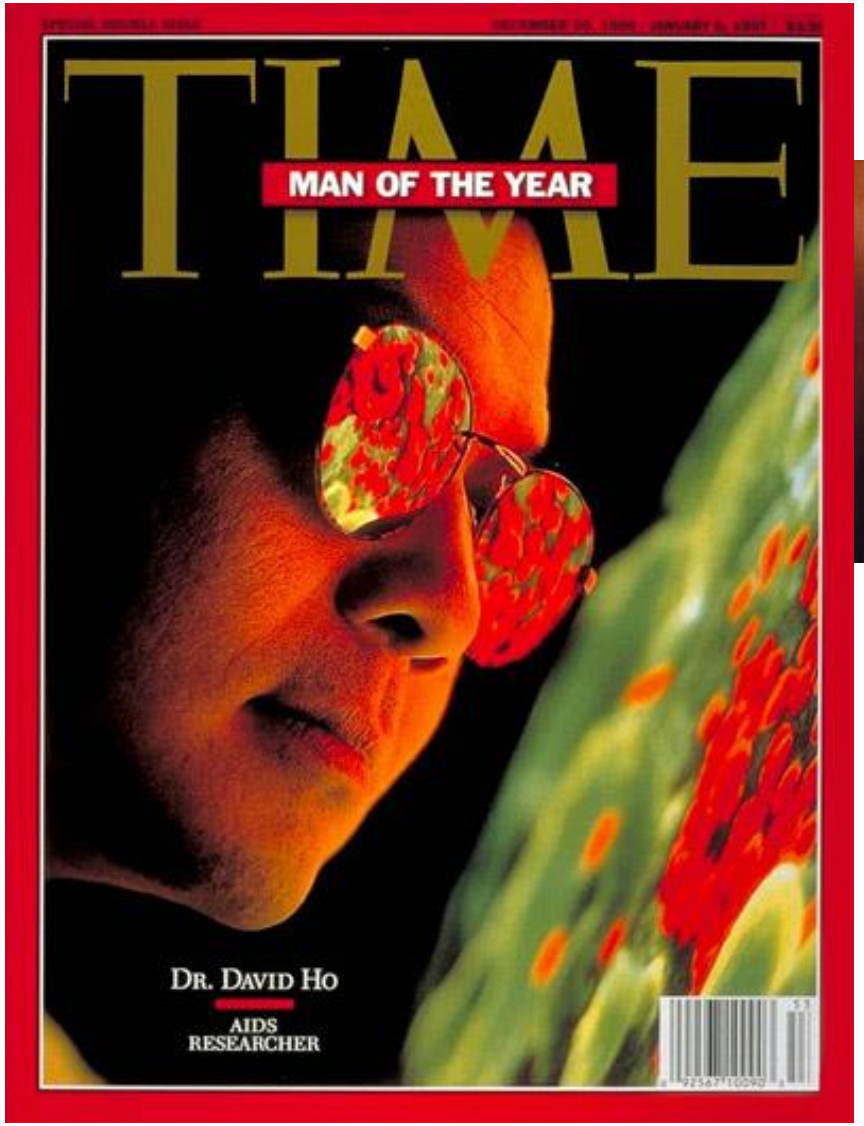


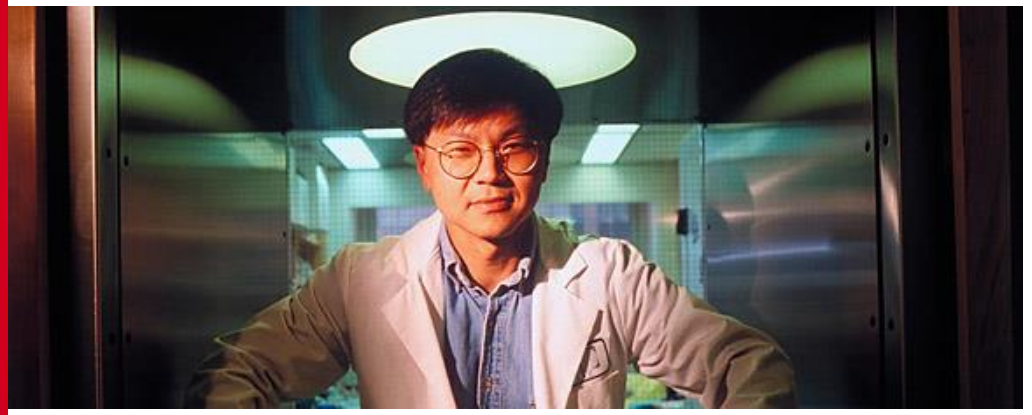
Optimal timing of therapeutic use unknown; proposed schematic based on medication type, potential for direct antiviral effect, mitigation of cytokine storm, or nonspecific adjuvant effect

JHMI Clinical Recommendations for Available Pharmacologic Therapies for COVID-19. Last updated May 19, 2020.

Slide credit: clinicaloptions.com

It’s the Virus Stupid !





Man of the Year 1996

Dr. David Ho

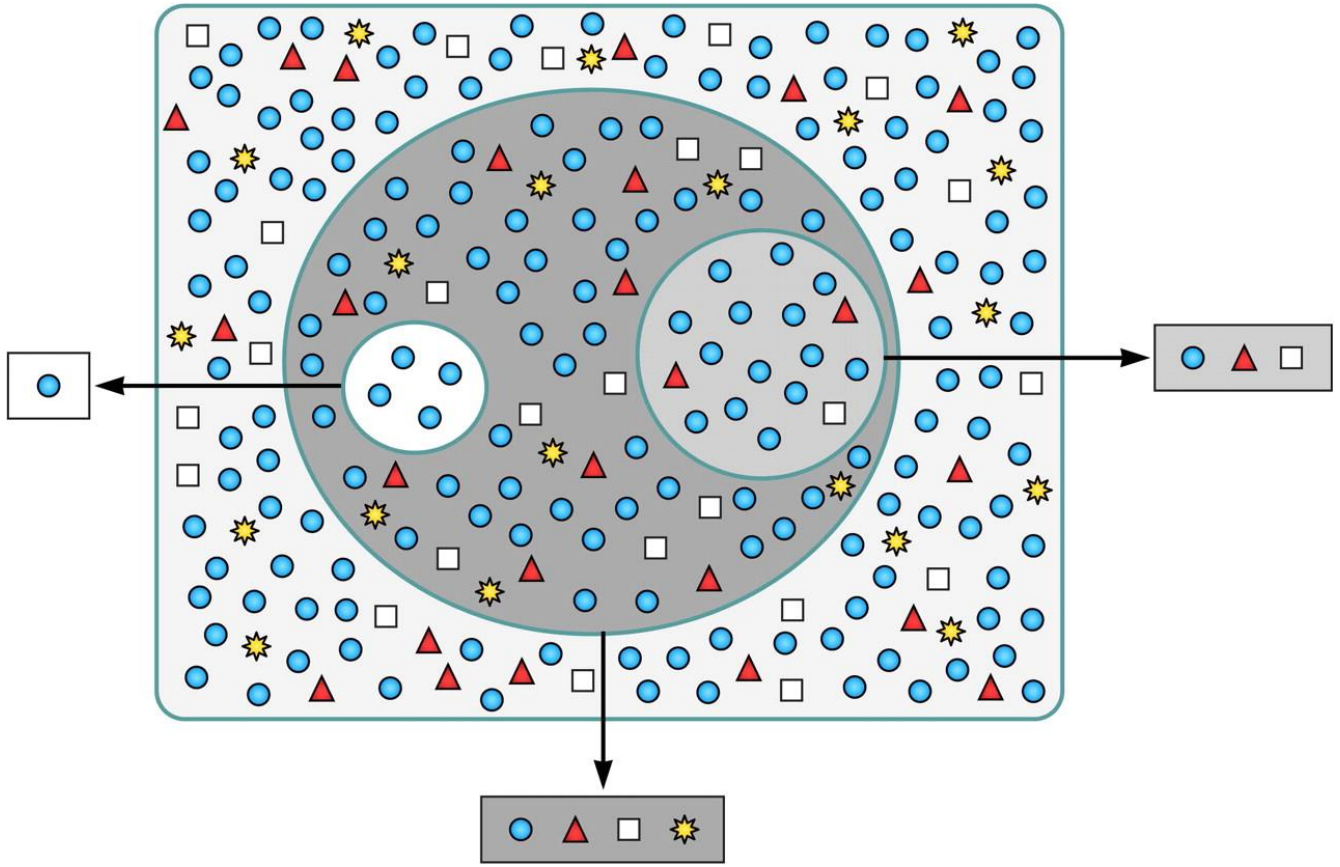
For helping lift a death sentence — for a few years at least, and perhaps longer — on tens of thousands of AIDS sufferers, and for pioneering the treatment that just might lead to a cure, David Da-i Ho, M.D., is TIME's Man of the Year

Viral Quasispecies

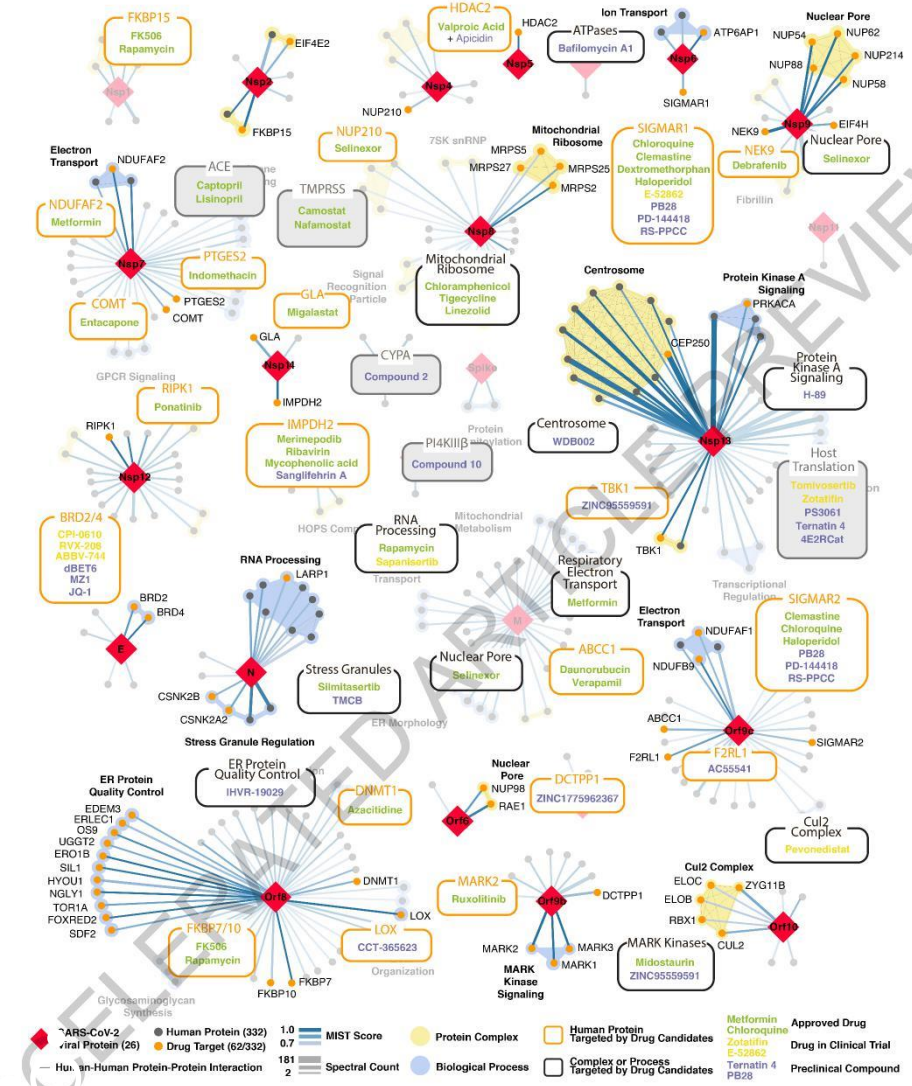
"The standard definition of a biological species does not apply to viruses. A more expansive and dynamic view of viral populations holds clues to understanding and defeating them"
Scientific American : July 1993

Manfred Eigen received his PhD at the University of Göttingen and has been former director of the Max Planck Institute for Biophysical Chemistry in Göttingen. He is an honorary doctor of the TU Braunschweig. From 1982 to 1993, Eigen was president of the German National Merit Foundation.
In 1967, Eigen was awarded, along with Ronald George Wreyford Norrish and George Porter, the Nobel Prize in Chemistry. They were distinguished for their studies of extremely fast chemical reactions induced in response to very short pulses of energy.
In addition, Eigen's name is linked with the theory of the chemical hypercycle, the cyclic linkage of reaction cycles as an explanation for the self-organization of prebiotic systems, which he described with Peter Schuster in 1977. Eigen is a member of the Board of Sponsors of *The Bulletin of the Atomic Scientists*. He founded two biotechnology companies, *Evotec* and *Direvo*.

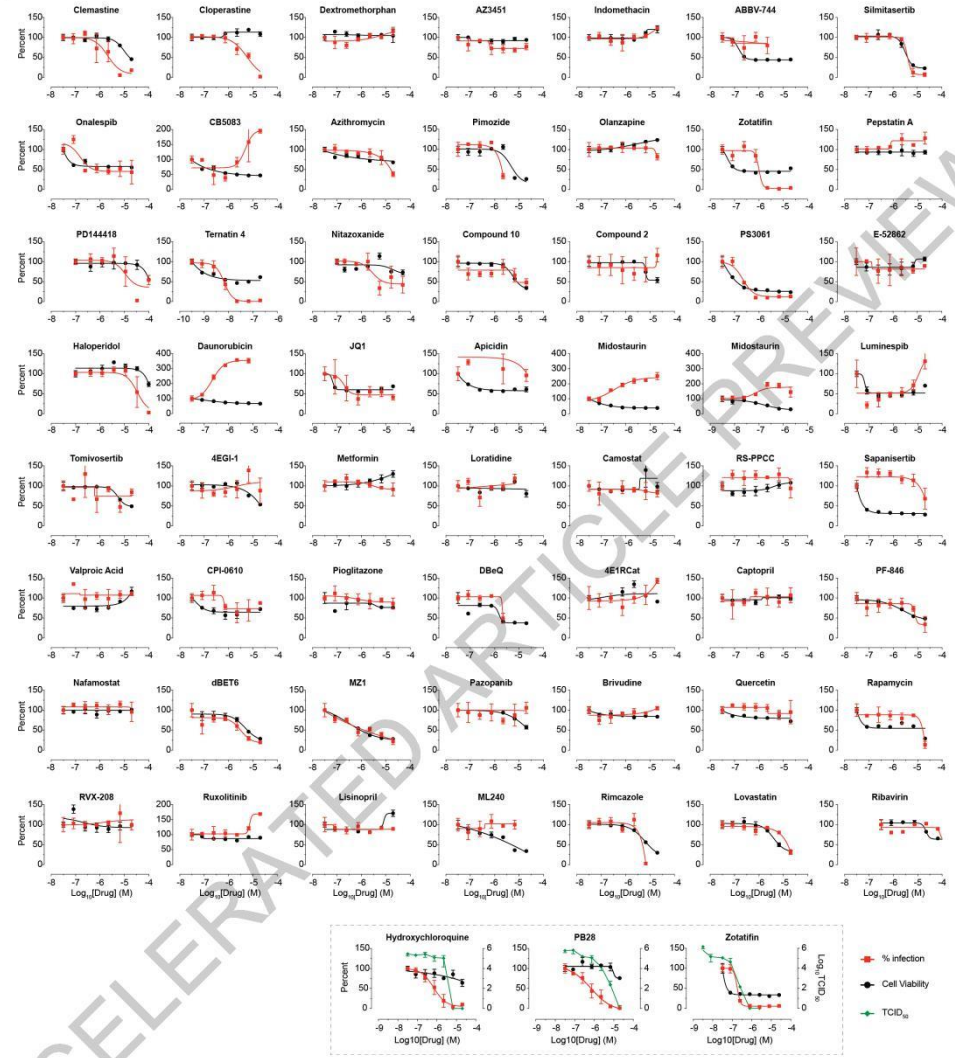
Population size may affect the evolutionary outcome. The large rectangle represents a viral quasispecies. Three types of particles harboring mutations that can confer resistance to a selective agent are distinguished (red triangles, white squares, and yellow stars) from other components of the mutant spectrum (blue circles). If a small population is analyzed (white inner circle on the left), no resistant variants will be found. Further viral replication of that subpopulation will be needed to generate the resistant mutants. If an intermediate-size population is analyzed (intermediate gray inner circle on the right), two of the resistant variants will be found. If a large population is analyzed (large circle), all relevant variants will be represented



Esteban Domingo et al. Microbiol. Mol. Biol. Rev. 2012;76:159-216



•an target network. PPIs of SARS-CoV-2 baits with approved drugs (green), clinical candidates (yellow), and preclinical candidates (purple) with experimental activities against the host proteins (white background) or previously known host factors (grey background) are shown.



•**CExtended Data Fig. 8 | Viral growth and cytotoxicity for compounds tested in New York.** Viral growth (percent infection; red) and cytotoxicity (black) results for compounds tested at Mount Sinai in New York. Zotatifin, hydroxychloroquine, and PB28 were also tested in Median Tissue Culture

•Infectious Dose assay ($TCID_{50}$; green). Zotafitin and Midostaurin were tested in two independent experiments and data are shown in two individual panels. Data=mean±SD; all n=3 biologically independent samples.

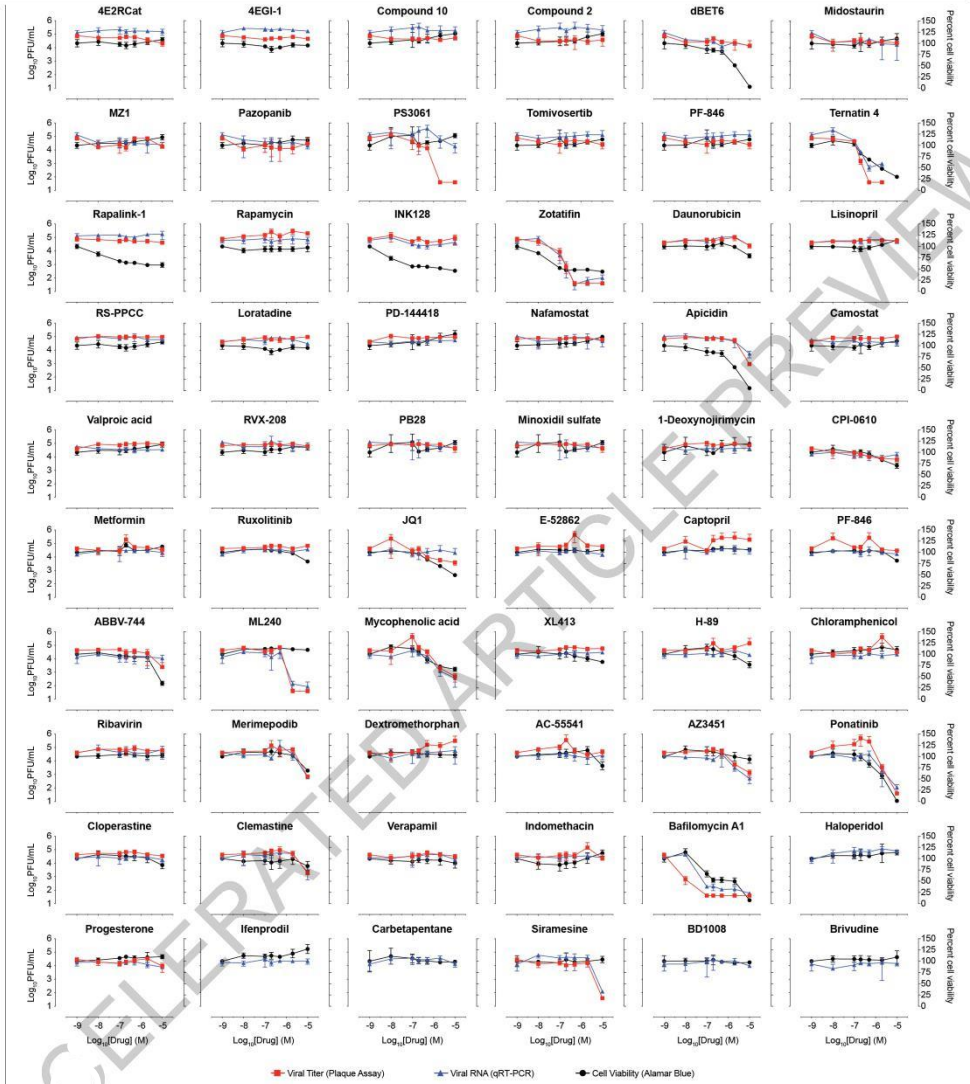


Fig. 9 | Virus plaque assays, qRT-PCR, and cell viability for compounds tested in Paris. Plaque assay (viral titer; red), qRT-PCR (viral RNA; blue) and cell viability (Alamar Blue; black) results for compounds tested at the Pasteur Institute in Paris. PF-846 was tested in two independent experiments and data are shown in two individual panels. Data=mean±SD; n=3 biologically independent samples for drug-treated cells and n=5 for PS3061, n=6 for DMSO controls.

Key Therapeutic Classes Under Investigation for Treatment of COVID-19

Antivirals

Baloxivir
Convalescent plasma
Favipiravir
(Hydroxy)chloroquine
Interferon
Lopinavir/ritonavir
Nitazoxanide
Oseltamivir
Remdesivir
Ribavirin

Immunomodulators

Corticosteroids
IL-1 inhibitors (eg, anakinra)
IL-6 inhibitors (eg, tocilizumab)
Intravenous immunoglobulin
JAK inhibitors (eg, baricitinib)

"Appropriate management strategies for patients with COVID-19 are a rapidly evolving therapeutic challenge, and the optimal agents (if any) to treat infection or prevent progression to critical illness remain ill-defined."

Barlow. Pharmacotherapy. 2020;40:416. McCreary. Open Forum Infect Dis. 2020;7:ofaa105. Sanders. JAMA. 2020;323:1824.

Slide credit: clinicaloptions.com

Key Data From Randomized Clinical Trials for COVID-19

Agent	N	Population	Comparator	Primary Outcome
Lopinavir/ritonavir[1]	199	Adults, severe	SOC alone	▪ No difference in time to clinical improvement
Lopinavir/ritonavir[2]	86	Adults, mild-to-moderate	Umifenovir or no antiviral	▪ No difference in rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid
Remdesivir[3]	237	Adults, severe	Placebo	▪ No difference in time to clinical improvement; study did not reach predetermined enrollment due to reduction in infections
Remdesivir, 5 days[4]	397	≥ 12 yrs, severe	Remdesivir, 10 days	▪ No difference in clinical status at Day 14 after adjustment for baseline severity
Favipiravir*[5]	240	Adults, pneumonia	Umifenovir	▪ No difference in clinical recovery rate of Day 7
Hydroxychloroquine*[6]	150	Adults, mild-to-moderate	SOC alone	▪ No difference in negative conversion of SARS-CoV-2 by Day 28

*Published as a preprint; not yet peer-reviewed.

1. Cao. NEJM. 2020;382:1787. 2. Li. Med. 2020;[Epub]. 3. Wang. Lancet. 2020;395:1569. 4. Goldman. NEJM. 2020;[Epub].
5. Chen. https://doi.org/10.1101/2020.03.17.20037432 6. Tang. https://doi.org/10.1101/2020.04.10.20060558

Slide credit: clinicaloptions.com

Key Data From Randomized Clinical Trials for COVID-19 (Cont.)

Agent	N	Population	Comparator	Primary Outcome
Remdesivir[1]	1063	Evidence of lower respiratory tract involvement	Placebo	▪ Median recovery time: 11 vs 15 days, *P* < .001 ▪ Mortality by Day 14: 7.1% vs 11.9% (NS)
Tocilizumab[2,3]	129	Moderate or severe pneumonia	Standard care alone	▪ Improvement in composite endpoint of death or need for ventilation at Day 14 with tocilizumab vs standard care
Sarilumab (200 or 400 mg)[4,5]	457	Severe or critical	Placebo	▪ CRP decline: 77% and 79% vs 21% ▪ IDMC recommended continuing phase III only in critical subgroup with 400 mg sarilumab vs placebo

1. Beigel. NEJM. 2020;[Epub]. 2. https://www.aphp.fr/contenu/tocilizumab-improves-significantly-clinical-outcomes-patients-moderate-or-severe-covid-19 3. NCT04331808. 4. NCT04315298. 5. https://newsroom.regeneron.com/news-releases/news-release-details/regeneron-and-sanofi-provide-update-us-phase-23-adaptive

Randomised Evaluation of COVid-19 thERapY (RECOVERY) Trial Among Hospitalised Patients

- Patients randomized to SOC plus: no additional treatment, **lopinavir/ritonavir, dexamethasone, hydroxychloroquine (HCQ), or azithromycin**
  - Factorial design with simultaneous allocation to no additional tx vs **convalescent plasma**
  - If progressive disease (hyper-inflammatory state), subsequent randomization to no additional treatment vs **tocilizumab**
- > 11,500 patients enrolled from > 175 NHS hospitals in UK

- **6/5/2020:** statement on closure of recruitment to HCQ arm for lack of clinical benefit
  - 28-day mortality: 25.7% with HCQ + SOC (n = 1542) vs 23.5% with SOC alone (n = 3132) (RR: 1.11; 95% CI: 0.98-1.26; *P* = .10)
- **6/8/2020:** recruitment to dexamethasone arm halted because sufficient patient numbers enrolled to establish potential benefit

https://www.recoverytrial.net. https://www.recoverytrial.net/files/hcq-recovery-statement-050620-final-002.pdf. https://www.recoverytrial.net/files/recovery_dexamethasone_statement_160620_final.pdf.

