Acute Respiratory Distress Syndrome, NLRP3 and IL-1β Activation Induced by COVID-19

Grand Rounds Presentation by
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The Coronavirus Patients Betrayed by Their Own Immune Systems

A “cytokine storm” becomes an all-too-frequent phenomenon, particularly among the young. But treatments are being tested.

Randy Cron:…” the cytokine storm keeps raging long after the virus is no longer a threat”

What is a cytokine storm?

Unusually high circulating levels of pro-inflammatory cytokines associated with organ damage

Pharmacologic blockade or neutralization of specific cytokines can reduce organ damage, particularly when treatment is initiated early in the disease
Therapeutic options to reduce the cytokine storm

Blocking IL-1\(\alpha\) and IL-1\(\beta\) with anakinra

Blocking IL-6R with tocilizumab

Blocking Upstream early with oral NLRP3 inhibitor
Increased chemokines and IL-18 from PBMC, but not IL-6. IL-6 is from Epithelial cells. The authors concluded:

Altogether, our data suggest that SARS-CoV-2 infection-induced excessive cytokine release correlates with lung tissue injury and COVID-19 pathogenesis.
One view of the evolving Cytokine Storm in COVID-19 infection
Virus infects type 2 epithelial cells. Death of cells. Release of IL-1α and cell contents. IL-1α induces chemokines and other cytokines from resident macrophages in the lungs. G-CSF and IL-1β enters circulation.

G-CSF; IL-1β release of immature neutrophil and monocytes (MDSC) adherence to endothelium; opening of endothelial junctions; infiltration of damaging MDSC; cytokine production: IL-1β, IL-6, IL-12, IL-18, TNFα, chemokines.

Processing of IL-1β and IL-18 by the NLRP3 inflammasome.

Neutrophils; monocytes (MDSC)

Bone marrow

Hemophagocytosis

Cytopenia

Macrophage Activation Syndrome

Liver hepatitis

Cytokine storm

IFNγ

IL-6 (IL-1β, TNFα, IL-8)

IL-12 + IL-18

Ferritin; D-dimer; CRP
Pro-inflammatory properties of immature neutrophils
Infiltrating the lung (also called Myeloid Derived Suppressor Cells)

Nitric Oxide (immunosuppressive)

PGE₂ (suppresses T-cell functions)

Reactive Oxygen Species (ROS) (generalized toxicity for immune responses)

cytokines and chemokines (produce IL-1β, IL-6, TNF, IL-10, IL-8)

Arginase-1 (immunosuppression)

PD-1/PD-L1 (highly immunosuppressive for immune responses)

IDO (indoleamine 2, 3-dioxygenase) (suppression of T-cell functions)
The Cytokine Storm is a cascade

SARS-Covid-19 enters macrophages via ACE2

- Activation of NLRP3
- Release of active IL-1β
- Induction of IL-6 from lung epithelium
  - IL-6
  - Liver
  - CRP

Liver

SARS-Covid-19 enters via ACE2 on airway epithelium

- Cell death
- Release of preformed IL-1α; HMGB1
- Lung resident macrophages
  - Activation of NLRP3
  - Release of active IL-1β
    - IL-1β
    - Induction of chemokines from lung epithelium
      - Immature neutrophils damage lungs
Activation of endothelium by COVID-19 binding

Urokinase plasminogen activator receptor
What is NLRP3 and what does it do?

NLRP3 is an intracellular complex of proteins. Upon activation by a fall in intracellular potassium, NLRP3 oligomerizes and converts inactive procaspase-1 to active caspase-1.
IL-1β precursor (inactive)

The inflammasome converts inactive pro-caspase-1 to active caspase-1 and thereby controls the processing and secretion of active IL-1β and IL-18.

Active IL-1β released from cell

IL-6, chemokines, cytokine storm

NLRP3 (Nucleotide-binding domain and Leucine-rich Repeat Pyrin containing 3)

IL-1β "inflammasome"

NLRP3 (cryopyrin)

ASC

PYR

NACHT

NAD

LRR

dapansutrile

NLRP-3

procaspase-1

p20 *cys

p10

active caspase-1

is a heterodimer

inactive caspase-1

FIIND

cardinal
OLT1177, a β-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation

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Viruses infect type 2 epithelial cells. Death of cells. Release of IL-1α. IL-1α induces chemokines and cytokines from resident macrophages. G-CSF and IL