Slide credit: clinicaloptions.com

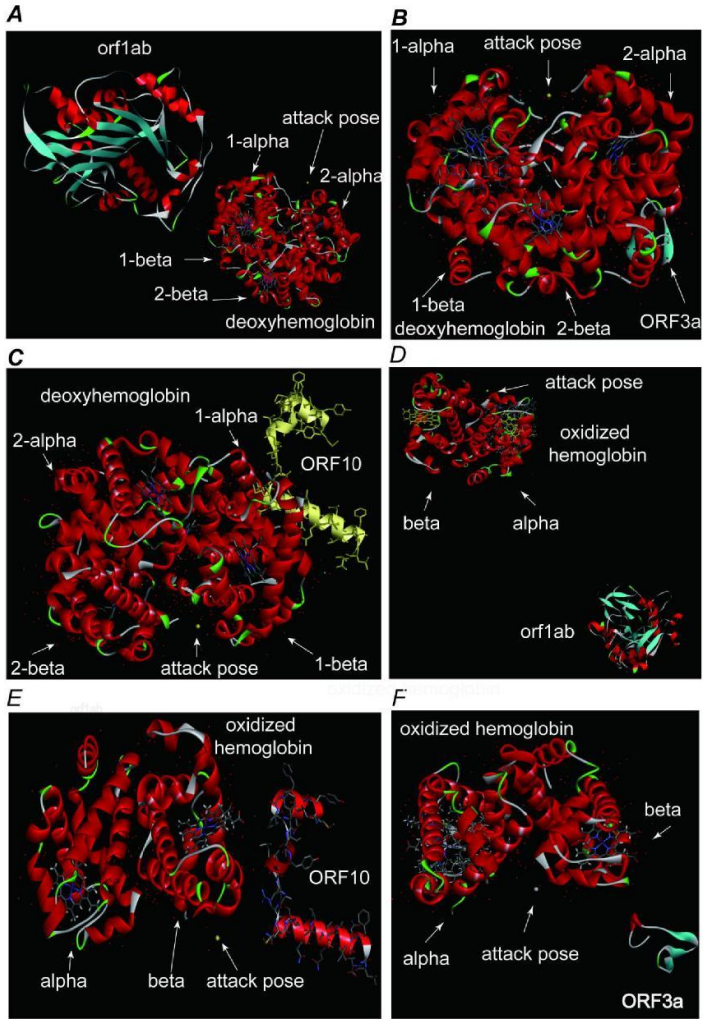
RECOVERY Trial: Partial Dexamethasone Results Reported by Press Release

- Data suggest 1 death prevented by treatment of ~ 8 ventilated patients or ~ 25 patients requiring oxygen alone

Outcome, %	Dexamethasone 6 mg QD PO or IV + SOC for 10 D (n = 2104)	SOC Only (n = 4321)	RR for Death With Dex + SOC vs SOC Alone (95% CI)	*P* Value
28-day mortality				
▪ Patients requiring ventilation	NR	41	0.65 (0.48-0.88)	.0003
▪ Patients requiring oxygen only	NR	25	0.80 (0.67-0.96)	.0021
▪ Patients requiring no respiratory intervention	NR	13	1.22 (0.86-1.75)	.14

https://www.recoverytrial.net. https://www.recoverytrial.net/files/recovery_dexamethasone_statement_160620_final.pdf.

Slide credit: clinicaloptions.com



Liu, Wenzhong
Li, Hualan

School of Computer Science and Engineering, Sichuan University of Science & Engineering, Zigong, 643002, China;

[2] School of Life Science and Food Engineering, Yibin University, Yibin,644000, China; * Correspondence: liuwz@suse.edu.cn

https://chemrxiv.org/ndownloader/files/22283226

Viral non-structural protein attack hemoglobin. ***A***. orf1ab attacks the deoxyhemoglobin. ***B***. ORF3a attacks the deoxyhemoglobin. ***C***. ORF10 attacks the deoxyhemoglobin. ***D***. orf1ab attacks the oxidized hemoglobin. ***E***. ORF10 attacks the oxidized hemoglobin. ***F***. ORF3a attacks the oxidized hemoglobin.

SARS-CoV-2: A Storm is Raging

Savannah F. Pedersen, Ya-Chi Ho

J Clin Invest. 2020. https://doi.org/10.1172/JCI137647.

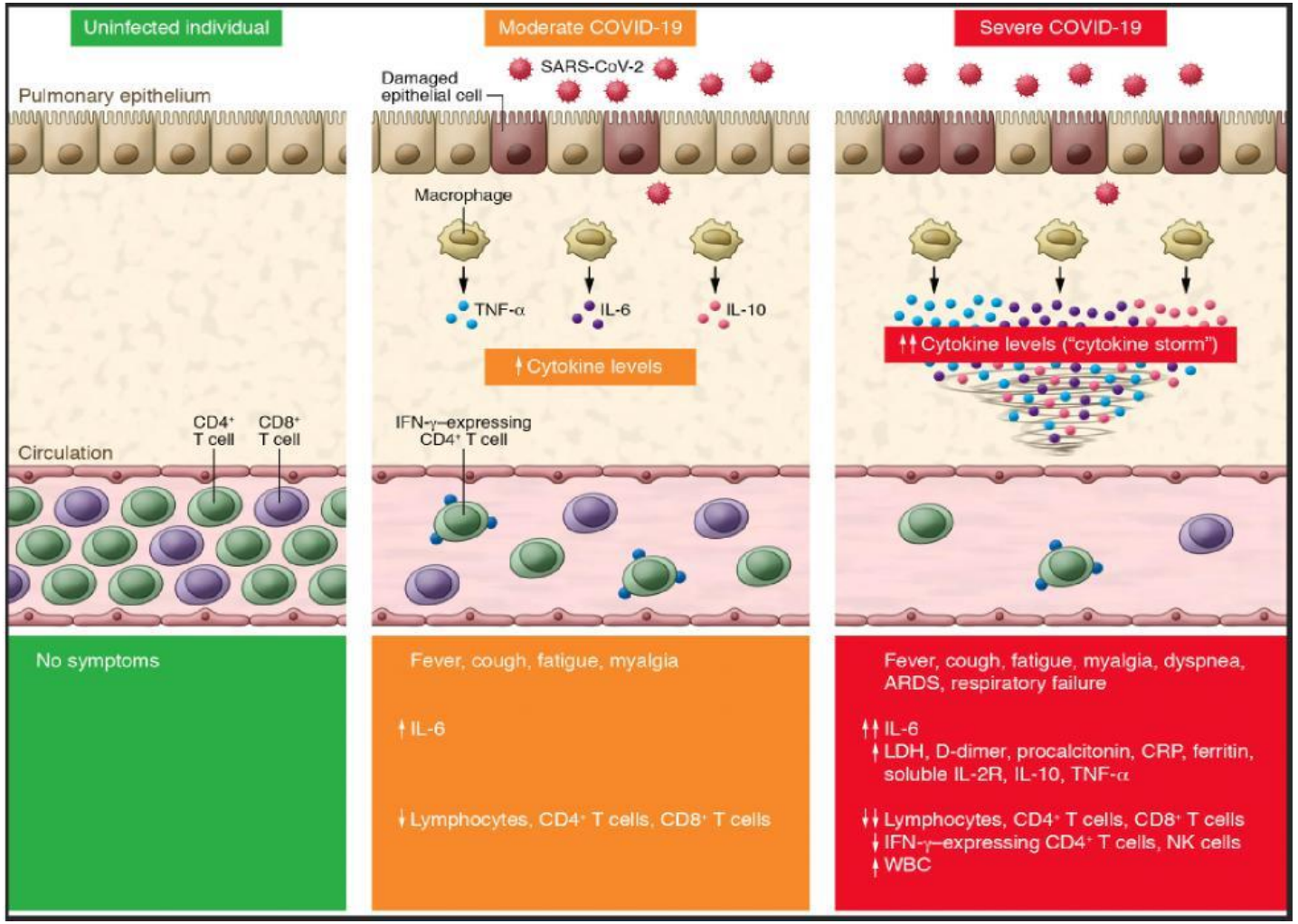
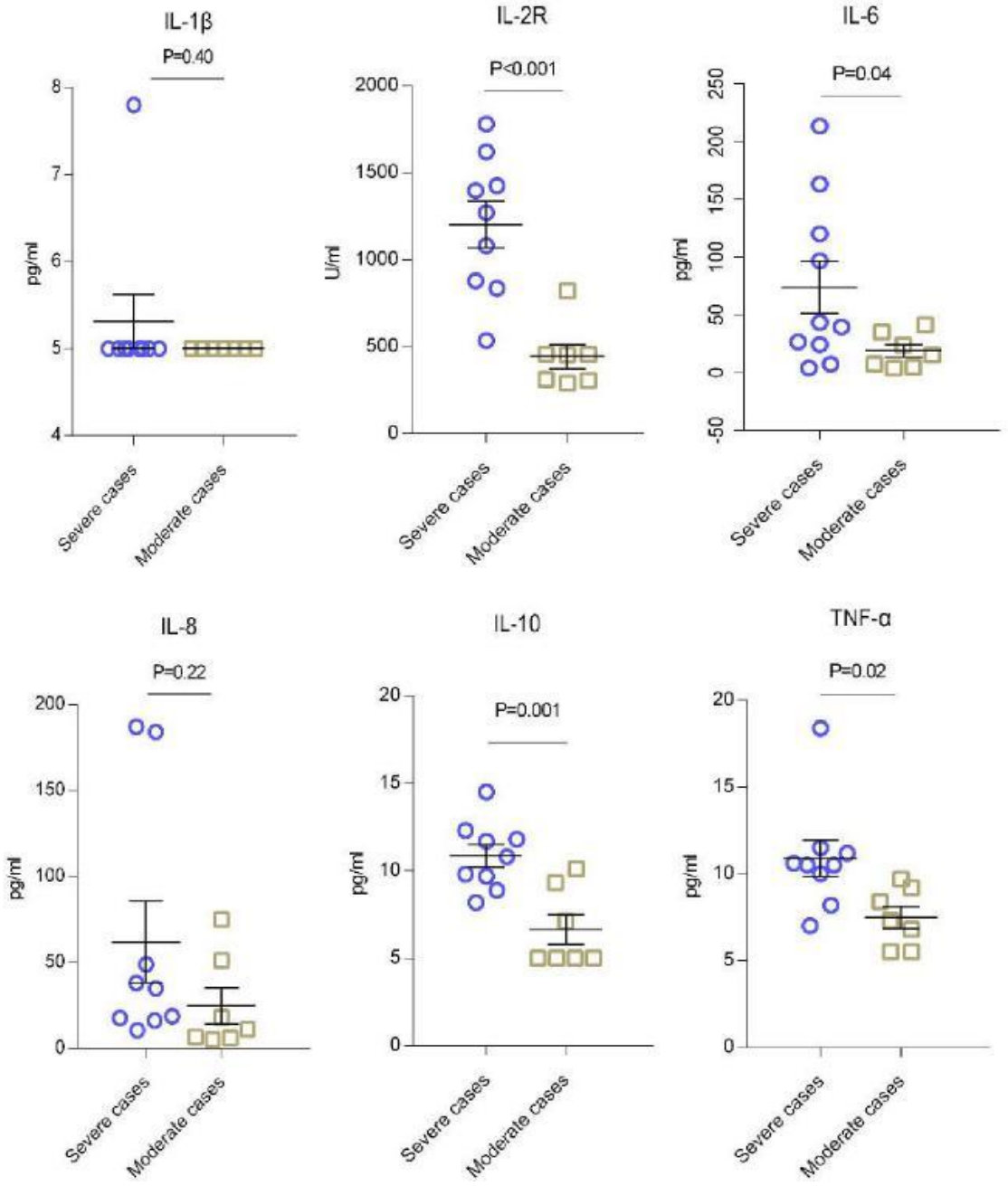


Figure 1. Cytokine storm and T cell lymphopenia is associated with COVID-19 disease severity. SARS-CoV-2 infection causes COVID-19. Compared to uninfected individuals (left panel), moderate COVID-19 cases exhibit an increase in IL-6 and a decrease in total T lymphocytes count, particularly $CD4^+$ T cells and $CD8^+$ T cells (middle panel). Severe COVID-19 cases have further increased production of IL-6, IL-2R, IL-10 and TNFα, while total T lymphocytes, particularly $CD4^+$ T cells and $CD8^+$ T cells, and IFNγ-expressing $CD4^+$ T cells markedly decrease (right panel). The level of cytokine storm and T cell lymphopenia is associated with pulmonary damage, respiratory distress and unfavorable outcome.

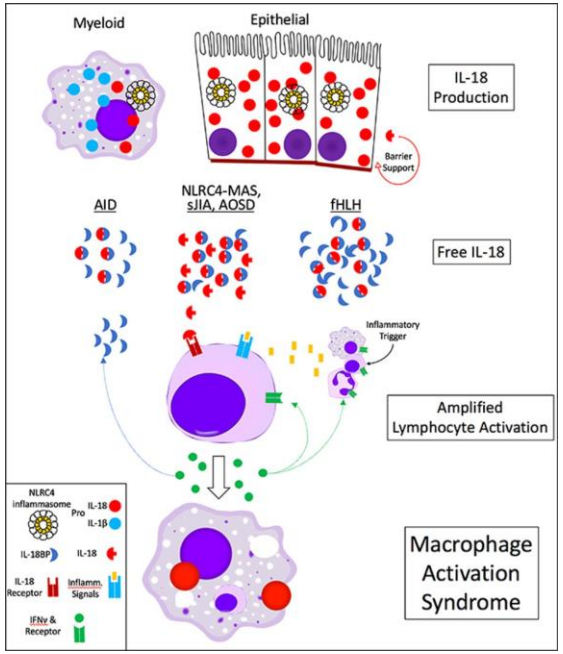


Clinical and immunologic features in severe and moderate Coronavirus Disease 2019

Guang Chen, ... , Jianping Zhao, Qin Ning

J Clin Invest. 2020. https://doi.org/10.1172/JCI137244.

Interleukin-18 diagnostically distinguishes and pathogenically promotes human and murine macrophage activation syndrome



Eric S. Weiss,Charlotte Girard-Guyonvarc'h,Dirk Holzinger,Adriana A. de Jesus,Zeshan Tariq,Jennifer Picarsic,Eduardo J. Schiffrin,Dirk Foell,Alexei A. Grom,Sandra Ammann,Stephan Ehl,Tomoaki Hoshino,Raphaela Goldbach-Mansky,Cem Gabay,Scott W. Canna, Interleukin-18 diagnostically distinguishes and pathogenically promotes human and murine macrophage activation syndrome, Blood, 2018,

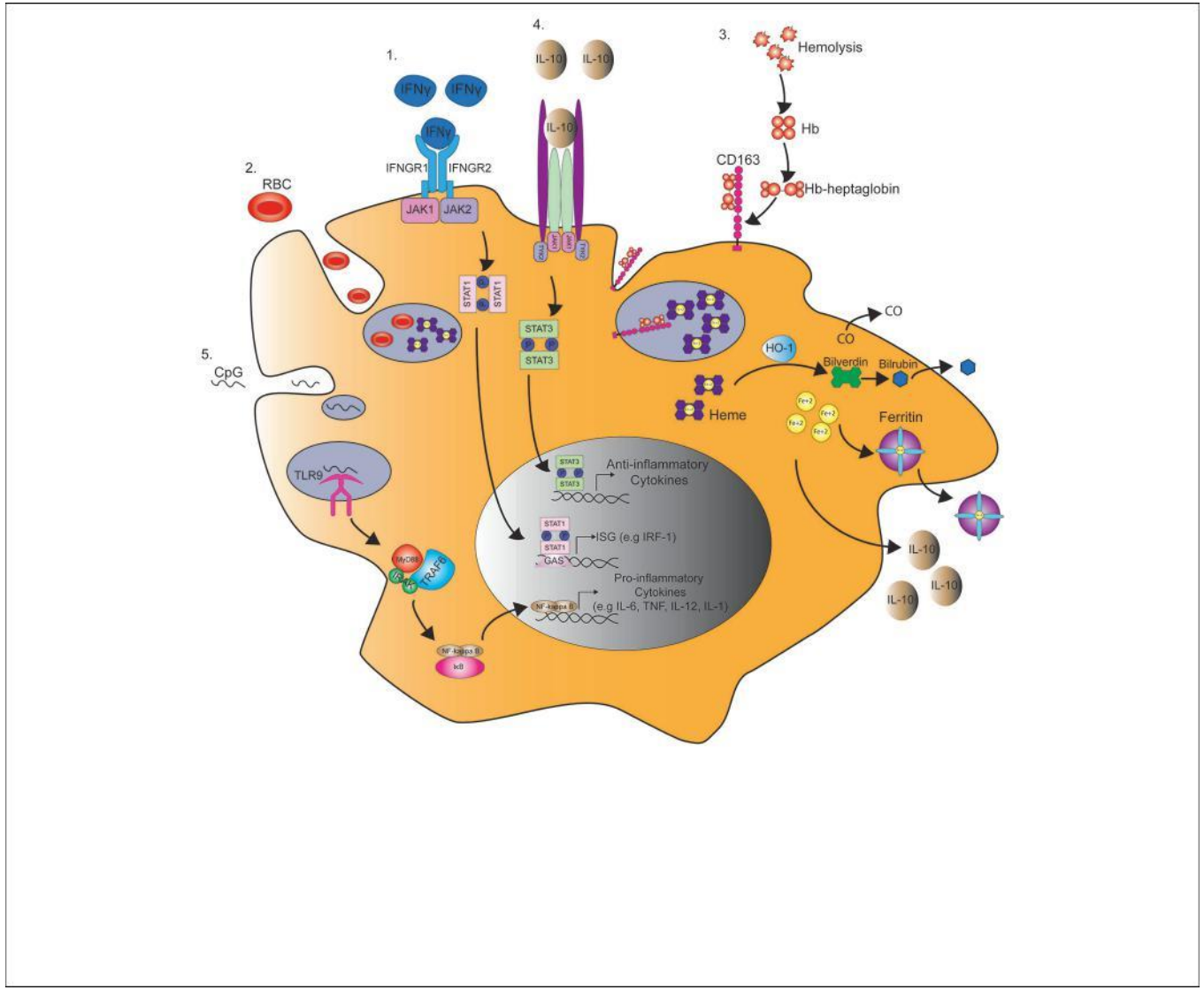


FIGURE 1 | Pathways regulating macrophage function in MAS. 1. IFNγ binds the IFNγ receptor (IFNGR) and subsequently induces the phosphorylation of STAT1 by JAK1/2 in the cytoplasm. STAT1 dimer then binds to γ-interferon activation site (GAS) and enhances the transcription of interferon-stimulated genes (ISG), such as interferon regulatory factor 1 (IRF1). 2. STAT1 activation by IFNγ also induces macropinocytosis leading to the engulfment and degradation of red blood cells (RBC) in a process known as hemophagocytosis. 3. Hemophagocytosis is also mediated by the uptake of hemoglobin (Hb)-heptaglobin complex by CD163. The Hb-heptaglobin complex is degraded in the lysosome followed by catalysis of heme by heme oxygenase-1 (HO-1) to carbon dioxide (CO), bilverdin, and iron (Fe^{2+}). Bilverdin is then converted to bilirubin by bilverdin reductase, and iron is bound to ferritin. 4. This process also leads to the production of IL-10 that through binding to IL-10 receptor induces STAT3 phosphorylation and the production of anti-inflammatory cytokines that counteract IFNγ signaling. 5. In a mouse model of MAS, serial injections of CpG induce the activation of toll-like receptor 9 (TLR9) in the macrophage endosome leading to the production of pro-inflammatory cytokines in a MyD88 and NFκB dependent manner.

The Immunology of Macrophage Activation Syndrome

Courtney B. Crayne [1], *Sabrin Albeituni*[2], *Kim E. Nichols*[2] *and Randy Q. Cron*[1]* [1] *Pediatric Rheumatology, University of Alabama Birmingham, Birmingham, AL, United States,* [2] *Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, United States*

(2019) The Immunology of Macrophage Activation Syndrome. Front. Immunol. 10:119. doi: 10.3389/fimmu.2019.00119

Haemophagocytic lymphohistiocytosis (HLH)—2004 diagnostic criteria

At least five of the following criteria should be met:
•Fever
•Splenomegaly
•Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood)
•Haemoglobin <90 g/L (haemoglobin <100 g/L in infants <4 weeks)
•Platelets $<100\times10^9$/L
•Neutrophils $<1.0\times10^9$/L
•Hypertriglyceridaemia and/or hypofibrinogenaemia
•Fasting triglycerides ≥3.0 mmol/L (ie, ≥265 mg/dL)
•Fibrinogen ≤1.5 g/L
•Hemophagocytosis in bone marrow or spleen or lymph nodes. No evidence of malignancy.
•Low or no NK cell activity (according to local laboratory reference)
•Ferritin ≥500 mg/L
•sCD25 (ie, soluble IL-2 receptor) ≥2400 U/mL

Alunno A, Carubbi F, Rodríguez-Carrio J
Storm, typhoon, cyclone or hurricane in patients with COVID-19? Beware of the same storm that has a different origin *RMD Open* 2020;**6:**e001295. doi: 10.1136/rmdopen-2020-001295

Summary of treatment for macrophage activation syndrome

Treatment	Medication
- First line	Corticosteroids: methylprednisolone, prednisolone, dexamethasone Cyclosporin A
- Alternative	Intravenous immunoglobulin Etoposide Cyclophosphamide Plasma exchange Anti-thymocyte globulin Biologic agents: IL-1 inhibitor

- Lerkvaleekul B, Vilaiyuk S. Macrophage activation syndrome: early diagnosis is key. *Open Access Rheumatol*. 2018;10:117-128. Published 2018 Aug 31. doi:10.2147/OARRR.S151013

Jiang et al. *Emerging Microbes & Infections* (2018) 7:77

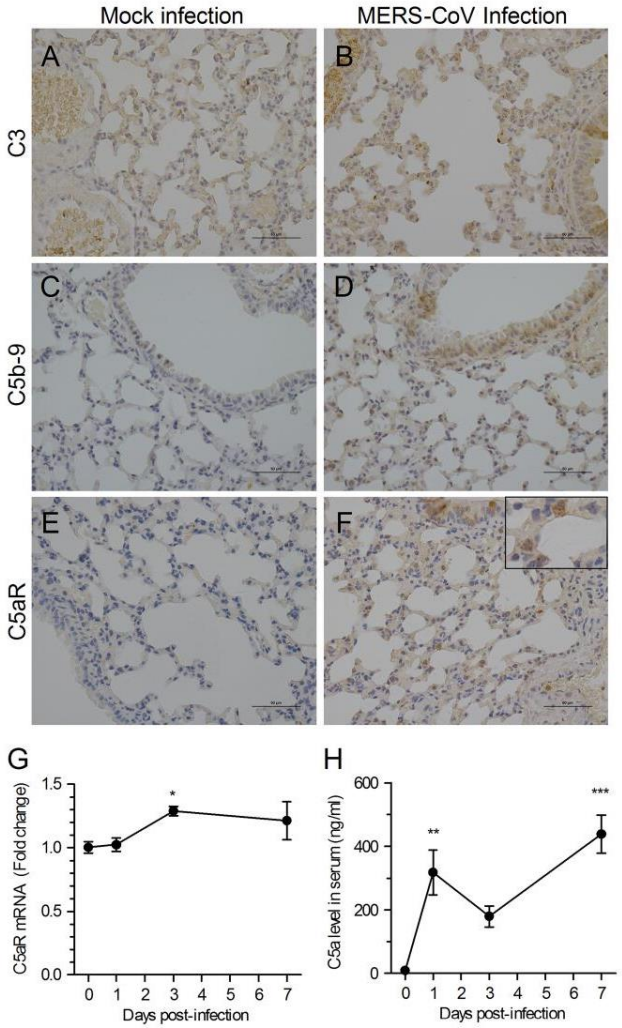


Fig. 1 MERS-CoV infection induces excessive complement activation in hDPP4-transgenic mice. **a–f** Representative images of lung tissue sections from MERS-CoV-infected or Mock-infected hDPP4-transgenic mice by immunohistochemical staining for C3, C5b-9, and C5aR (n = 5 per group). **g** Transcriptional expression of C5aR in lung tissues at different time points after MERS-CoV infection (n = 3–5 per group). **h** Concentration of C5a in sera at different time points after virus infection was measured by a quantitative enzyme-linked immunosorbent assay (ELISA). Data are expressed as the means ± SEM (n = 5 per group). 'P < 0.05, ''P < 0.01, and '''P < 0.001 (one-way analysis of variance (ANOVA) with Dunnett's post-test)

Jiang et al. *Emerging Microbes & Infections* (2018) 7:77

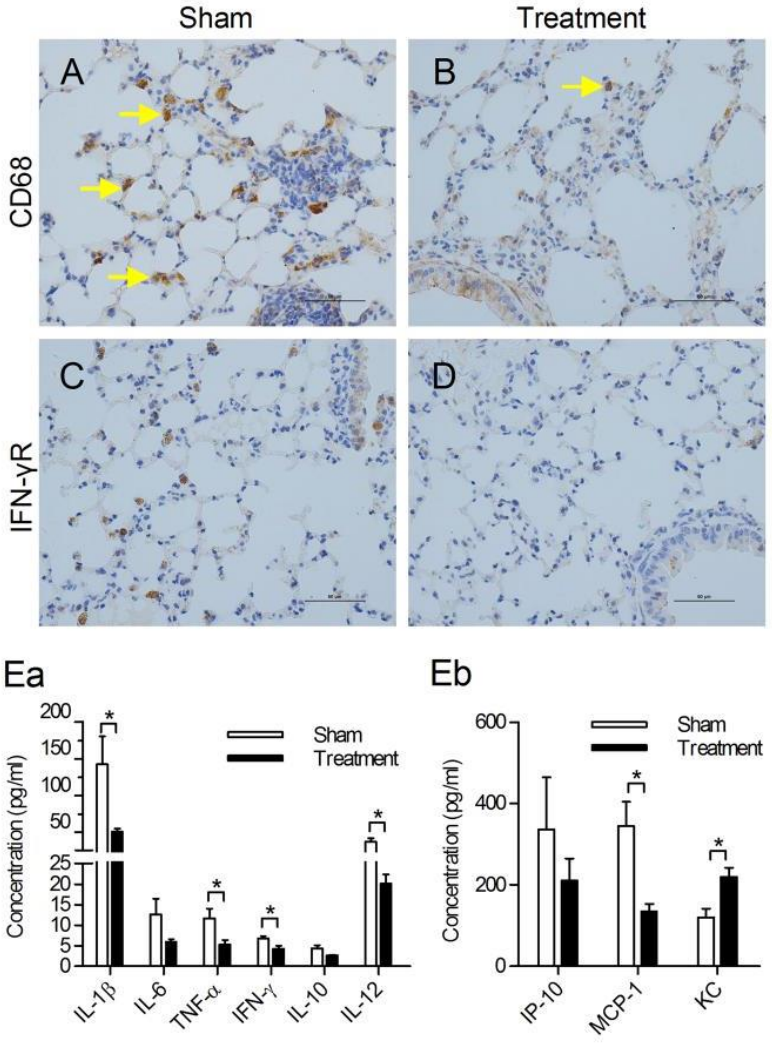


Fig. 2 C5a–C5aR blockade reduced local and systemic inflammatory responses in hDPP4-transgenic mice. a–d Infiltration of macrophage (**a**–**b**) and the expression of IFN-γ receptor (**c**–**d**) were assessed by immunohistochemical staining in the lungs 3 days after challenge ($CD68^+$macrophages were indicated by arrows). **e** Sera from the two groups of mice were collected on day 3 and assayed for the levels of IL-1β, IL-6, TNF-α, IFN-γ, IL-10, IL-12 (**e**a), KC, MCP-1, and IP-10 (**e**b). The results are the mean ± SEM ($n = 5$). *$P <$ 0.05 (Student's *t* test with Welch's correction)

Jiang et al. *Emerging Microbes & Infections* (2018) 7:77

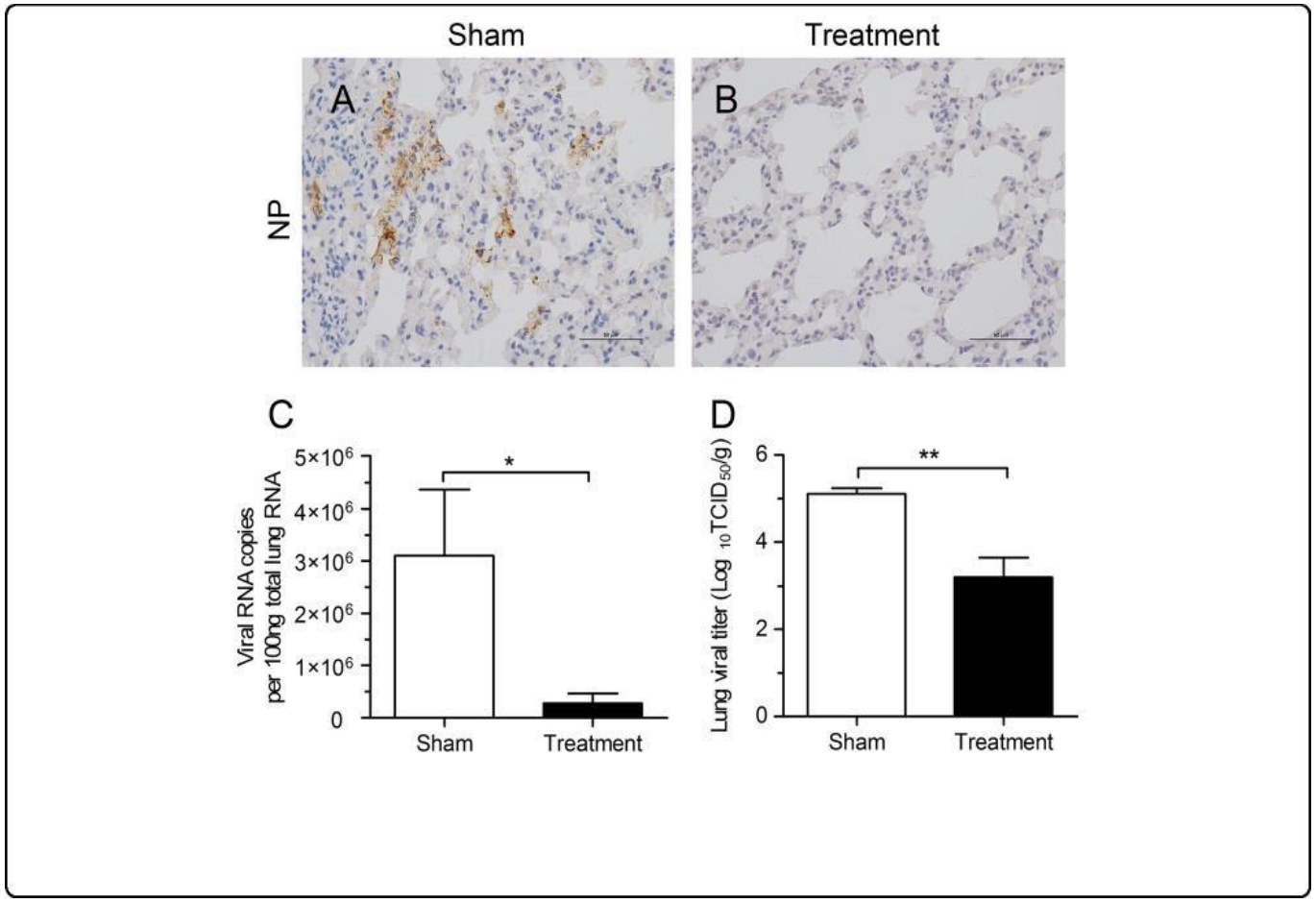


Fig. 3 C5a–C5aR blockade limits viral replication in lung tissue. a–b Representative images of immunohistochemical staining of MERS-CoV antigen in lungs on day 7 after challenge in the sham treatment and anti-C5aR Ab treatment groups. **c** Viral RNA copies in lung tissues in the sham treatment and anti-C5aR Ab treatment groups. **d** Virus titer in lungs on day 7 after challenge in the sham treatment and anti-C5aR Ab treatment groups. Data are expressed as the means ± SEM (n = 5 per group). *P < 0.05, **P < 0.01 (Student's t test with Welch's correction)

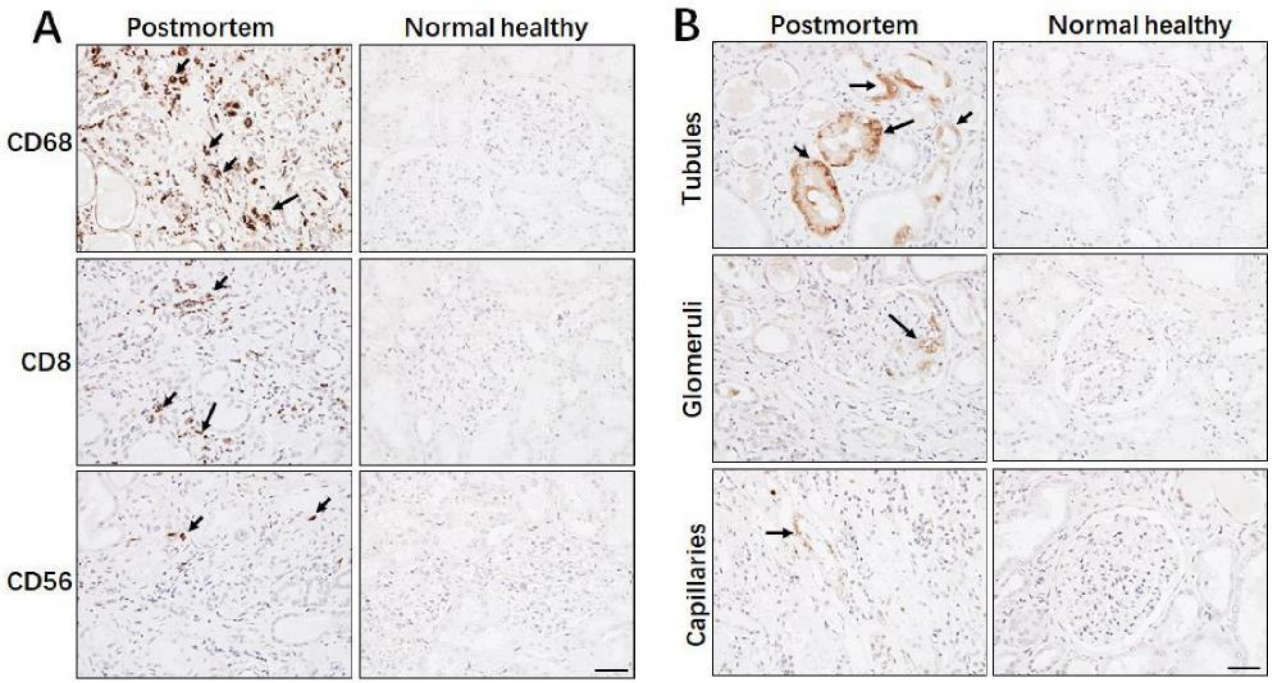
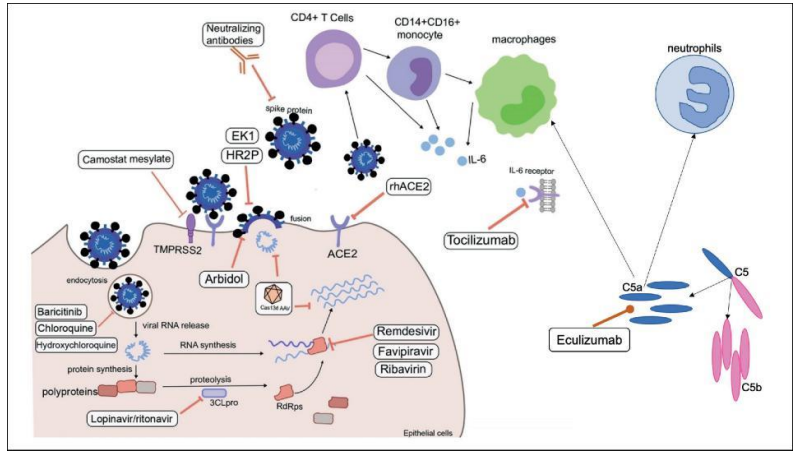


Figure 3 Immunohistochemistry analyzed lymphocytes and the complement C5b-9 in kidney tissues. The expression of **(A)** CD68, CD8 and CD56; **(B)** C5b-9 in kidney tissues from COVID-19 patients undergoing postmortem examination or normal healthy was detected by immunohistochemistry. Arrow indicated positive cells. Scale bar= 100 μM.

Human Kidney is a Target for Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection

Bo Diao[1#], Chenhui Wang[2#], Rongshuai Wang[3#], Zeqing Feng[2], Yingjun Tan[1], Huiming Wang[4], Changsong Wang[5], Liang Liu[6], Ying Liu[1], Yueping Liu[1], Gang Wang[1], Zilin Yuan[1] , Liang Ren[3#], Yuzhang Wu[2#], Yongwen Chen[2#]



•**Figure 1.** Pathogenesis and therapy targets.

•up to 4 weeks, and the final assessment at day 29. Screening and the day 1 visits can occur on the same day if necessary if the subject has met all inclusion and none of the exclusion criteria.

•Up to 4 weekly infusions of eculizumab at 900 mg were administered. Eculizumab is formulated at pH 7 and each 30 mL vial contains 300 mg of eculizumab, polysorbate 80 (6.6 mg) (vegetable ori-gin), sodium chloride (263.1 mg), sodium phosphate dibasic (53.4 mg), sodium phosphate monobasic (13.8 mg), and water for injection, USP. Eculizumab has to be administered *via* IV infusion *via* gravity feed, a syringe-type pump, or an infusion pump, and has been diluted to a final concentration of 5 mg/mL before administration. The diluted eculizumab has been IV administered over approximately 35 min-utes. The patients have been monitored for at least 1 hour following the infusion for signs or symptoms of an infusion reaction. The duration of each pa-tient's treatment with eculizumab was a minimum of 8 days and a maximum of 22 days.

•Patients could be treated with: 1. Confirmed severe COVID-19 requiring hospitalization; 2. Symptomatic, bilateral pneumonia confirmed by CT or X-ray at screening or within the 7 days prior to screening; 3. Severe pneumonia requir-ing oxygen supplementation (WHO 2020); 4. ≥18 year of age at the time of providing informed con-

•sent/assent; 5. willing and able to give written in-formed consent. Exclusion Criteria: 1. confirmed mild to moderate COVID-19, even if the patient is hospitalized; 2. the patient is not expected to survive > 24 hours.

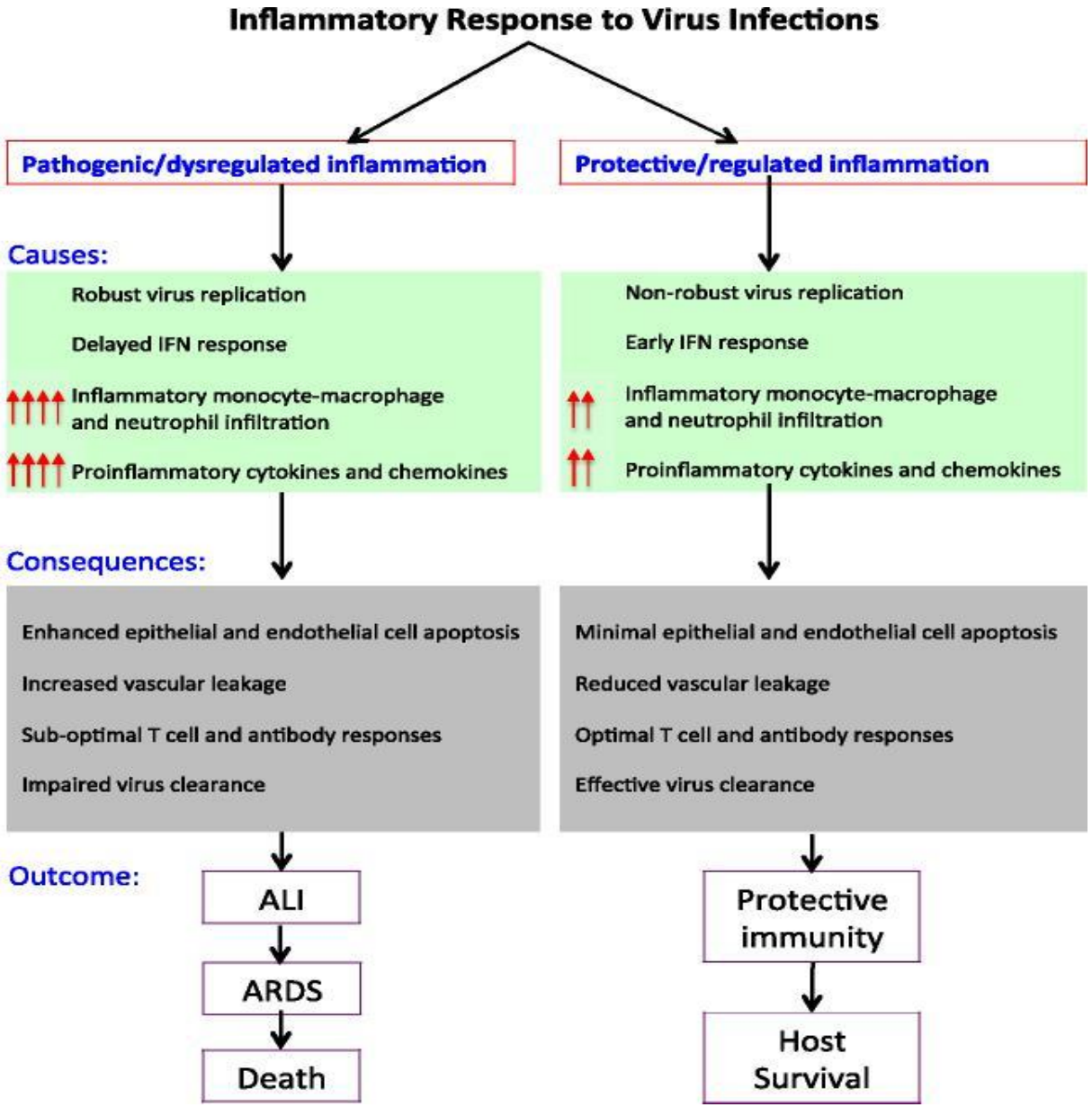
•Supportive therapy during treatment with ecu-lizumab consisted in:

- anticoagulant therapy with Enoxaparin 4000 IU/day *via* subcutaneous injection;
- antiviral therapy with Lopinavir 800 mg/day + Ritonavir 200 mg/day;
- hydroxychloroquine 400 mg/day;
- cefriaxone 2 g/day IV;
- vitamin C6 g/day for 4 days;
- CPAP (non-invasive ventilation).

•All chest CT examinations were performed with a Philips Ingenuity 64 scanner located in the "red" COVID area.

•Patients were examined in a supine position, wrapped in a waterproof sheet, with arms raised in respiratory apnea, if possible. Scanning range from the apex to the base. The scan parameters adopted were as follows: helical scan mode; tube voltage, 120 kV; mAs 30-200 mAs; matrix, at least 512 x 512; 1 mm thick reconstruction algorithm with high spatial frequency (for lung or bone); CT analysis: two radiologists have analyzed the CT images independently with lung window (width,

Fig. 2 Schematic representatic of protective versus pathogeni inflammatory responses to pathogenic hCoV infections



Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology

Rudragouda Channappanavar[1] & Stanley Perlman[1]

Semin Immunopathol (2017) 39:529–539

DOI 10.1007/s00281-017-0629-x

Reported Therapeutic mechanism Example cytokine target

IL-1 IL-1 receptor antagonist Anakinra, canakinumab

IL-6 Anti-IL-6R monoclonal Ab Tocilizumab

IL-18 IL-18 binding protein Not commercially available

CD28 CTLA4-Ig Abatacept

JAK1/2 JAK inhibitor Tofacitinib

Theoretical Proposed mechanism Example cytokine target

IL-10 Recombinant IL-10 protein None available

IL-33 Anti-IL-33R monoclonal Ab None available

IFNγ Anti-IFNγ monoclonal Ab None available

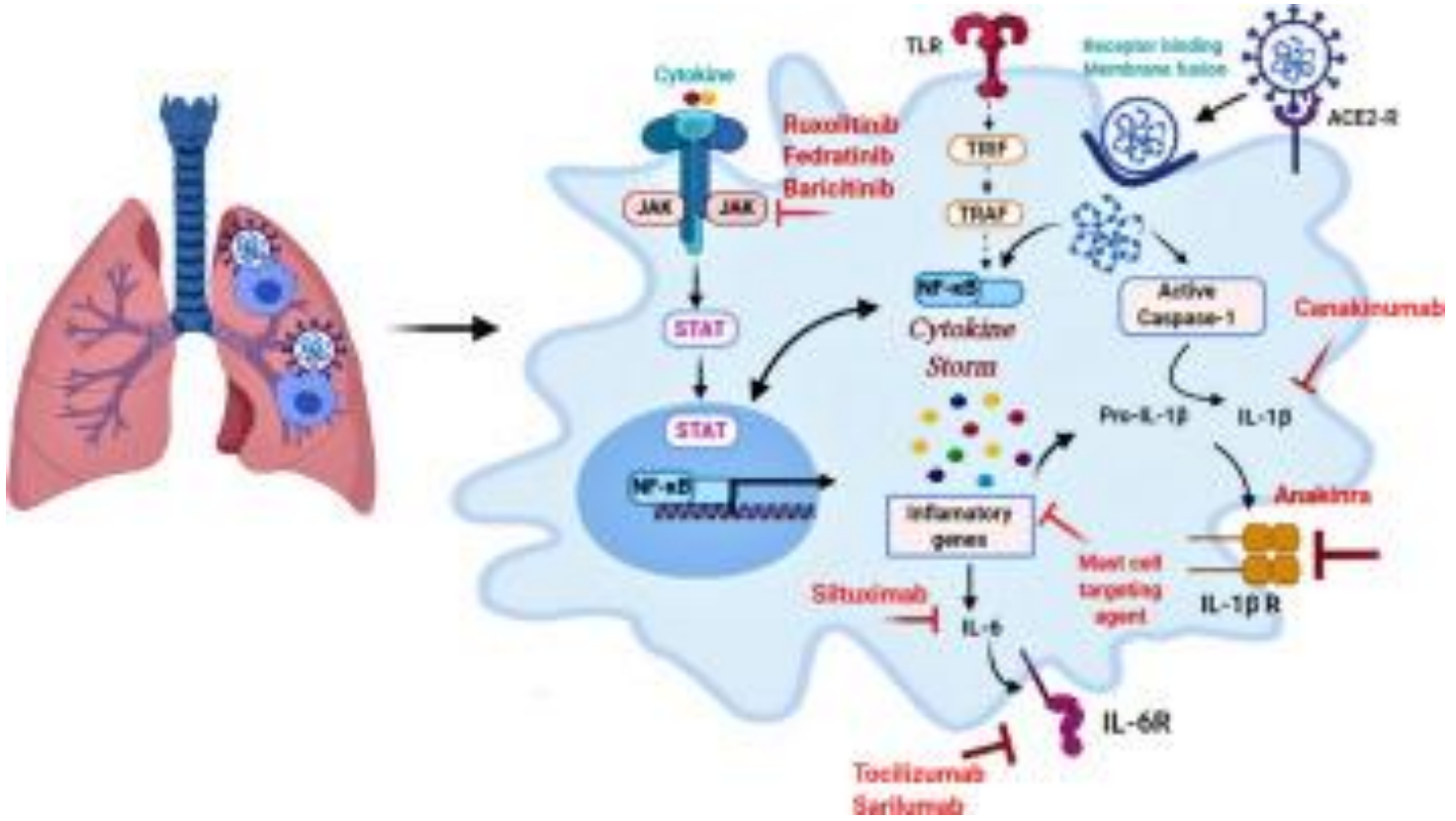
TNF, tumor necrosis factor; Ab, antibody; IL, interleukin; R, receptor; CTLA, cytotoxic T-lymphocyte-associated protein 4; Ig, immunoglobulin; JAK, Janus kinase; IFNγ, interferon-gamma.

The Immunology of Macrophage Activation Syndrome

Courtney B. Crayne [1], ***Sabrin Albeituni*** [2], ***Kim E. Nichols*** [2] **and** ***Randy Q. Cron*** [1]* [1] *Pediatric Rheumatology, University ofAlabama Birmingham, Birmingham, AL, United States,* [2] *Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN, United States*

(2019) The Immunology of Macrophage Activation Syndrome. Front. Immunol. 10:119. doi: 10.3389/fimmu.2019.00119

Therapeutic Implications of understanding the role of Cytokines



Abdin SM, Elgendy SM, Alyammahi SK, Alhamad DW, Omar HA. Tackling the cytokine storm in COVID-19, challenges, and hopes. *Life Sciences*. Published online July 11, 2020:118054. doi:10.1016/j.lfs.2020.118054

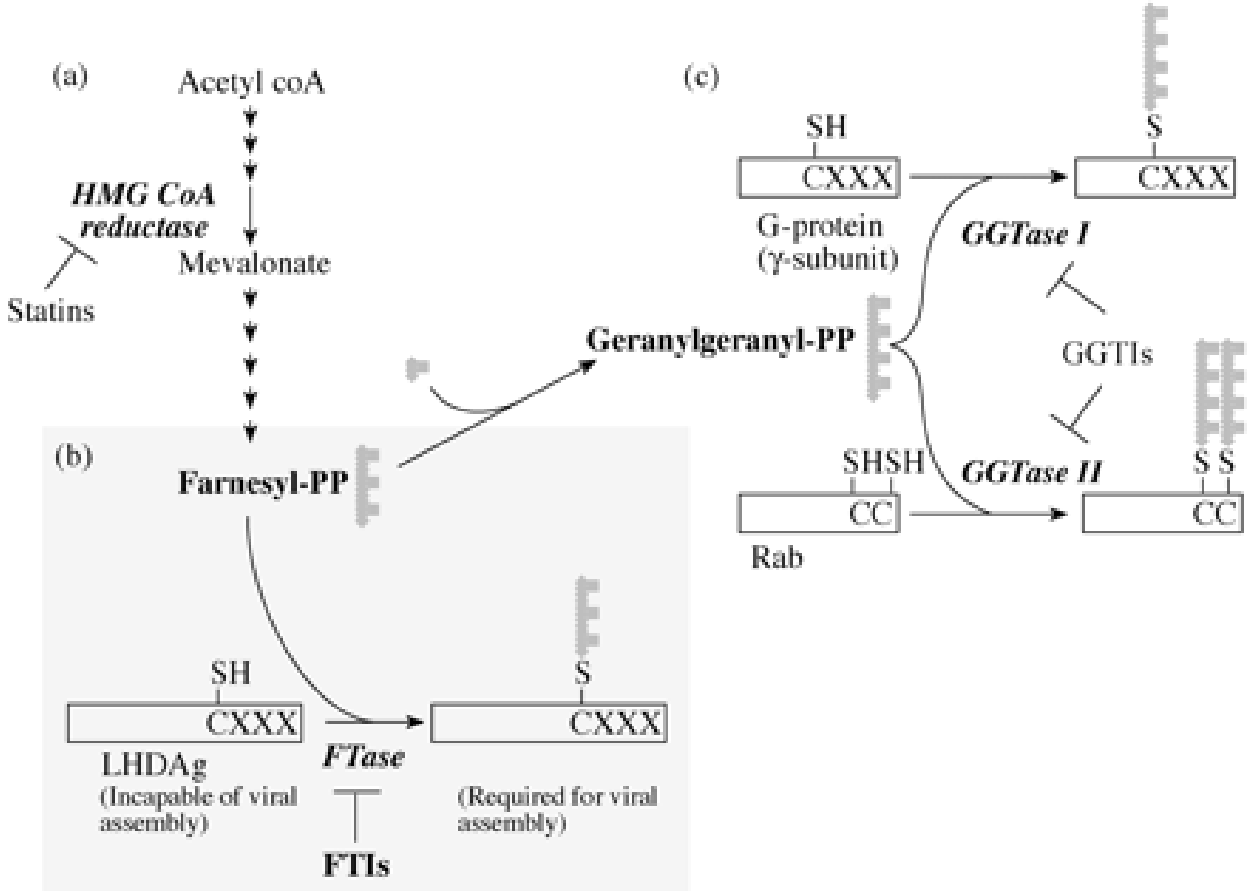
Therapeutic Implications of understanding the role of Cytokines

- Indiscriminate blockade of cytokines may be counterproductive
- An early interferon response and inflammatory cytokines are part of the host attempt to resist infection
- Delayed interferon response may lead to greater replication of the invading pathogen
- Lack of early clearance of pathogen is in part responsible for the cytokine storm
- Too early a blockade of cytokines may predispose to more infections
- Timely intervention with blockade of IL1; IL 6; Anti C5 maybe beneficial
- Combination of multiple locus blockade may be superior to a single agent
- Proper selection of individuals who may benefit is key
- Predictors of MAS/Cytokine storm in viral infections like COVID 19:
  - Absolute lymphocyte count less than 800
  - Ferritin greater than 800

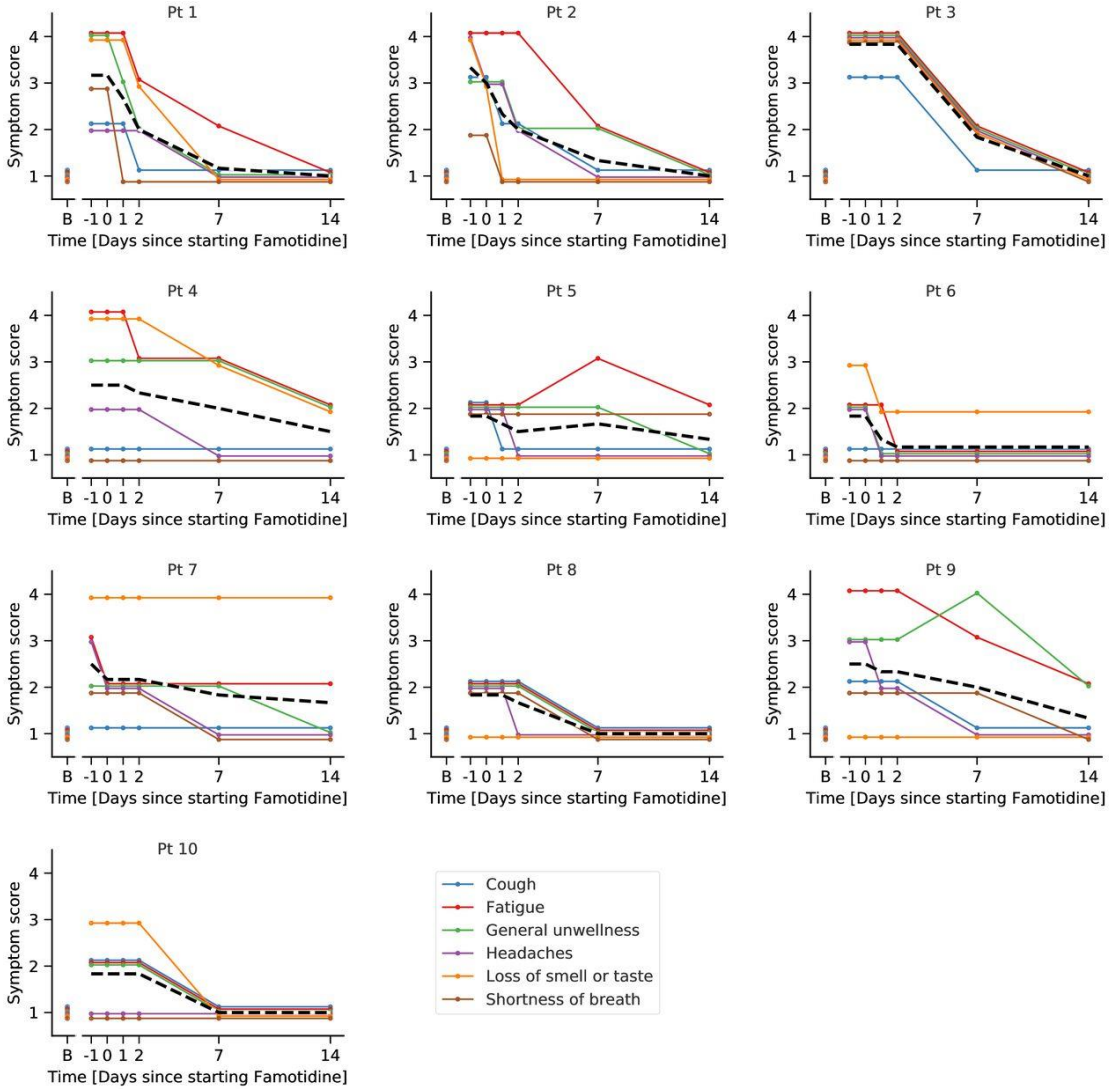
Yeldandi Rule

- The Yeldandi rule + any one of the following: Fever; Respiratory rate > 20; Hypotension; Evidence of organ dysfunction should be selected for early intervention with biologics to block cytokines in addition to standard treatment
- In future data from GWA may allow a genetic marker to be used for greater precision (Blood group A highest risk levels of von Willebrand Factor ?)
- **A controlled clinical trial is imperative**

Figure 1. Outline of major protein prenylation pathways and potential targets for antiviral therapy. (a) ...



J Antimicrob Chemother, Volume 52, Issue 6, December 2003, Pages 883–886, https://doi.org/10.1093/jac/dkg490



Janowitz T, Gablenz E, Pattinson D*, et al*
Famotidine use and quantitative symptom tracking for COVID-19 in non-hospitalised patients: a case series
Gut Published Online First: 04 June 2020. doi: 10.1136/gutjnl-2020-321852

From: **Is a "Cytokine Storm" Relevant to COVID-19?**

JAMA Intern Med. Published online June 30, 2020. doi:10.1001/jamainternmed.2020.3313

Table. Plasma Levels of Interleukin-6 Reported in COVID-19 Compared With Levels Previously Reported in ARDS[a]

COVID-19	Total population No.	Total population IL-6 levels, pg/mL	Severe disease No.	Severe disease IL-6 levels, pg/mL	Measurement platform
Zhou et al[4]	191	7 (5-11)	54[b]	11 (8-14)	CL
Wu et al[1]	123	7 (6-9)	84[c]	7 (6-11)	CL
Mo et al[5]	155	45 (17-96)	85[d]	64 (31-165)	CL
Qin et al[2]	452	21 (6-47)	286[e]	25 (10-55)	CL
Cummings et al[6]	NR	NR	237[f]	26 (11-69)	CL

ARDS	Total population No.	Total population IL-6 levels, pg/mL	Hypoinflammatory No.	Hypoinflammatory IL-6 levels, pg/mL	Hyperinflammatory No.	Hyperinflammatory IL-6 levels, pg/mL	Measurement platform
ALVEOLI[7]	521	238 (94-741)[f]	386	154 (67-344)	135	1525 (584-3802)	ELISA
FACTT[8]	884	130 (46-411)[f]	638	86 (34-216)	246	578 (181-2621)	ELISA
SAILS[9]	720	443 (173-1513)[f]	451	282 (115-600)	269	1618 (517-3205)	ELISA

Abbreviations: ALVEOLI, Assessment of Low Tidal Volume and Elevated End-Expiratory Pressure to Obviate Lung Injury; ARDS, acute respiratory distress syndrome; CL, clinical laboratory; CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; FACTT, Fluids And Catheters Treatment Trial; ICU, intensive care unit; IL-6, interleukin-6; NR, not reported; SAILS, Statins for Acutely Injured Lungs From Sepsis.

[a] Presented values are the medians with interquartile ranges. The top segment of the Table reports data from selected COVID-19 cohorts (n > 100) and their corresponding severe subgroups. The bottom segment reports data from 3 National Heart, Lung, and Blood Institute ARDS network randomized clinical trials. Values are reported for the total cohorts and in subgroups stratified by ARDS phenotypes (hypoinflammatory and hyperinflammatory). The mean (SD) IL-6 levels for the ARDS trials were as follows: ALVEOLI, 2051 (8208) pg/mL; FACTT, 1048 (3348) pg/mL; and SAILS, 2363 (10 940) pg/mL.

[b] Nonsurvivors.

[c] ARDS.

[d] Refractory hypoxemia.

[e] Acute hypoxemic respiratory failure.

[f] Requiring ICU admission.

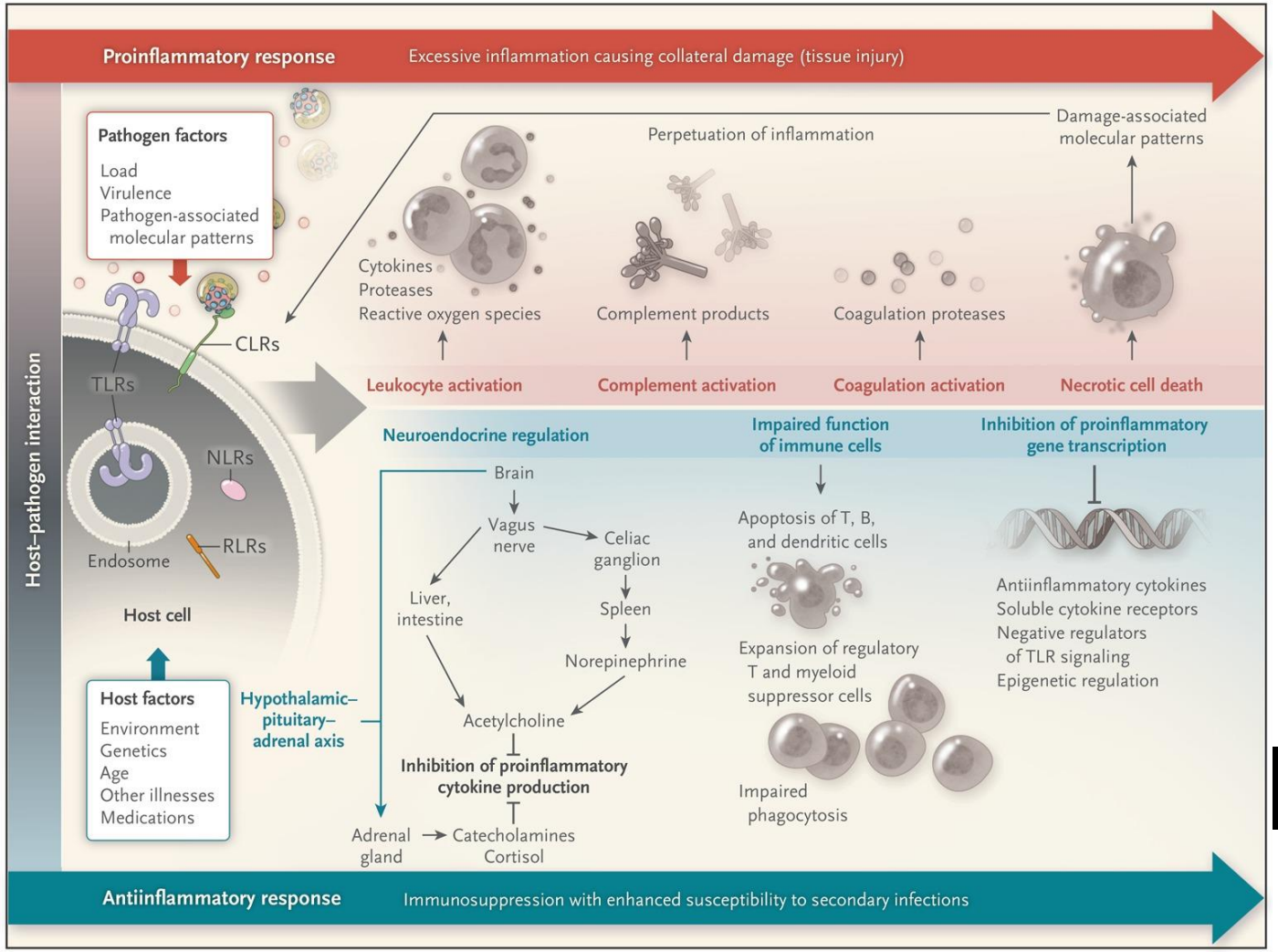
Sinha P, Matthay MA, Calfee CS. Is a "Cytokine Storm" Relevant to COVID-19? *JAMA Intern Med.* Published online June 30, 2020. doi:10.1001/jamainternmed.2020.3313

Table Title:

Plasma Levels of Interleukin-6 Reported in COVID-19 Compared With Levels Previously Reported in ARDS[a]

Review Article: Critical Care Medicine

Severe Sepsis and Septic Shock



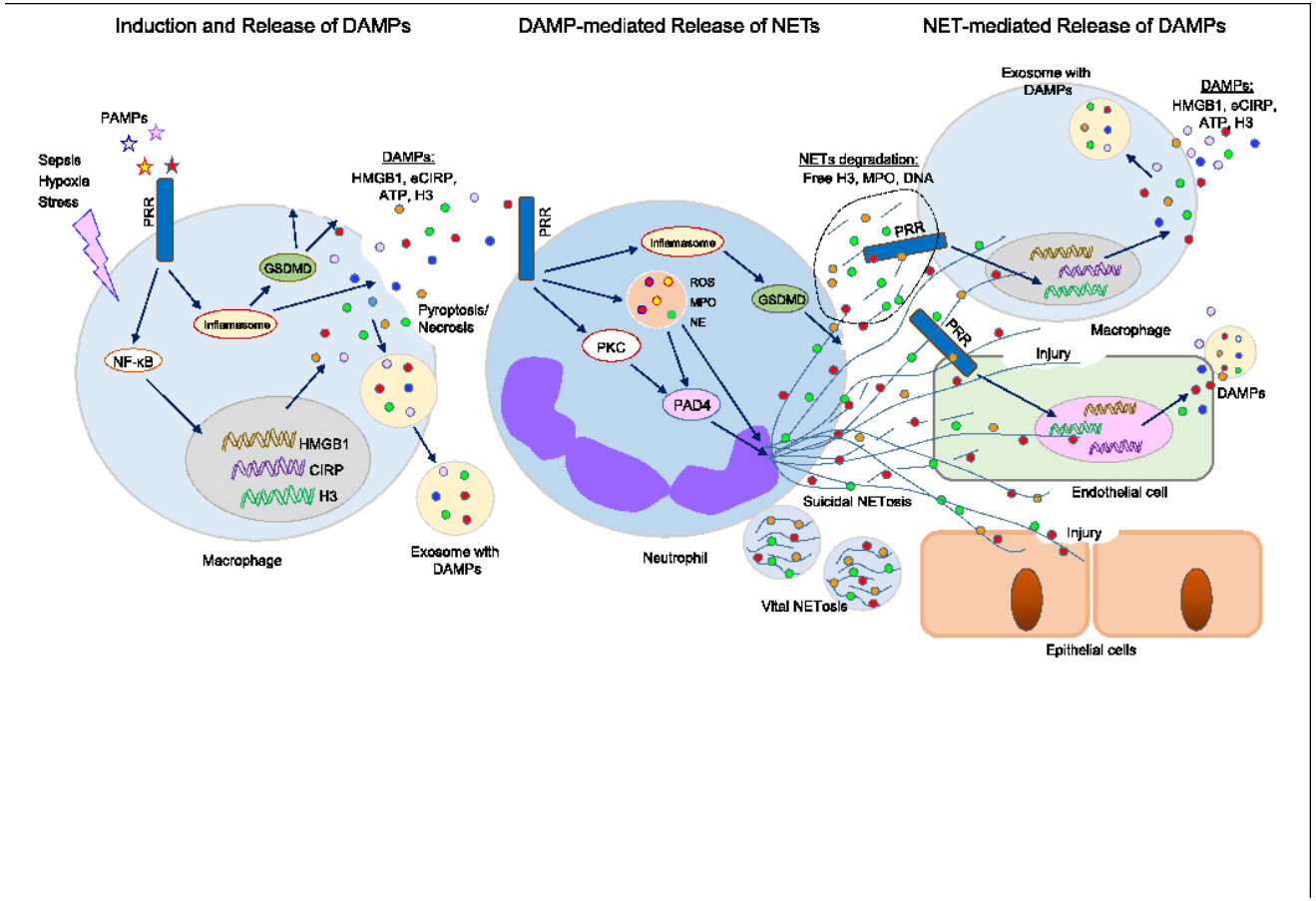
N Engl J Med
Volume 369(9):840-851
August 29, 2013

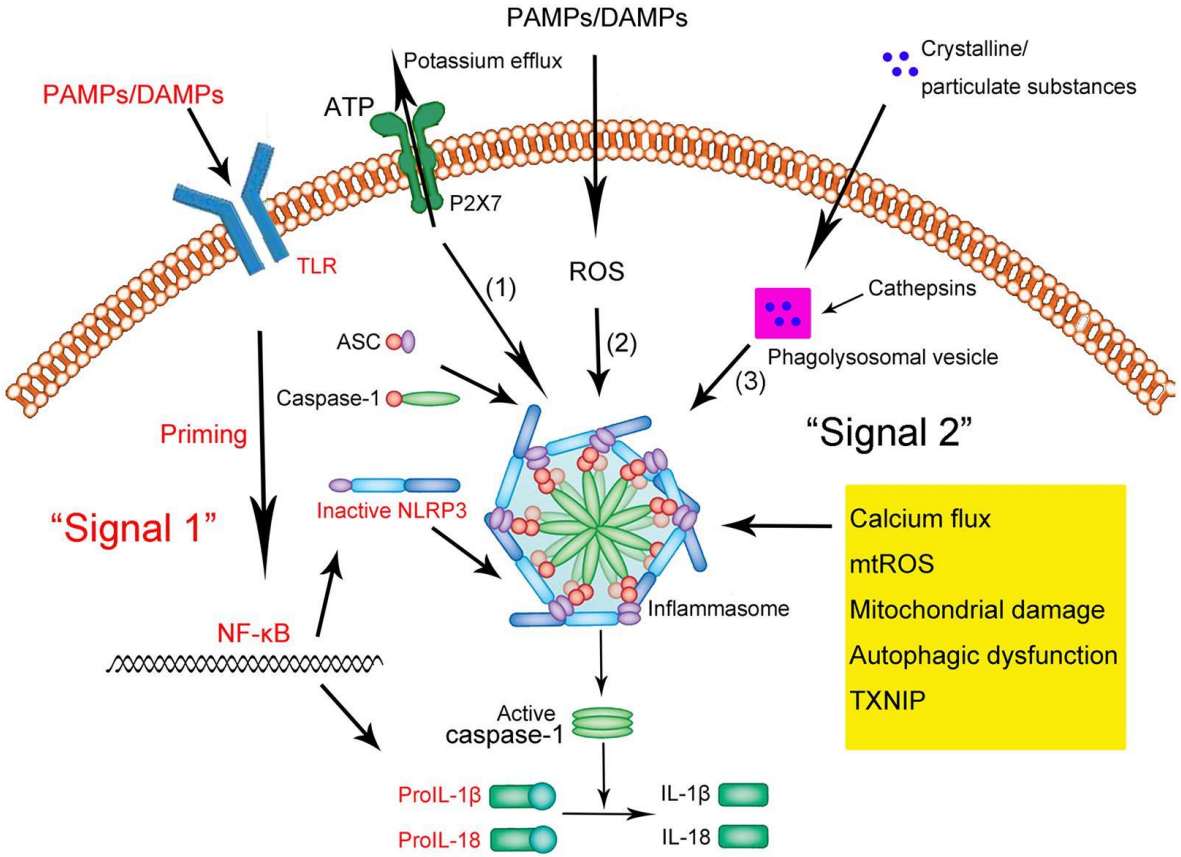
The NEW ENGLAND JOURNAL of MEDICINE

Derek C. Angus, M.D., M.P.H., and Tom van der Poll, M.D., Ph.D.

Denning et al. DAMPs, NETs, and Sepsis

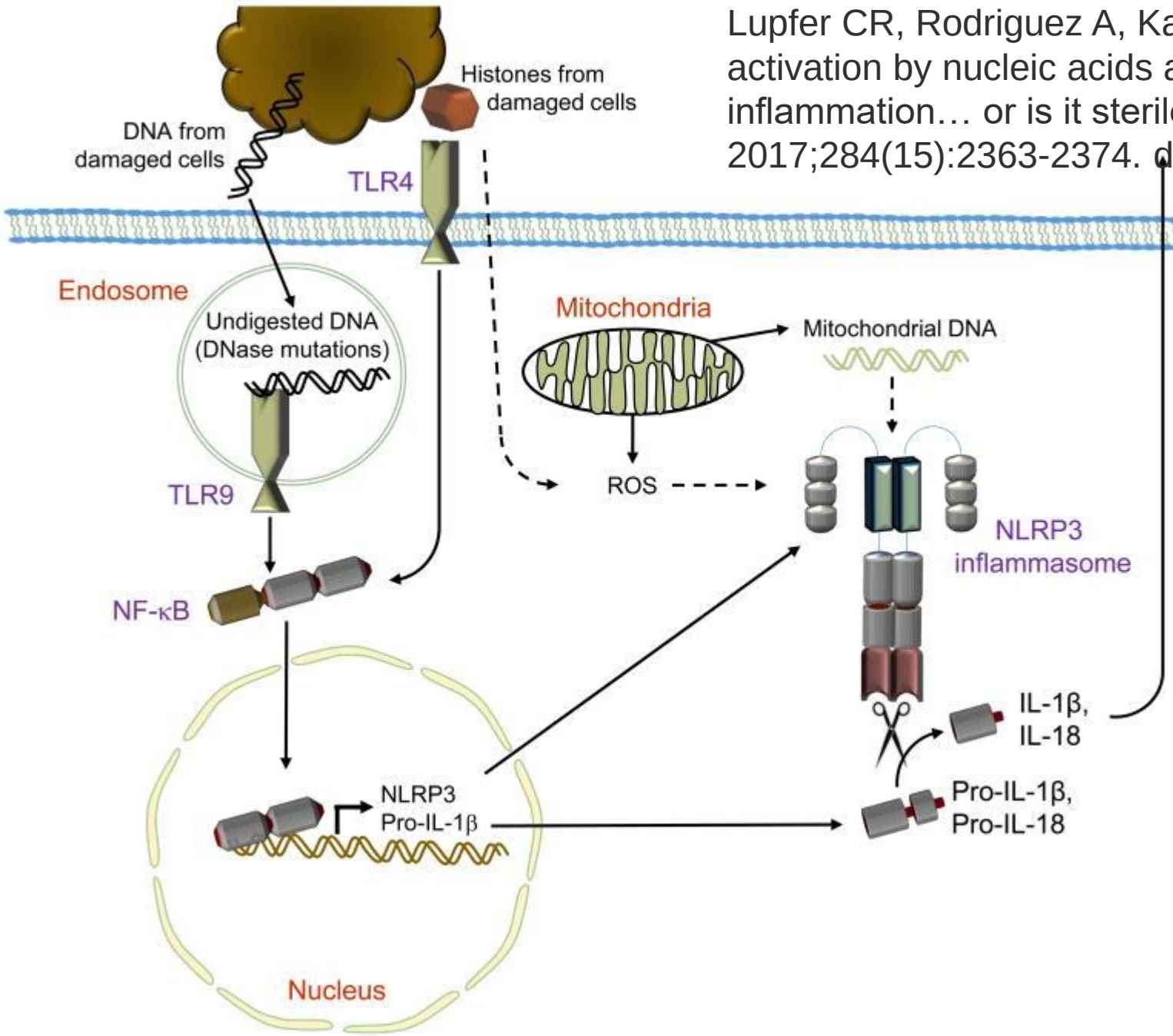
FIGURE 1 | Cross talks between DAMPs and NETs in sepsis. Sepsis or hypoxia activates immune reactive cells, including macrophages, and neutrophils. In bacterial sepsis, PAMPs interact with PRR on macrophages to activate NF-KB, leading to increased expression of DAMPs (HMGB1, CIRP, H3) at transcriptional and translational levels. These intracellular DAMPs are then released extracellularly through different mechanisms, such as inflammasome-mediated GSDMD activation, which causes increased membrane pore formation to release intracellular DAMPs, or pyroptosis-, necroptosis-, or exosome-mediated pathways. These DAMPs can in turn recognize PRR on surrounding neutrophils and activate PAD4, GSDMD to promote NET formation. NETs components such as H3, MPO, or DNA can further activate immune cells and endothelial cells to release increased levels of DAMPs to augment the inflammatory cascade. In epithelial cells, extracellular histones derived from NETs promote cell/tissue injury, resulting in increased severity of ALI. DAMPs, damage-associated molecular patterns (DAMPs); NETs, neutrophil extracellular traps; PAMPs, pathogen-associated molecular patterns; PRR, pattern recognizing receptors; GSDMD, gasdermin D; HMGB1, high mobility group box 1; CIRP, cold-inducible RNA-binding protein; PAD4, peptidoglycan arginine deiminase 4; ALI, acute lung injury





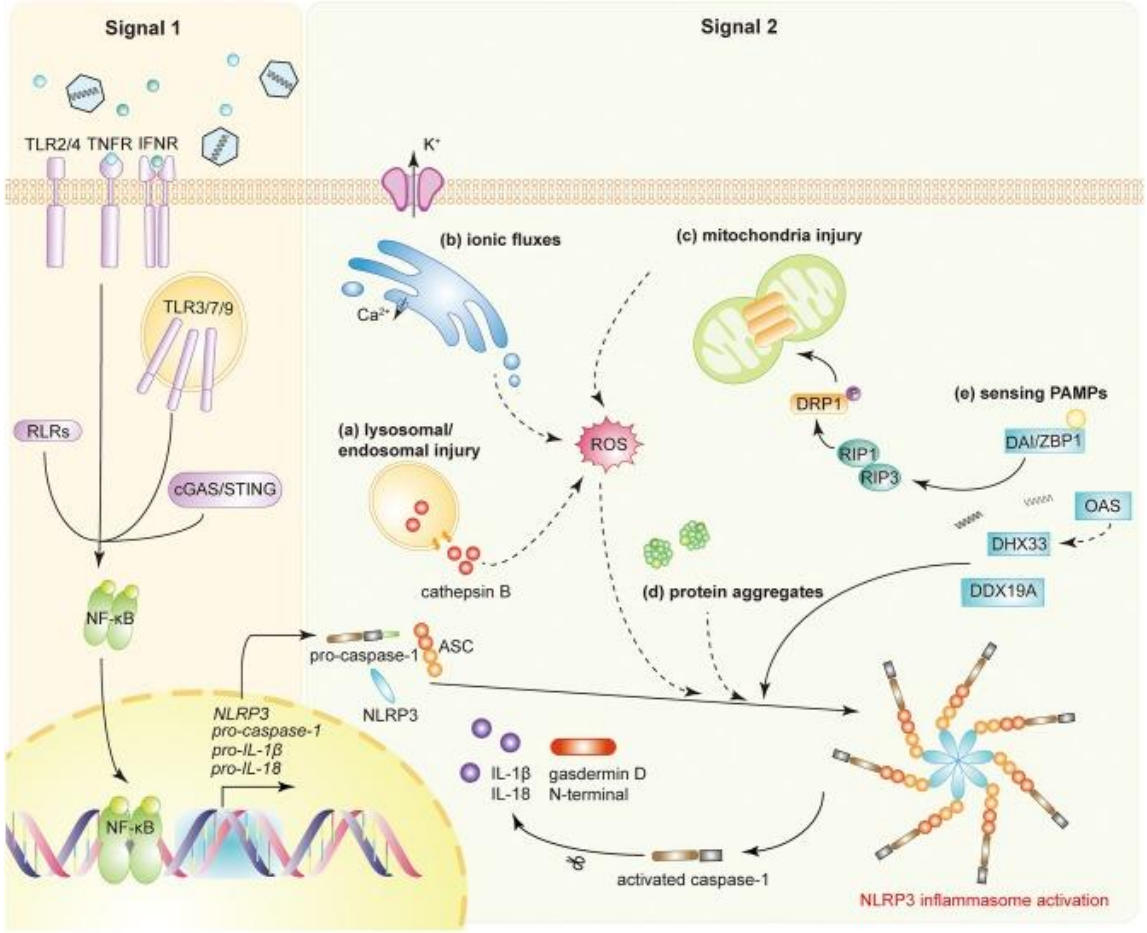
Schematic illustration of the NLRP3 inflammasome activation. Upon exposure to pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs), Toll-like receptors (TLRs) are phosphorylated and subsequently activate NF-κB. In the nucleus, NF-κB promotes the transcription of NLRP3, proIL-1β, and proIL-18, which, after translation, remain in the cytoplasm in inactive forms. Thus, this signal (depicted in red as "Signal 1") is a priming event. A subsequent stimulus (shown as "Signal 2" in black) activates the NLRP3 inflammasome by facilitating the oligomerization of inactive NLRP3, apoptosis-associated speck-like protein (ASC), and procaspase-1. This complex, in turn, catalyzes the conversion of procaspase-1 to caspase-1, which contributes to the production and secretion of the mature IL-1β and IL-18. Three models have been proposed to describe the second step of inflammasome activation: (1) Extracellular ATP can induce K^+/potassium efflux through a purogenic P2X7-dependent pore, which, leads to the assembly and activation of the NLRP3 inflammasome. Calcium flux is also involved in this process. (2) PAMPs and DAMPs trigger the generation of ROS that promote the assembly and activation of the NLRP3 inflammasome. (3) Phagocytosed environmental irritants form intracellular crystalline or particulate structures leading to lysosomal rupture (magenta box) and release of lysosomal contents like cathepsin B. These induce NLRP3 inflammasome assembly and activation. In addition, other factors and mechanisms have been implicated in the assembly and activation of the NLRP3 inflammasome, including mitochondrial damage, autophagic dysfunction, and thioredoxin-interacting protein (TXNIP).

Shao B-Z, Xu Z-Q, Han B-Z, Su D-F, Liu C. NLRP3 inflammasome and its inhibitors: a review. *Front Pharmacol*. 2015;6. doi:10.3389/fphar.2015.00262



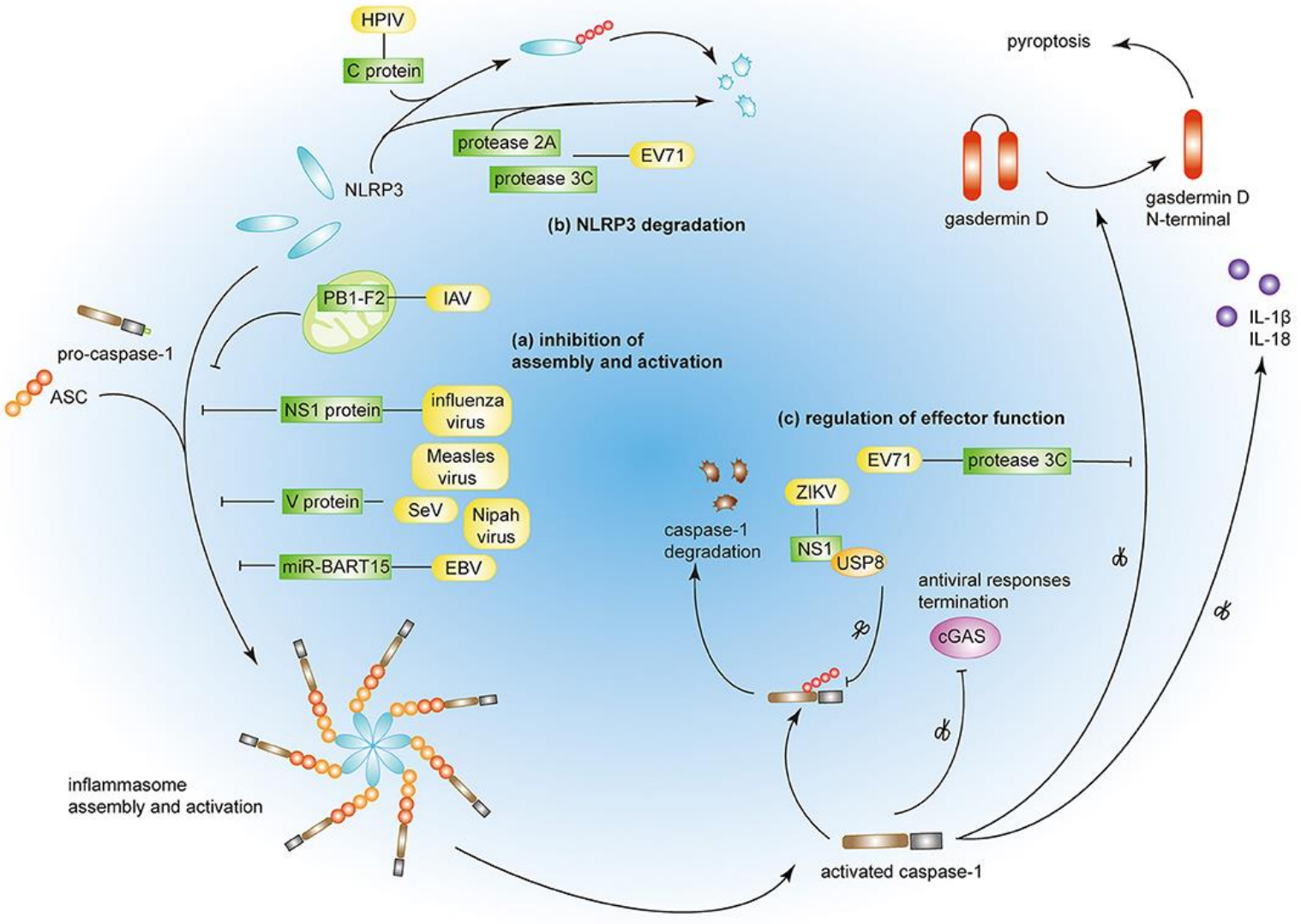
Lupfer CR, Rodriguez A, Kanneganti TD. Inflammasome activation by nucleic acids and nucleosomes in sterile inflammation… or is it sterile?. *FEBS J*. 2017;284(15):2363-2374. doi:10.1111/febs.14076

NLRP3 activation by mitochondrial DNA and histonesNLRP3 inflammasome activation requires two signals in the form of priming the expression of NLRP3 and pro-IL-1β as well as a second damage signal for NLRP3 activation. During sterile inflammation, DNA and histones derived from damaged host cells can prime the NLRP3 inflammasome through TLR-9 or TLR4-mediated increases in NLRP3 and pro-IL-1β expression. The NLRP3 inflammasome can be activated by the presence of cytoplasmic nucleic acids. During sterile inflammation, mitochondrial damage releases mitochondrial DNA (mtDNA) into the cytoplasm where it activates NLRP3. The mechanism of mtDNA-mediated NLRP3 activation is not clear but likely hinges on unknown adaptor proteins or the common signals of potassium efflux or reactive oxygen species generation. NLRP3 activation can also result from histones' ability to damage the cell membrane, but the exact mechanisms are unknown.



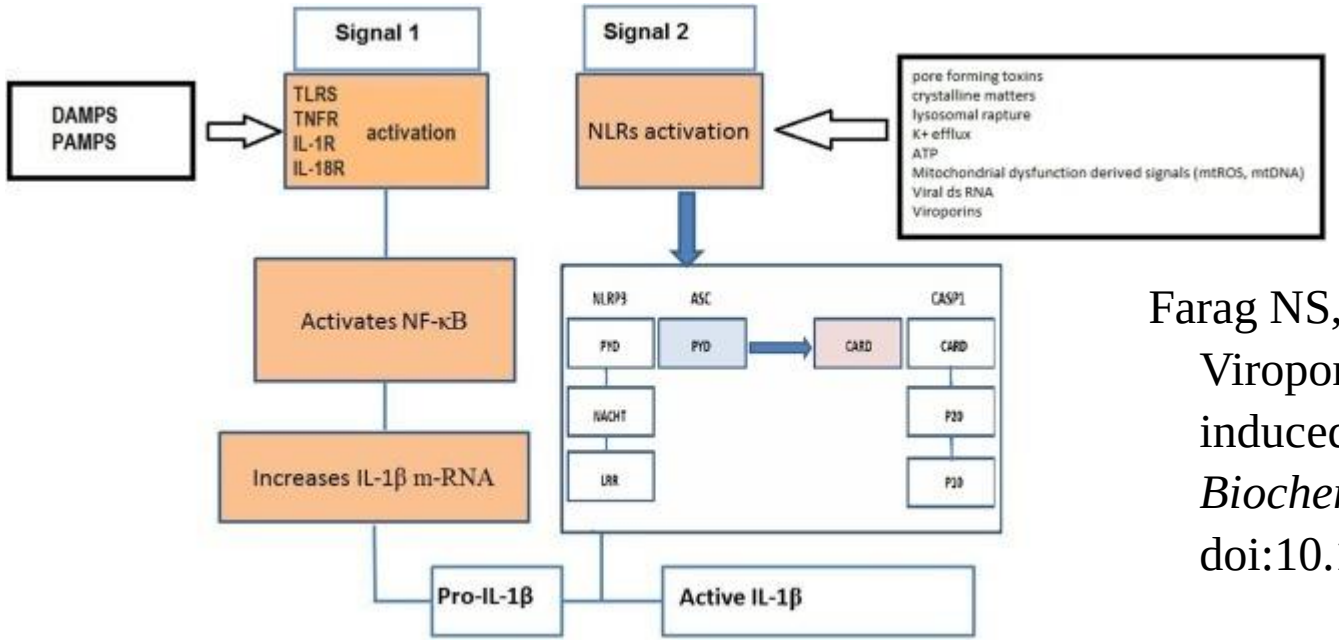
Zhao C, Zhao W. NLRP3 Inflammasome-A Key Player in Antiviral Responses. *Front Immunol*. 2020;11:211. Published 2020 Feb 18. doi:10.3389/fimmu.2020.00211

NLRP3 inflammasome activation during viral infections. Activation of the NLRP3 inflammasome requires two signals. Signal 1 (priming signal): the activation of PRRs, TNFR, or IFNR induces NF-κB activation, triggers the transcription of NLRP3, pro-caspase-1, pro-IL-1β, and pro-IL-18. Signal 2 (activation signal): multiple DAMPs and PAMPs induce NLRP3 inflammasome assembly and activation. DAMPs include **(a)** lysosomal or endosomal injury, **(b)** aberrant ionic fluxes, **(c)** mitochondrial injury, and **(d)** protein aggregates. **(e)** With the help of DAI/ZBP1, DHX33, OAS, or DDX19A, NLRP3 is activated by sensing viral proteins and RNA. NLRP3 inflammasome activation leads to the auto-cleavage of pro-caspase-1. Caspase-1 then mediates the proteolytic process of pro-IL-1β, pro-IL-18, and gasdermin D (GSDMD).



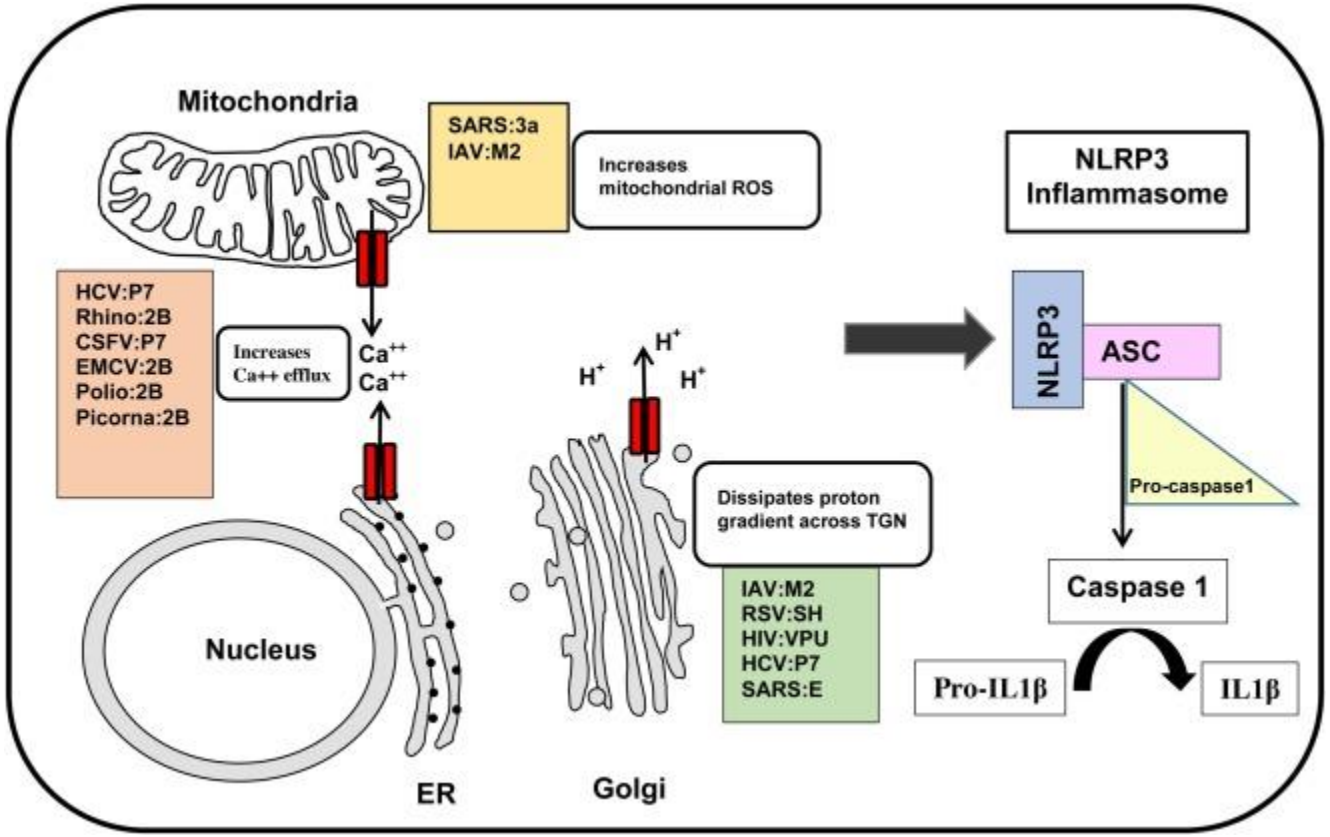
Viral immune evasion strategies by targeting the NLRP3 inflammasome. (a) Influenza virus NS1 protein, measles virus, SeV, and Nipah virus V proteins prevent NRLP3 inflammasome assembly. PB1-F2 of IAV and miR-BART15 of EBV inhibit NLRP3 inflammasome activation. (b) EV71 proteases 2A and 3C and HPIV C protein induce NLRP3 protein degradation. (c) EV71 protease 3C and ZIKV NS1 protein modulate the effector function of the NLRP3 inflammasome by targeting GSDMD and caspase-1, respectively.

Zhao C, Zhao W. NLRP3 Inflammasome-A Key Player in Antiviral Responses. *Front Immunol*. 2020;11:211. Published 2020 Feb 18. doi:10.3389/fimmu.2020.00211



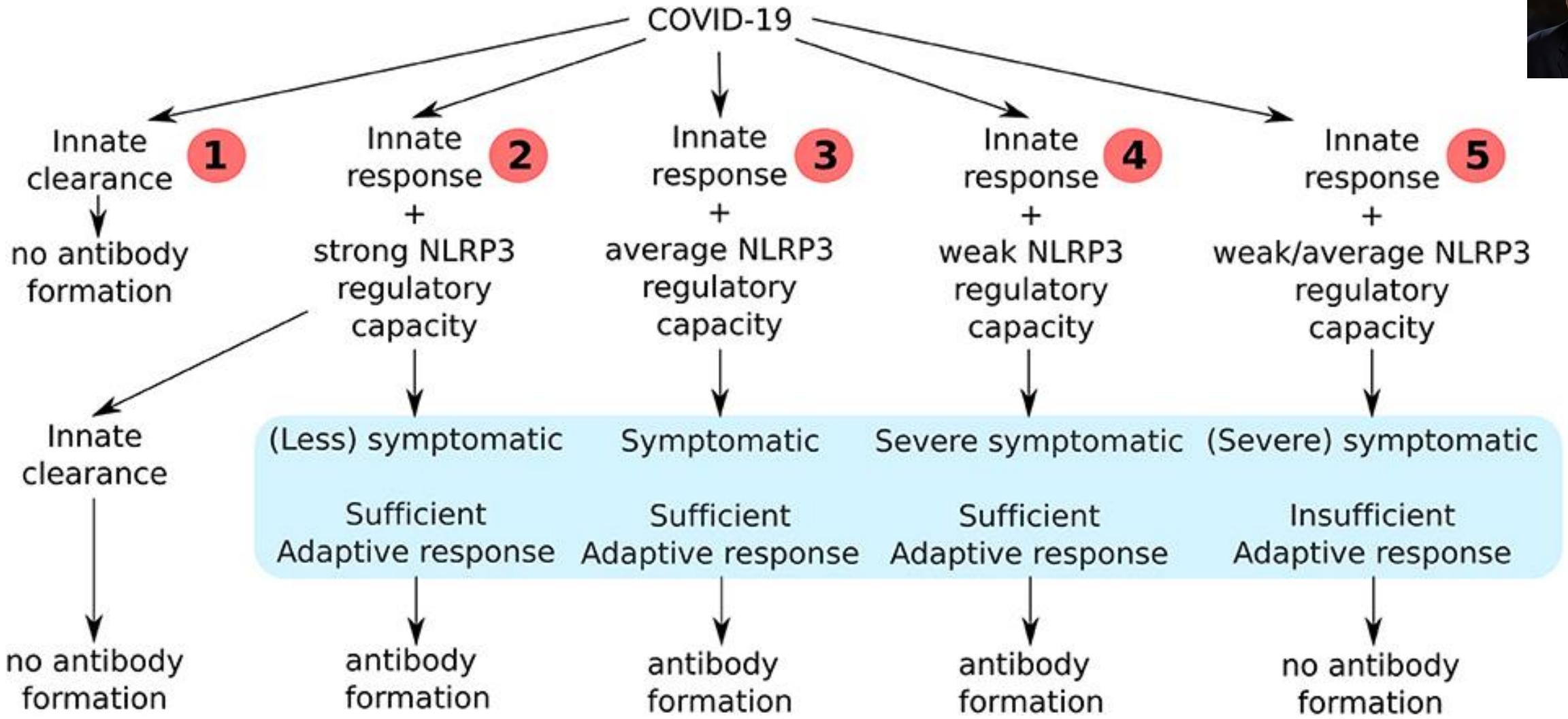
Farag NS, Breitinger U, Breitinger HG, El Azizi MA. Viroporins and inflammasomes: A key to understand virus-induced inflammation. *The International Journal of Biochemistry & Cell Biology*. 2020;122:105738. doi:10.1016/j.biocel.2020.105738

Signals required for activation and release of IL-1β and IL-18. The first signal can be triggered by various PAMPs or DAMPS recognized by Toll-like receptor (TLR), IL-1 receptor (IL-1R), IL-18 receptor (IL-18R) or tumor necrosis factor receptor (TNFR). The activation of such receptors leads to the activation of NF-κB which induces the synthesis of pro-IL-1β. The second signal is provided by the activation of the inflammasome complex and caspase-1 leading to IL-1β processing. NLRP3 inflammasome detects signs of metabolic stress, including elevated extracellular glucose, monosodium urate (MSU) crystals, ATP and changes in the intracellular ion composition caused by viral encoded ion channels; viroporins activity and certain bacterial toxins, such as nigericin and maitotoxi. NLRP3 oligomerization leads to PYD domain clustering and presentation for homotypic interaction with the PYD- and CARD-containing adaptor ASC, whose CARD domain in turn recruits the CARD of procaspase-1. Procaspase-1 clustering permits autocleavage and formation of the active caspase-1 p10/p20 tetramer. Caspase-1 is activated within the inflammasome multiprotein complex through interaction with ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD), a bipartite adapter protein that bridges NLRs and caspase-1.

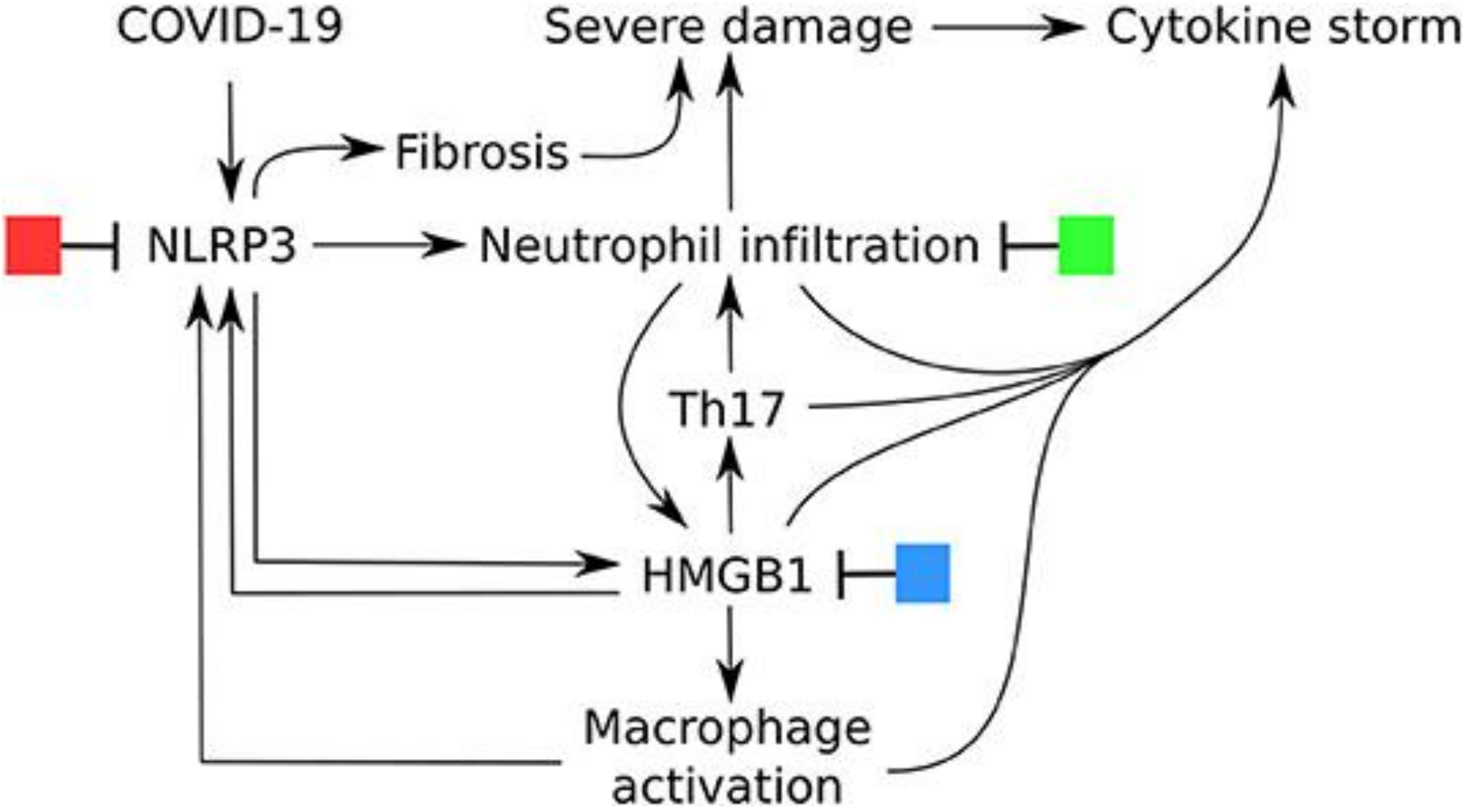


Farag NS, Breitinger U, Breitinger HG, El Azizi MA. Viroporins and inflammasomes: A key to understand virus-induced inflammation. *The International Journal of Biochemistry & Cell Biology*. 2020;122:105738. doi:10.1016/j.biocel.2020.105738

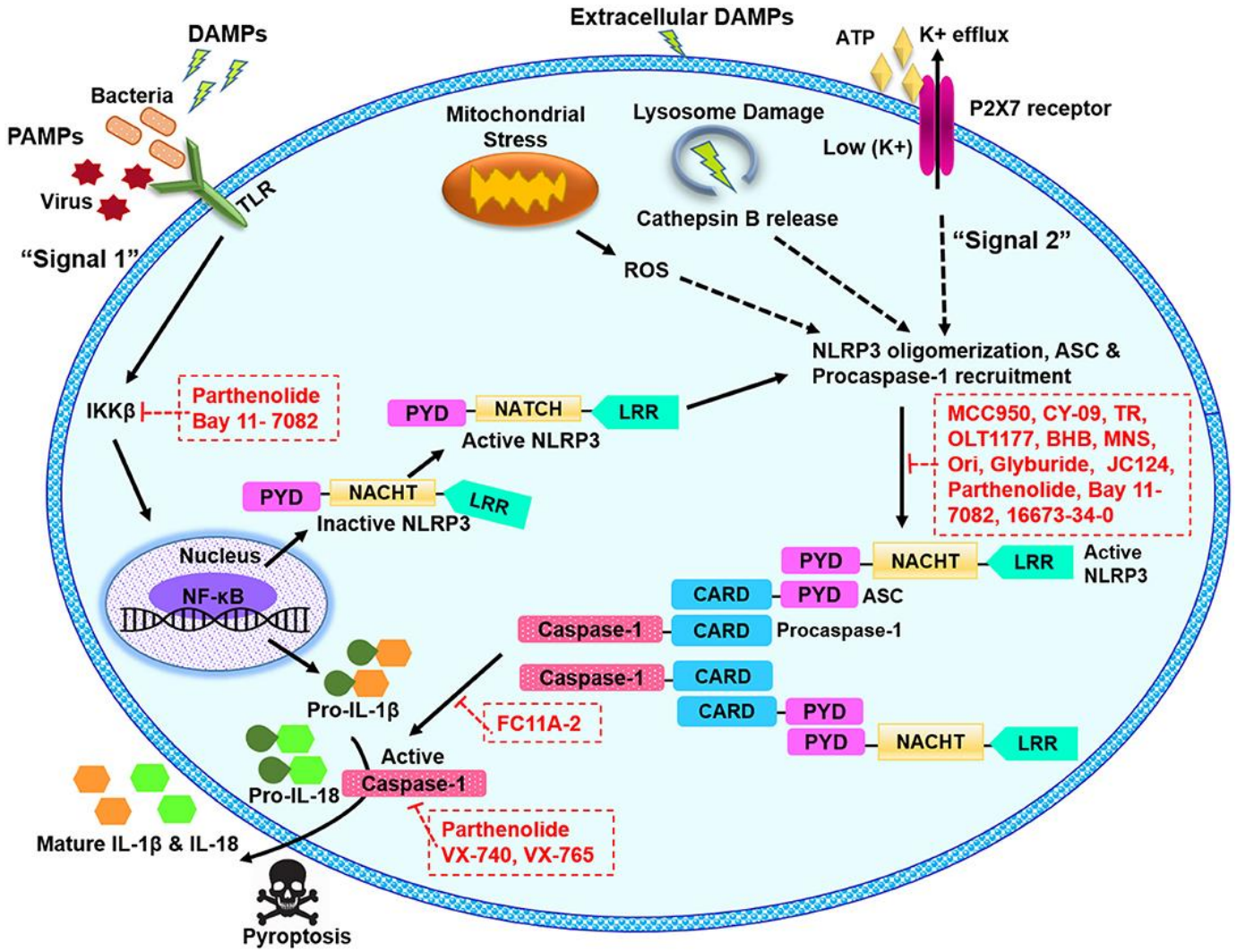
Viroporins activity and activation of inflammasomes. Viroporins activities can be clustered into three main groups that have been linked to activation of NLRP3 inflammasomes. The first group of viroporins pumps protons and dissipates proton gradient across trans-golgi network, eg.M2 of influenza A virus. The second group manipulates Ca^{2+} homeostasis, stimulating Ca^{2+} flux from intracellular storages to the cytosol providing the second signal for NLRP3 activation and IL-1β production such as 2B of polio and rhino virus. The third group increases mitochondrial stress and affects ROS production such as 3a of corona virus.



van den Berg DF, te Velde AA. Severe COVID-19: NLRP3 Inflammasome Dysregulated. *Front Immunol*. 2020;11. doi:10.3389/fimmu.2020.01580



van den Berg DF, te Velde AA. Severe COVID-19: NLRP3 Inflammasome Dysregulated. *Front Immunol.* 2020;11. doi:10.3389/fimmu.2020.01580

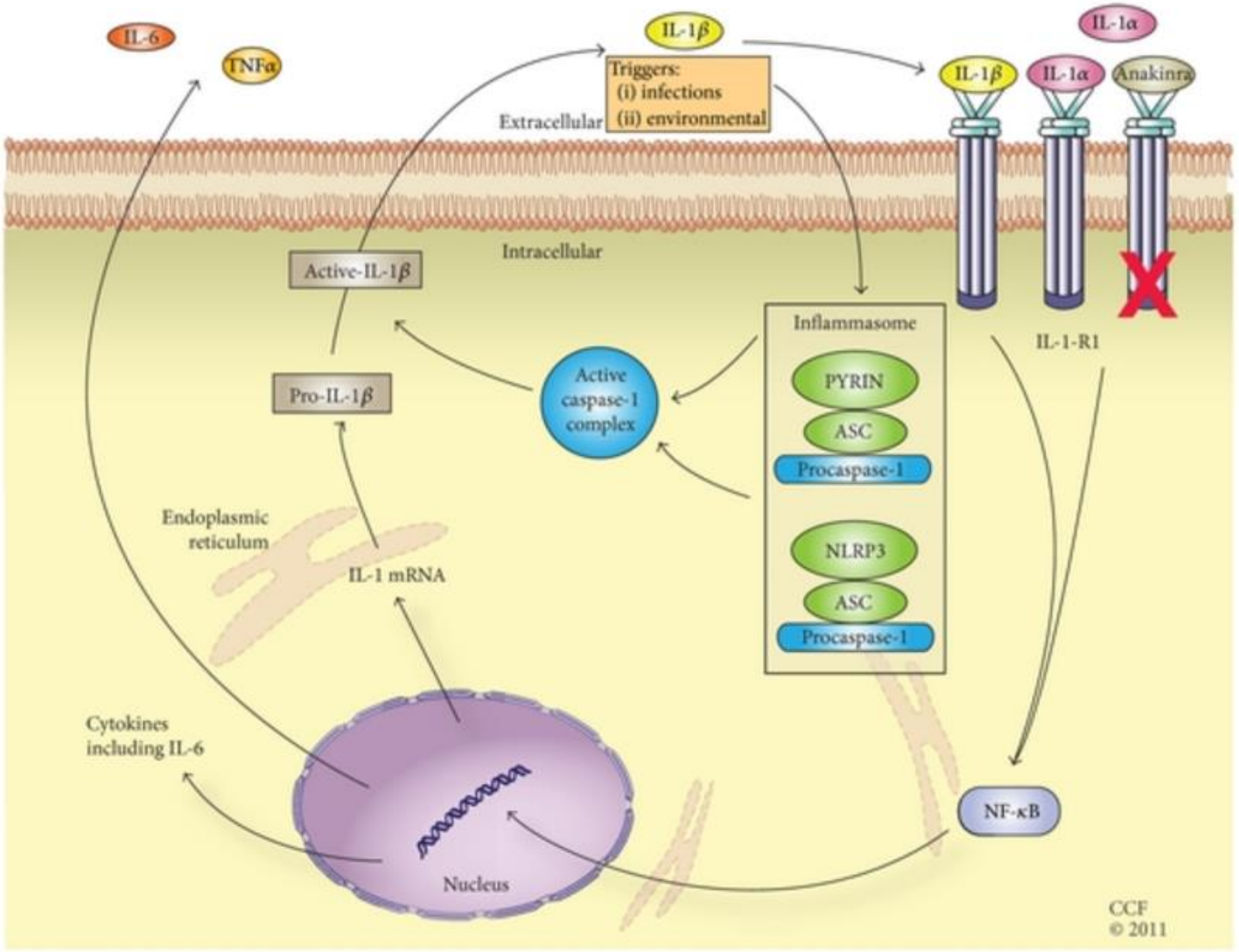


Zahid A, Li B, Kombe AJK, Jin T, Tao J.

Pharmacological Inhibitors of the NLRP3 Inflammasome.

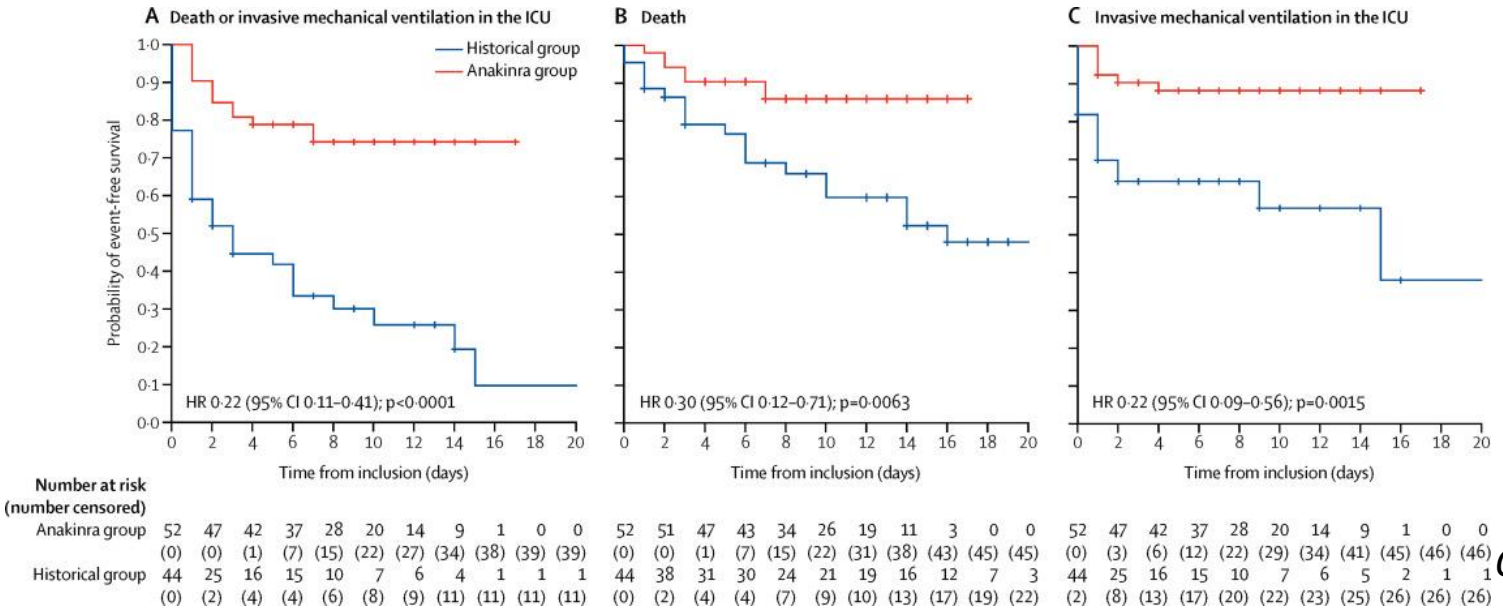
Front Immunol. 2019;10. doi:10.3389/fimmu.2019.02538

Schematic illustration of NLRP3 inflammasome pathway and potential blockade sites of various pharmacological inhibitors. The signal 1 or the priming signal is mediated by pathogenic PAMPs from bacteria or virus, or sterile DAMPs resulting in NF-κB-dependent upregulation of NLRP3 and pro-IL-1β expression. The signal 2 or activation signal mediated by numerous PAMP or DAMP stimulation, promotes the NLRP3 oligomerization, and recruitment of ASC and pro-caspase-1, leading to the activation of NLRP3 inflammasome complex. NLRP3 can be activated in response to extracellular ATP and K^+ efflux through the ATP-gated P2X7 channel, in response to cathepsin B release from damaged lysosomes or in response to reactive oxygen species (ROS) released from damaged mitochondria. NLRP3 inflammasome activation results in active caspase-1, which cleaves the proforms of IL-1β and IL-18 into their mature forms. ASC, apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain; ATP, adenosine triphosphate; BHB, β-Hydroxybutyrate; CARD, caspase recruitment domain; DAMPS, danger or damage associated molecular patterns; IL, interleukin; LRR, leucine-rich repeat; MNS, methylenedioxy-β-nitrostyrene; NACHT, central nucleotide-binding and oligomerization; NF-κB, nuclear factor kappa B; Ori, oridonin; P2X7, P2X purinergic receptor 7; PAMPS, pathogen associated molecular patterns; PYD, pyrin domain; ROS, reactive oxygen species; TLR, toll-like receptor; TR, tranilast.



Mechanism of action of anakinra. Both IL-1α and IL-1β act through IL-1 receptor 1 to stimulate the production of inflammatory cytokines and TNFα that lead to the inflammatory cascade. The inflammasome is a complex of distinct proteins which together convert inactive prointerleukin-1β to active IL-1β. Environmental and infectious triggers can mediate the formation of the inflammasome. Anakinra blocks IL-1 receptor 1, antagonizing the effects of both IL-1α and IL-1β. ASC: Apoptosis associated speck-like protein containing caspase activation and recruitment domain, IL: interleukin, IL-1-R1: interleukin-1 receptor 1, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, NLRP3: NOD-Like Receptor containing pyrin domain 3.

Baskar S, Klein A, Zeft A. The Use of IL-1 Receptor Antagonist (Anakinra) in Idiopathic Recurrent Pericarditis: A Narrative Review. *Cardiology Research and Practice*. 2016;2016:1-6. doi:10.1155/2016/7840724

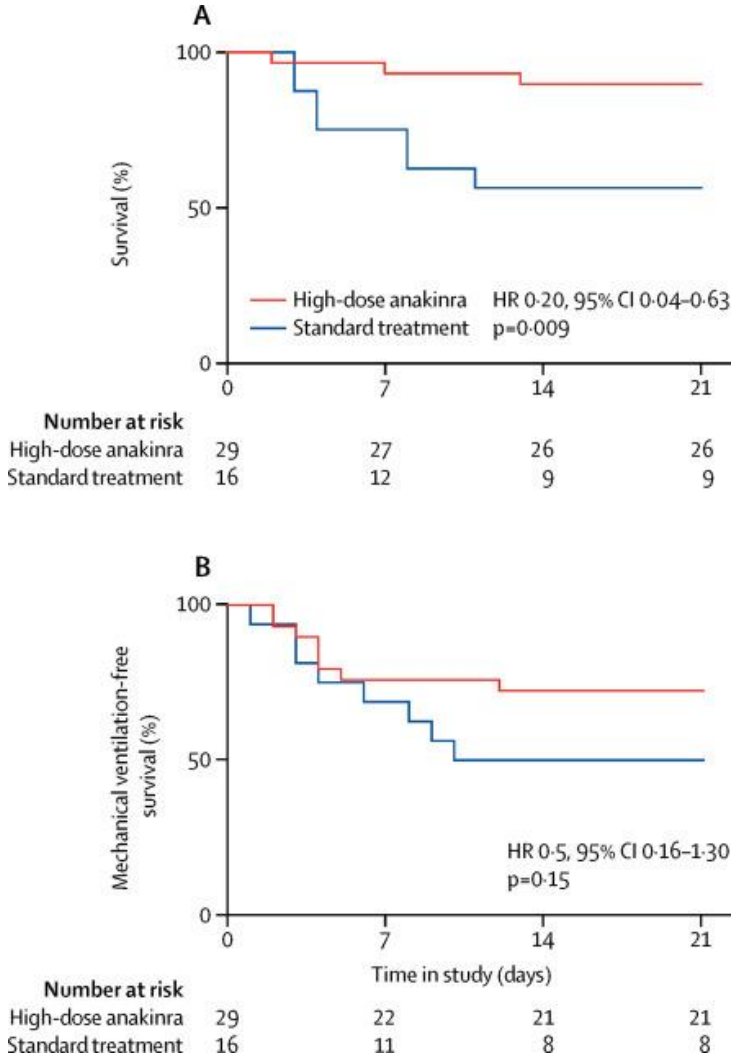


Kaplan-Meier cumulative estimates of probability of death or invasive mechanical ventilation in the ICU (A), death (B) and invasive mechanical ventilation in the ICU (C) in the anakinra group compared with the historical group

Anakinra for severe forms of COVID-19: a cohort study

Thomas Huet, MD, Hélène Beaussier, Pharm D, Olivier Voisin, MD, Stéphane Jouveshomme, MD, Gaëlle Dauriat, MD, Isabelle Lazareth, MD, Emmanuelle Sacco, PhD, Jean-Marc Naccache, MD, Yvonnick Bézie, Pharm D, Sophie Laplanche, Pharm D, Alice Le Berre, MD, Jérôme Le Pavec, MD, Sergio Salmeron, MD, Prof Joseph Emmerich, MD, Prof Jean-Jacques Mourad, MD, Prof Gilles Chatellier, MD, Gilles Hayem, MD

The Lancet Rheumatology
Volume 2 Issue 7 Pages e393-e400 (July 2020)
DOI: 10.1016/S2665-9913(20)30164-8



Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study

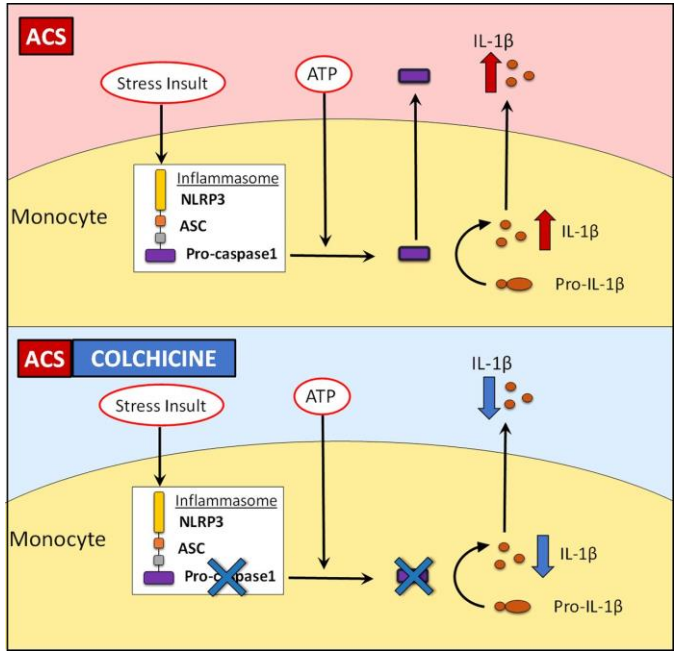
Giulio Cavalli, MD, Giacomo De Luca, MD, Corrado Campochiaro, MD, Emanuel Della-Torre, MD, Marco Ripa, MD, Diana Canetti, MD, Chiara Oltolini, MD, Barbara Castiglioni, MD, Chiara Tassan Din, MD, Nicola Boffini, MD, Alessandro Tomelleri, MD, Nicola Farina, MD, Annalisa Ruggeri, MD, Prof Patrizia Rovere-Querini, MD, Giuseppe Di Lucca, MD, Sabina Martinenghi, MD, Raffaella Scotti, MD, Moreno Tresoldi, MD, Prof Fabio Ciceri, MD, Prof Giovanni Landoni, MD, Prof Alberto Zangrillo, MD, Paolo Scarpellini, MD, Prof Lorenzo Dagna, MD

The Lancet Rheumatology
Volume 2 Issue 6 Pages e325-e331 (June 2020)
DOI: 10.1016/S2665-9913(20)30127-2

Survival and mechanical ventilation-free survival at 21 days Plots show survival (A) and mechanical ventilation-free survival (B) at 21 days of patients with COVID-19, ARDS, and hyperinflammation managed outside the intensive care unit with CPAP and high-dose (n=16). For mechanical ventilation-free survival (B), death and mechanical ventilation were considered equivalent to treatment failure. COVID-19=coronavirus disease 2019. ARDS=acute respiratory distress syndrome. CPAP=continuous positive airway pressure. HR=hazard ratio. anakinra (n=29) or receiving CPAP and standard treatment only

Clin Sci (Lond) Volume 130 Issue 14 2016 1237-1246 10.1042/CS20160090

Figure 7



Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation

Table 1. Baseline Characteristics (continued)

Characteristic	No. (%)		Difference (95% CI)[a]
	Control group (n = 50)	Colchicine group (n = 55)	
Aspartate aminotransferase, median (IQR), U/L	34 (23 to 52)	30 (21 to 42)	4 (−2 to 10)
Alanine aminotransferase, median (IQR), U/L	35 (18 to 49)	25 (17 to 43)	4.5 (−3 to 12)
Lactate dehydrogenase, median (IQR), U/L	280 (224 to 405)	251 (196 to 350)	30 (−13 to 73)
Creatine phosphokinase, median (IQR), U/L	80 (55 to 133)	80 (49 to 164)	0 (−23 to 23)
C-reactive protein, median (IQR), mg/dL	4.0 (1.2 to 9.5)	3.6 (1.0 to 6.7)	9.5 (−6 to 25)
High-sensitivity cardiac troponin, median (IQR), ng/mL	0.007 (0.0035 to 0.0185)	0.008 (0.004 to 0.0123)	0.0005 (−2.0 to 3.0)
D-dimer, median (IQR), µg/ml	0.60 (0.40 to 1.01)	0.52 (0.28 to 0.94)	0.0815 (−0.0082 to 0.0245)

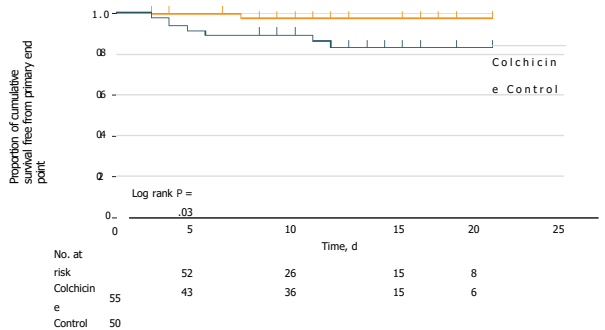
Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); COVID-19, coronavirus disease 2019; D-dimer, dimerized plasma fragment D; GFR, glomerular filtration rate; IQR, interquartile range; NA, not applicable; Pao_2, partial pressure of oxygen.

SI conversion factors: To convert alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, and lactate dehydrogenase to microkatals per liter, multiply by 0.0167; C-reactive protein to milligrams per liter, multiply by 10.0; D-dimer to nanomoles per liter, multiply 5.476; high-sensitivity cardiac troponin to micrograms per liter, multiply by 1.0; glucose to millimoles per liter, multiply by 0.0555; hemoglobin to grams per liter, multiply by 10.0; lymphocyte and white blood cell count to $\times 10^9$ per liter, multiply by 0.001; platelet count to $\times 10^9$ per liter, multiply by 1.0; and potassium and sodium to millimoles per liter, multiply by 1.0.

[a] Values in 95% CI of the difference represent rate differences for categorical variables and differences of the medians (Hodges-Lehmann estimate) for continuous variables.

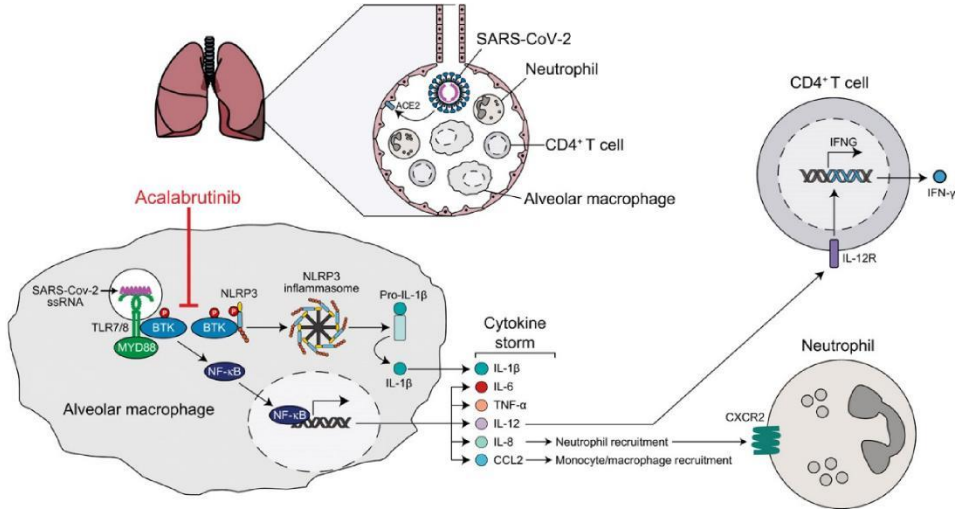
[b] Darunavir or cobicistat, remdesevir, and human interferon a1b were not used for any patient.

Figure 2. Kaplan-Meier Curves for Survival From the Primary Clinical End Point

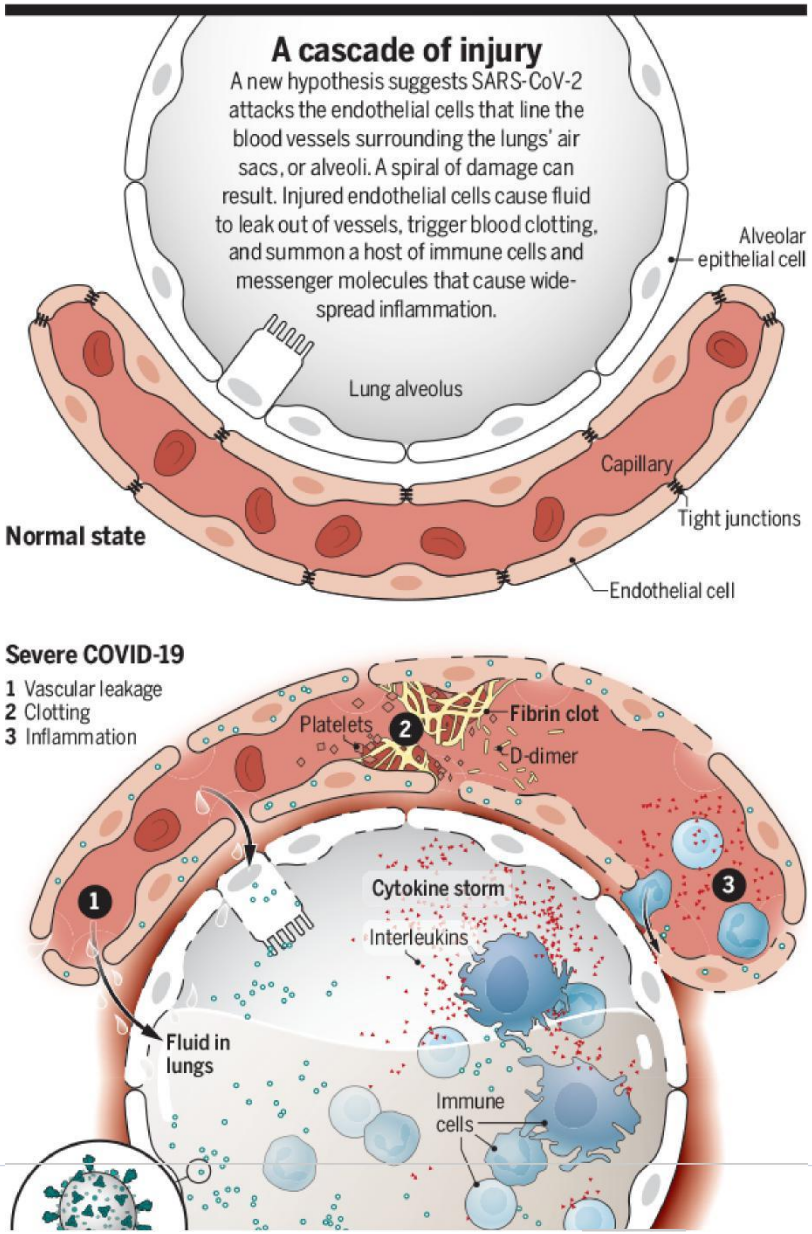


0.11 (95% CI, 0.01-0.96; *P* = .046). Kaplan-Meier event-free survival curves are depicted in **Figure 2**. Cumulative event-free 10-day survival was 83% vs 97% in the control and colchicine groups, respectively (Gehan statistic, 4.9; *P* = .03). Of the 7 patients who met the primary clinical end point in the control group, 1 (14.3%) needed noninvasive mechanical ventilation (bilevel positive airway pressure), 5 (71.4%) were intubated and ventilated mechanically (3 [42.9%] died shortly after intubation), and 1 (14.3%) died suddenly in the ward of cardiorespiratory arrest. The patient in the colchicine group who met the end point needed invasive mechanical ventilation and died subsequently in the intensive care unit.

The primary outcome measure of the biochemical phase, hs cTn concentration, increased from a median (IQR) of 0.073 (0.037 to 0.0149) ng/mL at baseline to a peak median (IQR) value of 0.0084

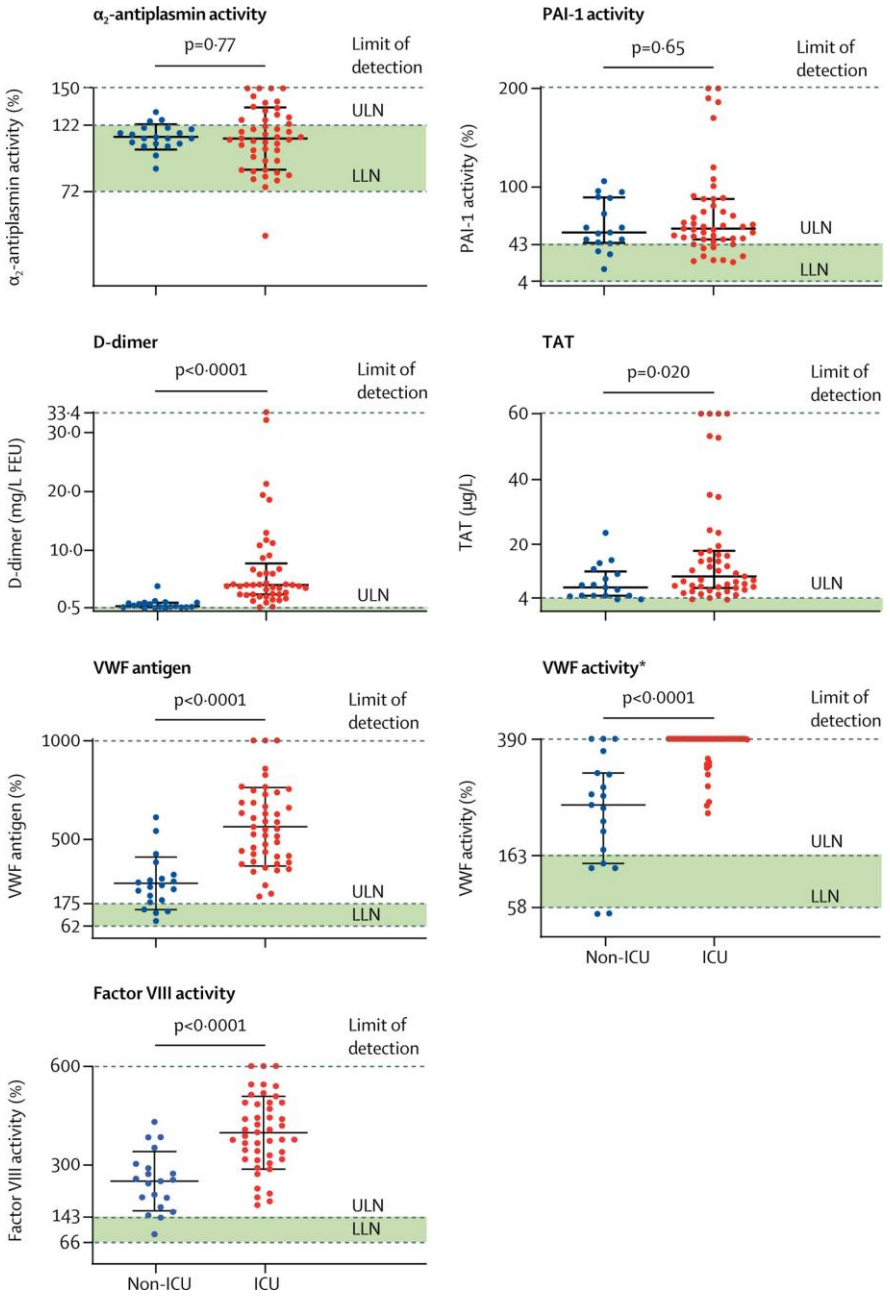


•**Fig. 1. Model of BTK-dependent hyper-inflammation in severe COVID-19.** Binding of SARS-CoV2 to ACE2 on respiratory epithelia initiates infection. Hypothetically, macrophages may participate in the COVID-19 inflammatory response by phagocytic uptake of viral particles or cellular debris containing viral single-stranded RNA (ssRNA). ssRNA can bind to TLR7 and TLR8, thereby recruiting and activating BTK and MYD88 (*51*, *52*). Downstream of TLR engagement, BTK-dependent NF-κB activation results in the production of pro-inflammatory cytokines and chemokines (*53*), a "cytokine storm" that could increase the recruitment of monocytes/macrophages and neutrophils during the late phase of severe COVID-19 infection. BTK inhibitors such as acalabrutinib block TLR-dependent NF-κB activation in macrophages (*20*, *21*), thereby dampening the production of pro-inflammatory mediators, as occurs in an influenza-induced lung injury model (*27*). During severe COVID-19, the heightened levels of IL-1₁₃ in several COVID-19 patients (*11*, *12*) indicates the formation of an NLRP3 inflammasome that converts pro-IL-1₁₃ to mature IL-1₁₃ (*54*). BTK binds to and phosphorylates NLRP3, thereby promoting its oligomerization and assembly into an inflammasome (*24–26*). BTK inhibitors such as acalabrutinib inhibit inflammasome-mediated production of IL-1₁₃, as observed in a model of influenza-induced lung injury (*27*). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; ACE2, angiotensin-converting enzyme 2; TLR, Toll-like receptor; MyD88, myeloid differentiation primary response 88; BTK, Bruton tyrosine kinase; NF-κB, nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; ASC, Apoptosis-associated speck-like protein containing a caspase recruitment domain; ORF3a, open reading frame 3a; IFN-γ, interferon gamma; IL, interleukin; IL-12R, IL-12 receptor; CCL2, C-C motif chemokine ligand 2; CXCL1, C-X-C motif chemokine ligand 1; CXCR2, C-X-C motif chemokine receptor 2.



6/5/2020 Science Magazine - June 5, 2020 - Blood vessel injury may spur disease's fatal second phase including interleukins, which raise local blood pressure and weaken cell junctions. Damage to the endothelial cells also exposes the membrane underneath them.

https://www.sciencemagazinedigital.org/sciencemagazine/05_june_2020

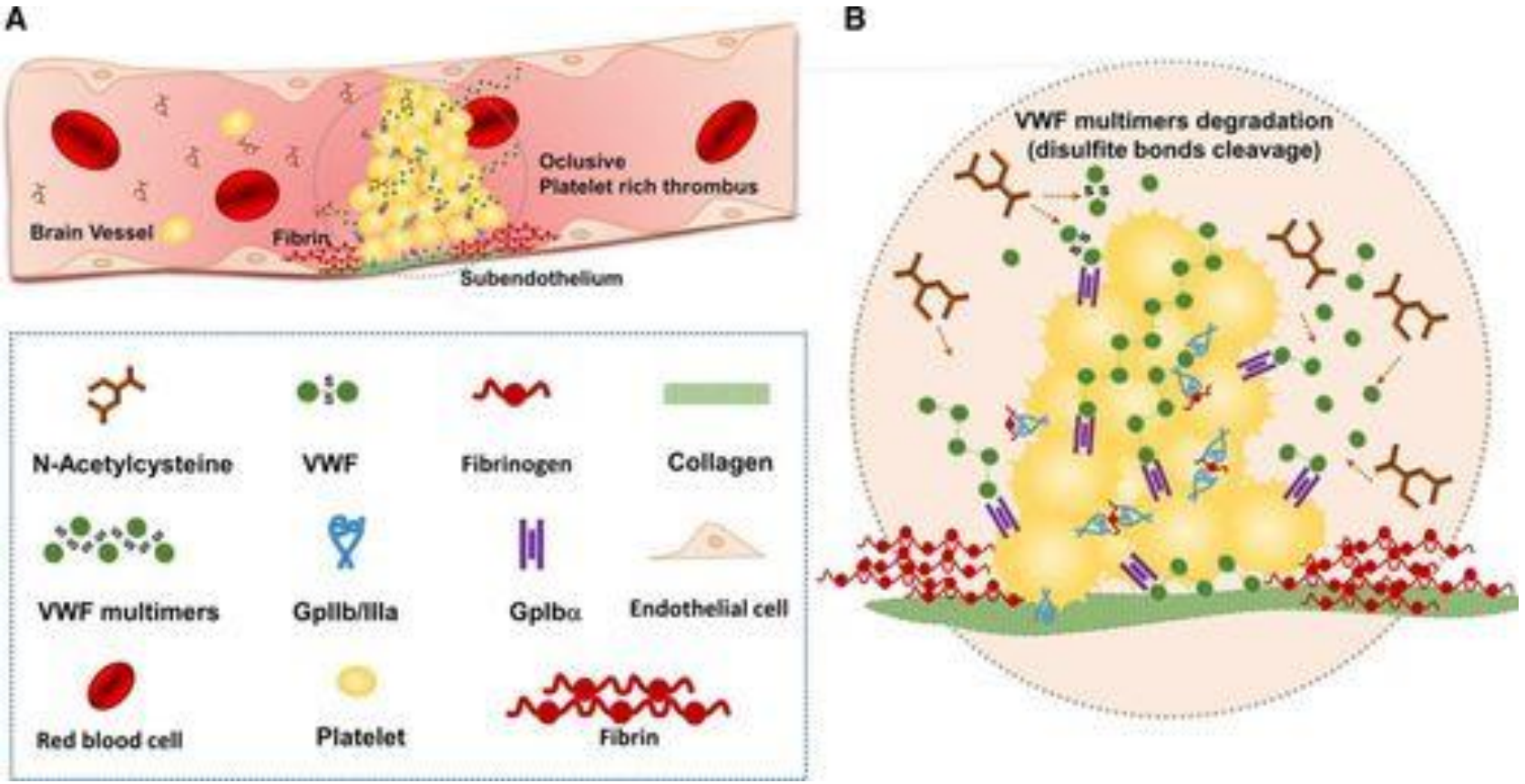


Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study

George Goshua, MD, Alexander B Pine, MD, Matthew L Meizlish, MPhil, C-Hong Chang, PhD, Hanming Zhang, PhD, Parveen Bahel, MS, Audrey Baluha, RN, Noffar Bar, MD, Robert D Bona, MD, Adrienne J Burns, PA-C, Charles S Dela Cruz, MD, Anne Dumont, RN, Stephanie Halene, MD, Prof John Hwa, MD, Jonathan Koff, MD, Hope Menninger, PA-C, Natalia Neparidze, MD, Christina Price, MD, Jonathan M Siner, MD, Christopher Tormey, MD, Prof Henry M Rinder, MD, Hyung J Chun, MD, Alfred I Lee, MD

The Lancet Haematology

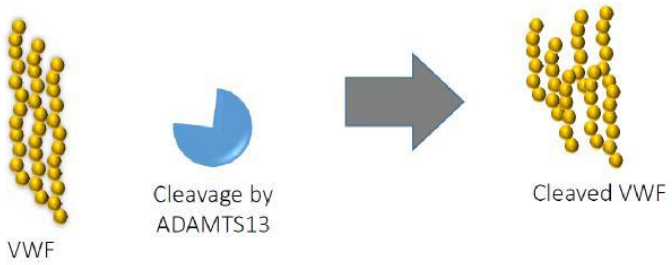
DOI: 10.1016/S2352-3026(20)30216-7



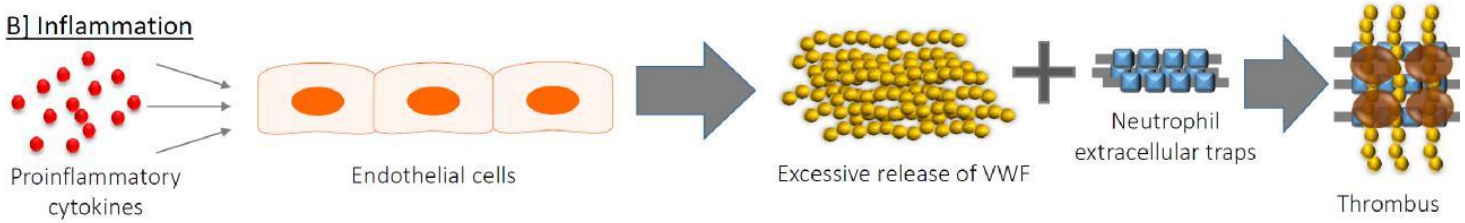
Schematic representation of the main findings.A, Schematic representation. **B**, *N*-acetylcysteine reduces the disulfide bonds of the von Willebrand Factor multimers that maintain platelets linked in arterial thrombi, thereby inducing thrombus dissolution. VWF indicates von Willebrand Factor.

Sara Martinez de Lizarrondo. Circulation. Potent Thrombolytic Effect of N-Acetylcysteine on Arterial Thrombi, Volume: 136, Issue: 7, Pages: 646-660, DOI: (10.1161/CIRCULATIONAHA.117.027290)

A] Hemostasis



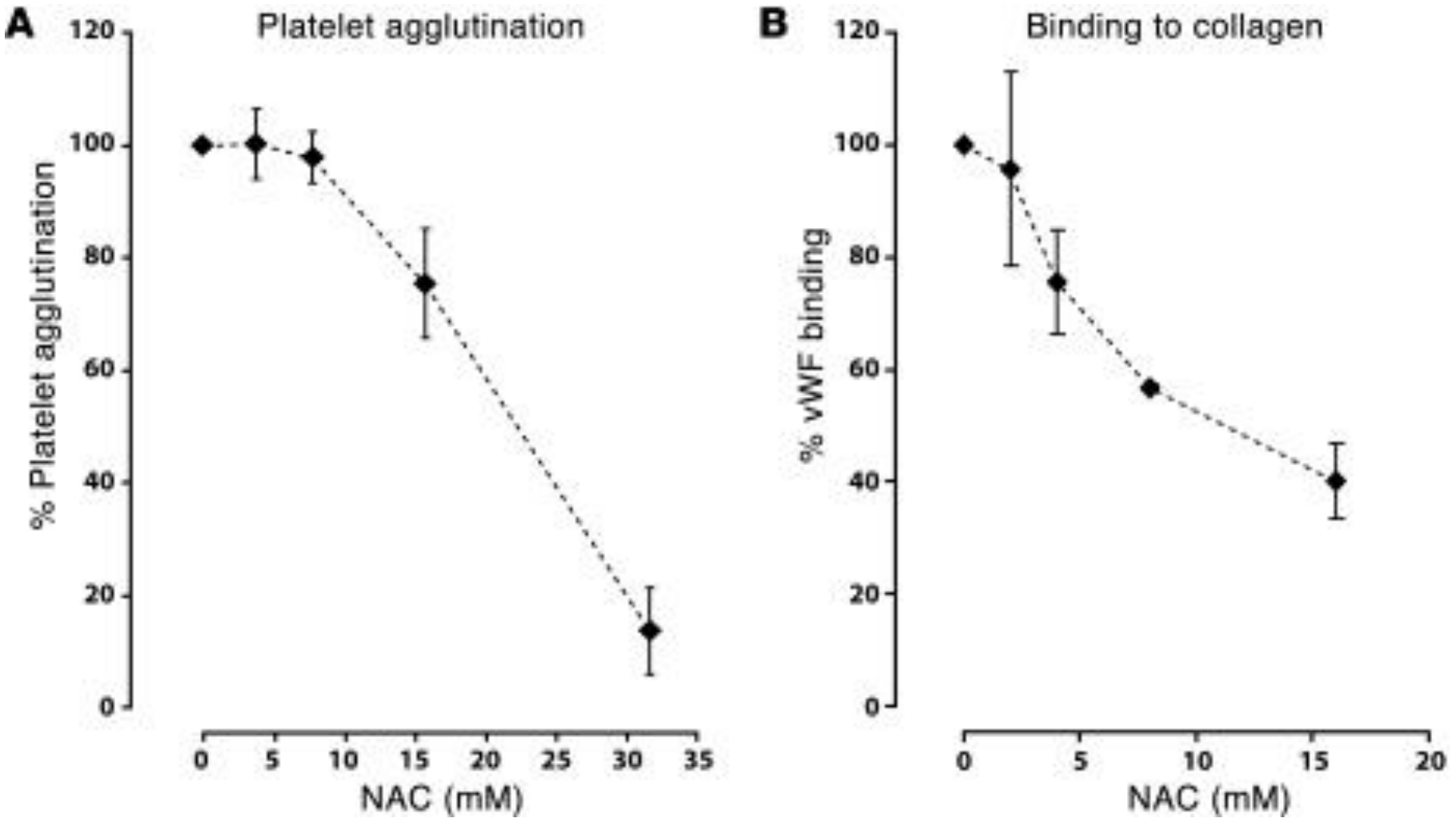
B] Inflammation



C] Cleavage of VWF by ADAMTS13 is prevented by the following mechanisms

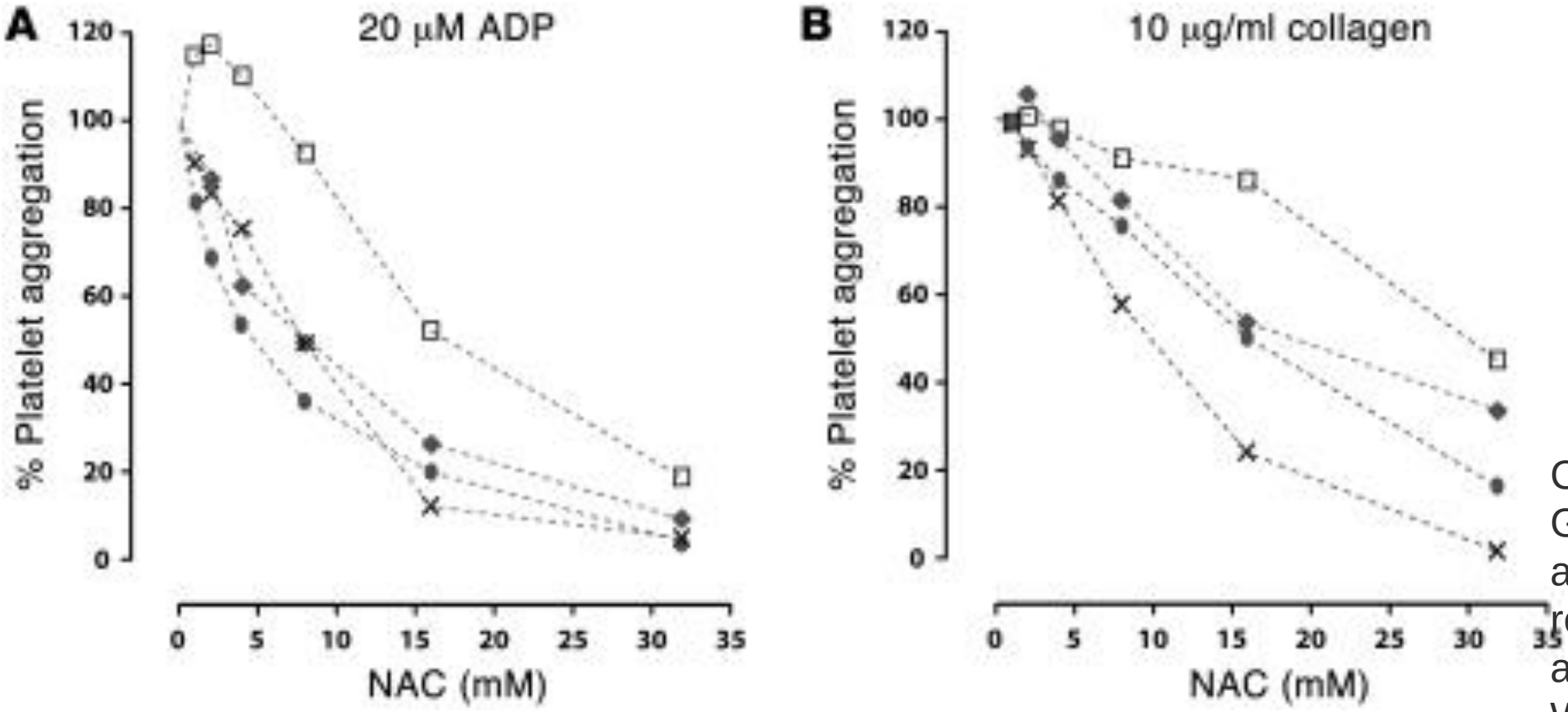
1) Binding of thrombospondin-1 released from α-granules of activated platelets to A2-A3 domain of VWF harboring the proteolytic cleavage site of ADAMTS13
2) Binding of α-defensins released from neutrophils to A2 domain of VWF
3) Oxidation of Met 1606 residue in the ADAMTS13 cleavage site of VWF by reactive oxygen species
4) Proteolytic cleavage of ADAMTS13 by granulocyte elastases, plasmin, and thrombin that are elevated in inflammatory conditions

Katneni, U.K.; Alexaki, A.; Hunt, R.; Schiller, T.; DiCuccio, M.; Buehler, P.W.; Ibla, J.C.; Kimchi-Sarfaty, C. Consumptive Coagulopathy and Thrombosis during Severe COVID-19 Infection: Potential Involvement of VWF/ADAMTS13. *Preprints* **2020**, 2020050385 (doi: 10.20944/preprints202005.0385.v1)



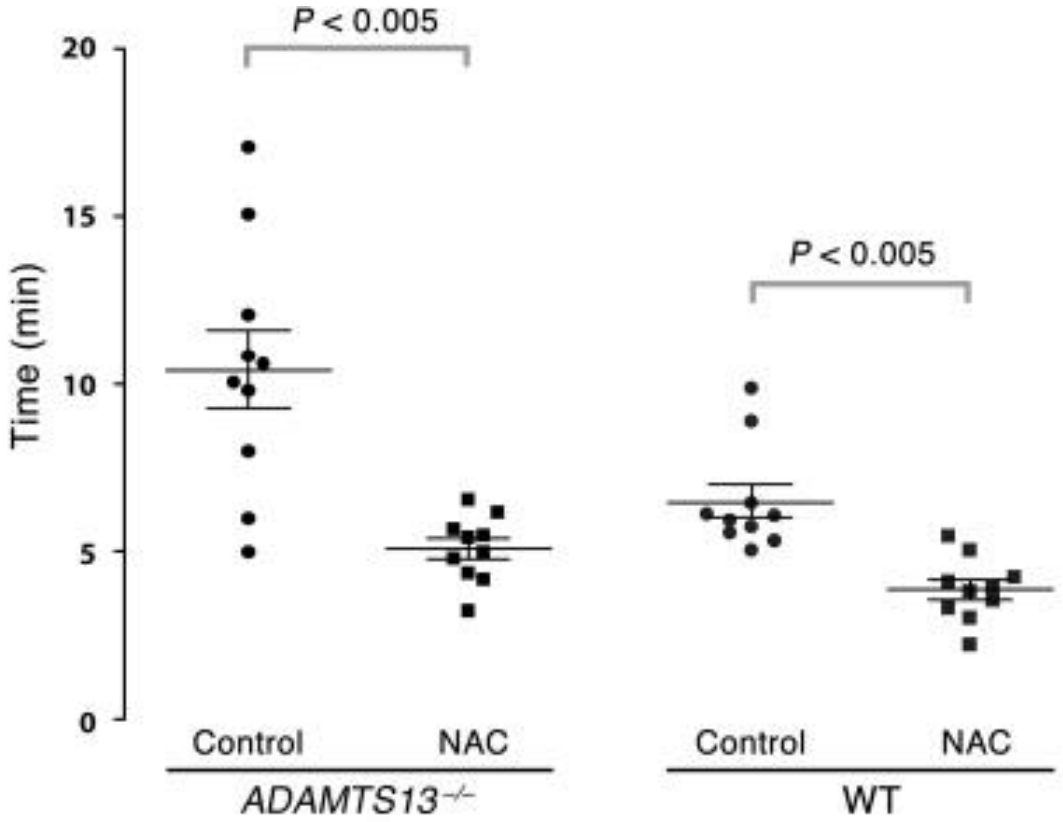
Chen J, Reheman A, Gushiken FC, et al. N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. *J Clin Invest*. 2011;121(2):593-603. doi:10.1172/JCI41062

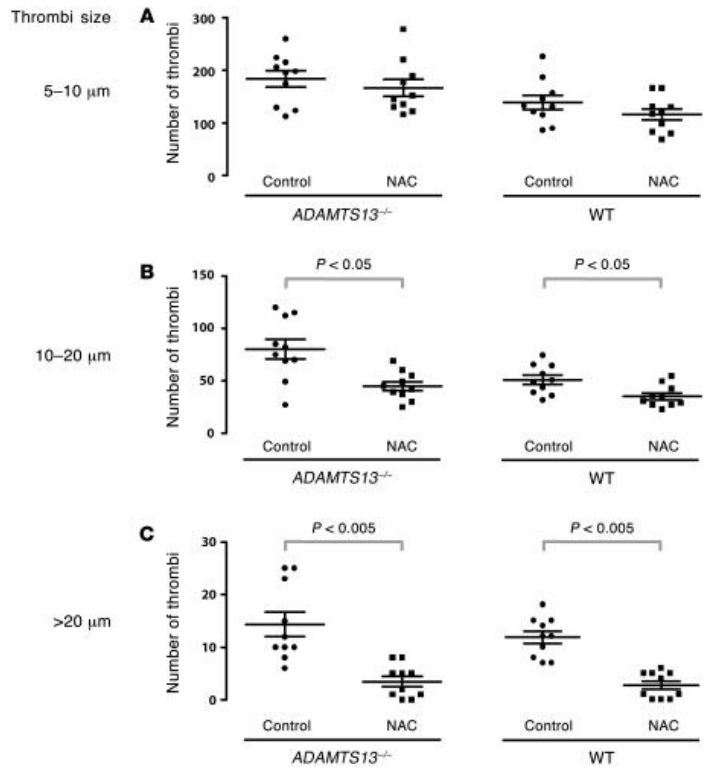
NAC inhibits the ability of vWF to bind platelets and collagen.



Chen J, Reheman A, Gushiken FC, et al. N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. *J Clin Invest*. 2011;121(2):593-603. doi:10.1172/JCI41062

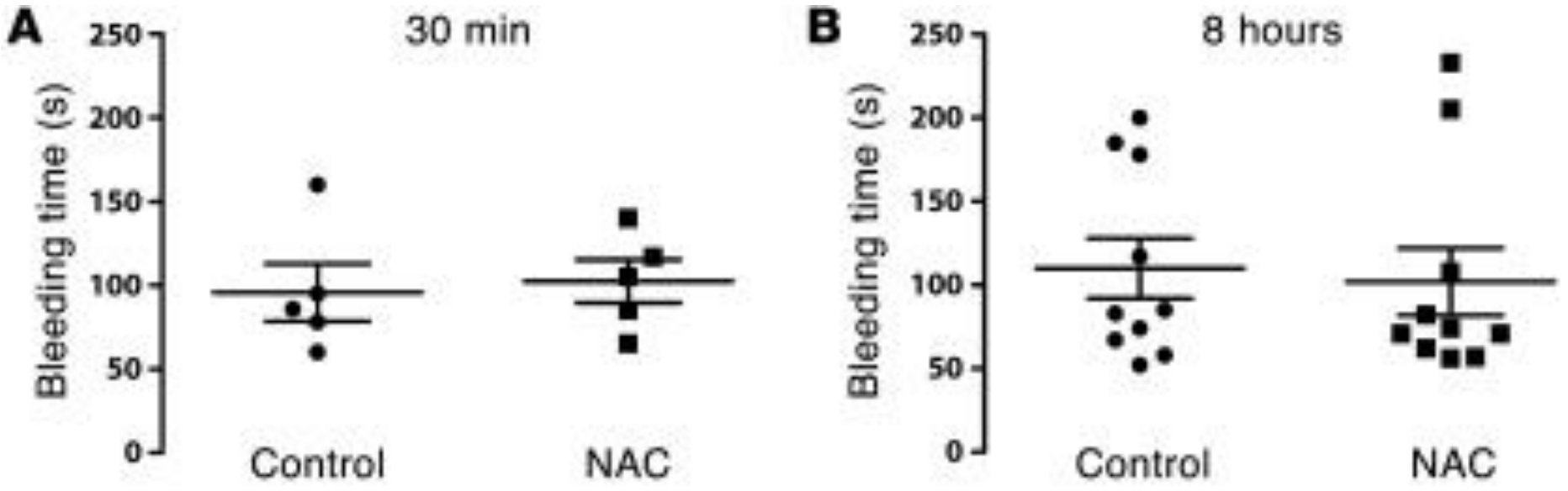
NAC inhibits ADP- and collagen-induced platelet aggregation.





Chen J, Reheman A, Gushiken FC, et al. N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. *J Clin Invest*. 2011;121(2):593-603. doi:10.1172/JCI41062

Time to thrombus resolution in calcium ionophore–treated mesenteric venules. Upon calcium ionophore stimulation of the mesenteric venules, platelets immediately began to accumulate on the vessel wall. Adhesion was monitored, and the time required for the fluorescence value to return to baseline was measured. Platelet adhesion persisted longer in mice deficient in *ADAMTS13* than in wild-type mice. NAC treatment in either *ADAMTS13*$^{-/-}$ or wild-type mice significantly shortened the time required for platelet adhesion to return to baseline. The mean (± SEM) values for time to thrombus resolution were as follows. *ADAMTS13*$^{-/-}$: control, 10.4 ± 1.2 minutes; NAC, 5.1 ± 0.3 minutes; wild type: control, 6.5 ± 0.5 minutes; NAC, 3.8 ± 0.3 minutes. 10 mice were examined in each group. The data were analyzed using Student's *t* test, and the adjusted *P* values are indicated.



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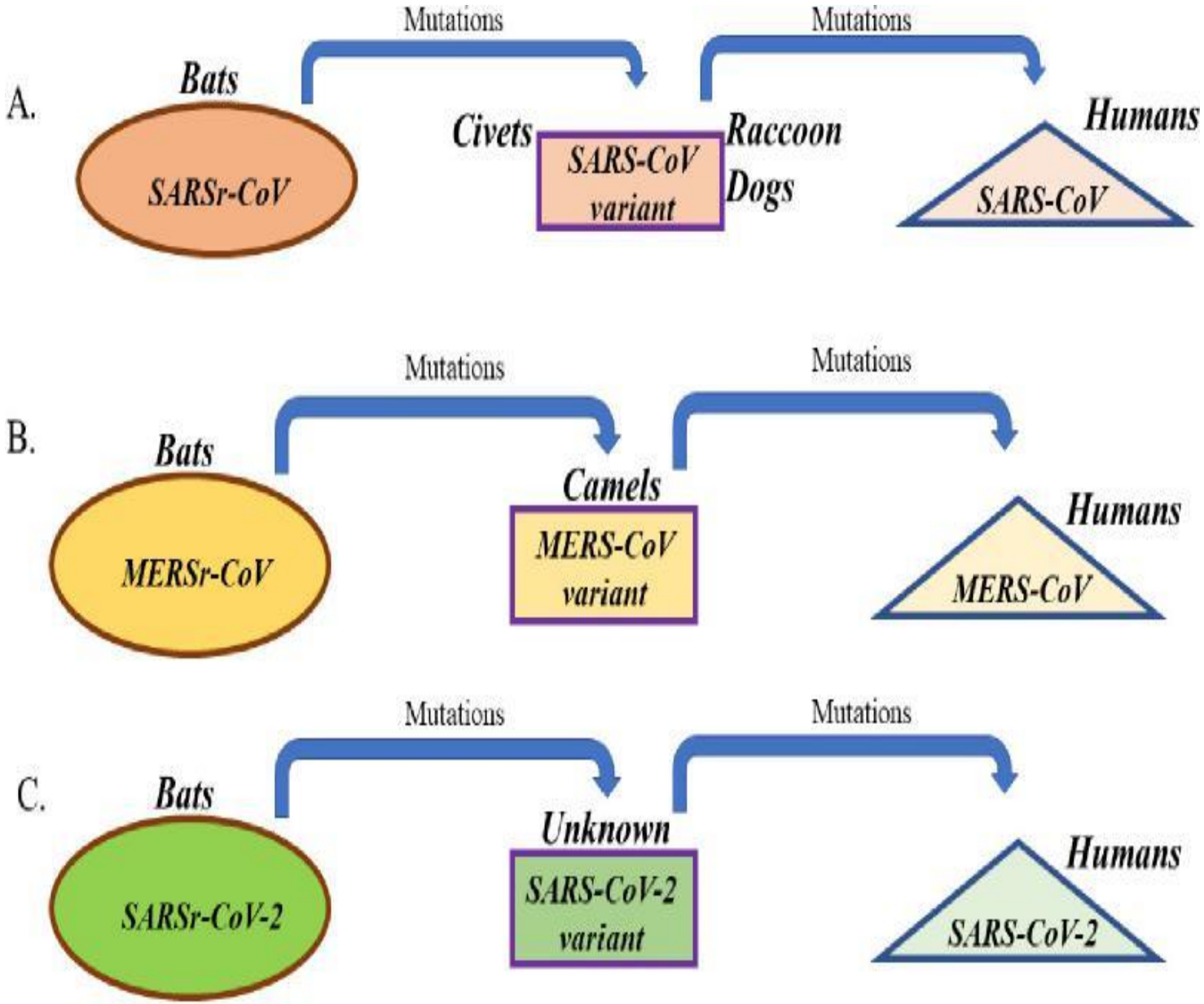
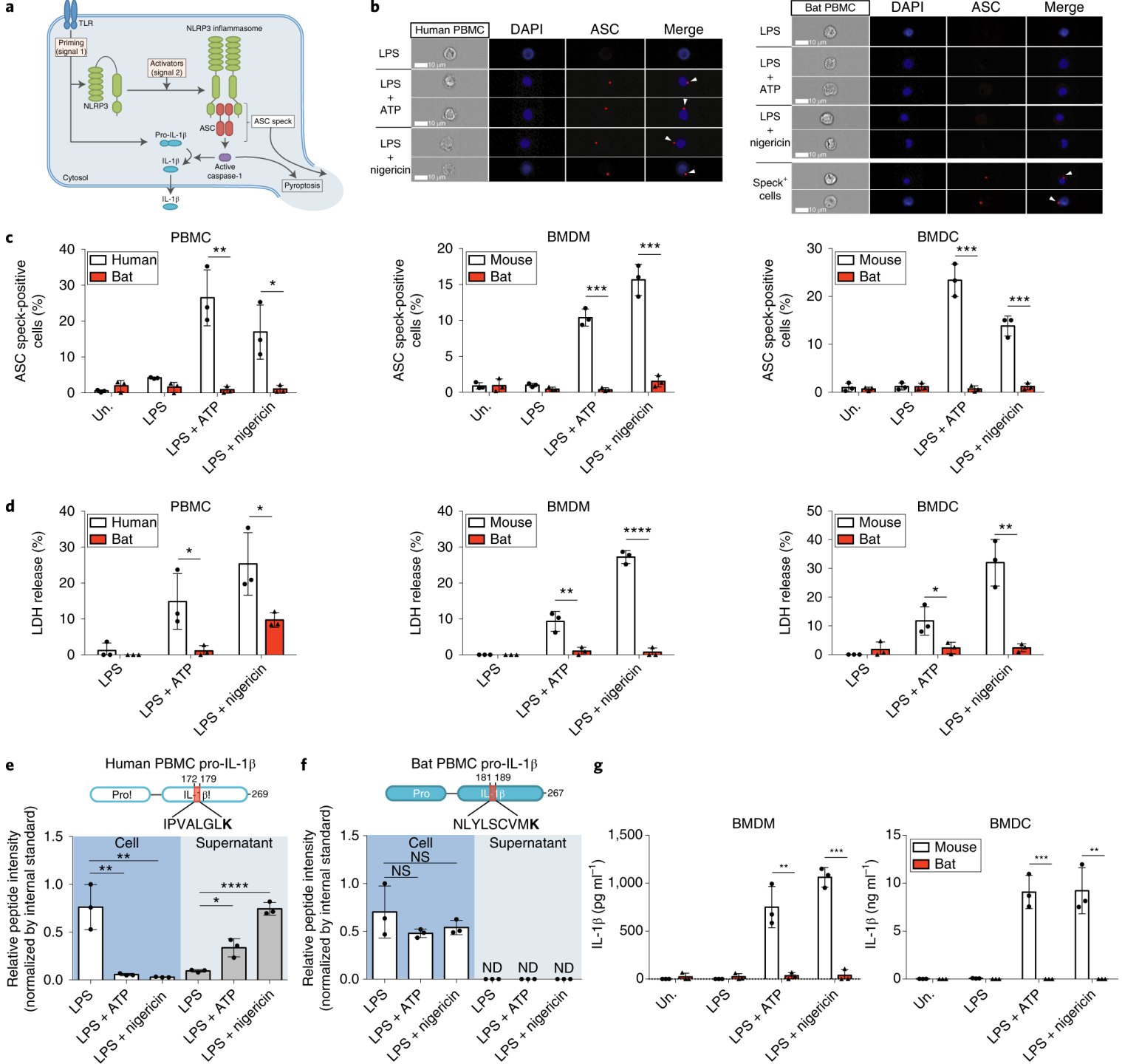
NAC has no effect on bleeding times in mice. Tail-vein bleeding times were examined in 6- to 8-week-old wild-type C57BL/6 mice. Either PBS (control) or NAC (0.8 mg/g) was administered through the tail vein 30 minutes or 8 hours before the bleeding time was determined. Data represent mean ± SEM. The number of mice examined in each group was 5 for **A** and 10 for **B**. No significant differences in bleeding times were observed between treated and untreated mice. The data were analyzed using the Mann-Whitney nonparametric procedure. The adjusted *P* values for the comparisons between control and NAC-treated animals were 0.47 and 0.86, for the 30 minute and 8 hour analyses, respectively.



WHY are Coronavirus infections not lethal for Bats ?

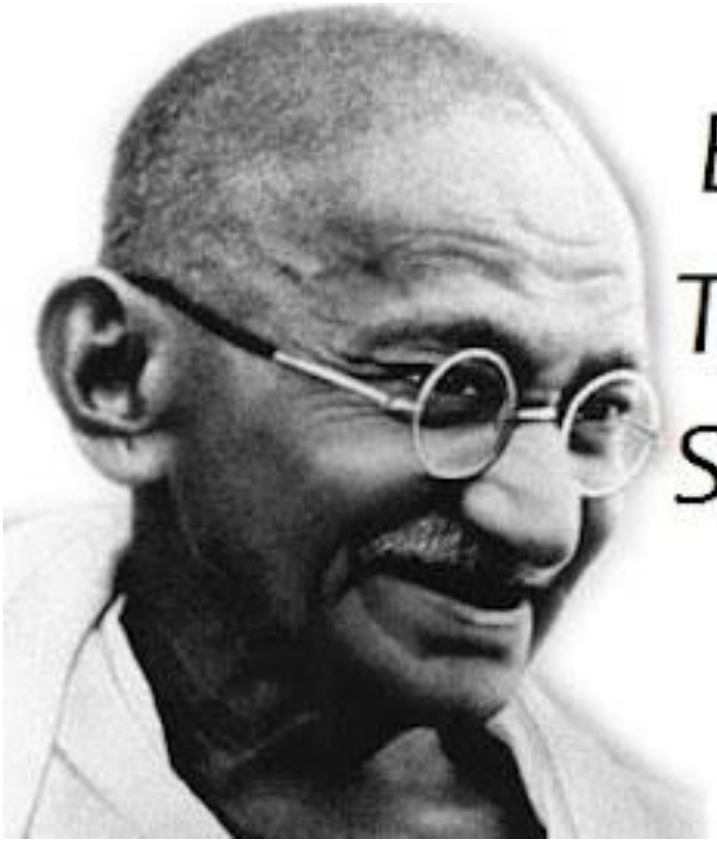


Ahn, M., Anderson, D.E., Zhang, Q. *et al.* Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. *Nat Microbiol* **4,** 789–799 (2019). https://doi-org.proxy.cc.uic.edu/10.1038/s41564-019-0371-3

Therapeutic Implications of a new understanding of the pathophysiology of COVID 19

- SARS CoV-2 does not cause a problem in the majority of humans because they are like BATS ????
- In all humans the viral replication is eventually controlled by the host immune systems
- Anti-Virals relevant only in early stages and probably need combination of antivirals with different sites of action
- In a minority of individuals there is an exaggerated NLRP 3 response reminiscent of the hereditary inflammatory disorders like Familial Mediterranean Fever or other Cryopyrin Associated Periodic Syndromes (Familial Cold autoinflammatory syndrome; Muckle-Wells Syndrome; Neonatal onset multisystem inflammatory disease)
- Young individuals Multi System Inflammatory Disorder including Kawasaki Disease is mediated by NLRP 3
- Systemic Vasculitis due to direct infection and activation of endothelial inflammation is common
- Initial stages have severe platelet aggregation and platelet thrombi that cannot be controlled due to deficiency of Von-Willebrand factor proteases this serves as the initiator of VTE
- In late stages ALI and AKI is mediated by the terminal attack complex of complement with multiple triggers causing complement activation and a concomitant deficiency of complement control proteins

The Yeldandi Protocol: Look for markers of inflammation in all symptomatic individuals : Lymphopenia; High CRP; High LDH; High Ferritin; If Oxygen saturation low look for D dimer, evidence for TMA
Early stage : Colchicine; NAC; Famotidine + Antiviral if possible but not imperative
Moderate illness: Colchicine; NAC; Famotidine; Dexamethasone; Atorvastatin of 40 mg daily
Hypoxic Illness: Colchicine consider Anakinra; Famotidine; NAC; Atorvastatin; Dexamethasone. Add Rivaroxaban if D dimer high
Refractory Hypoxemia with LDH > 500 and Platelets decreased 25% below baseline Eculizumab would be ideal to address complement induced ALI and AKI, Can Fresh Frozen Plasma help to replace ADAMTS 13 and Complement control proteins?



Be The Change
That You Want to
See In The World.

Power is an Illusion; Authority is a delusion; RESPONSIBILITY is REALITY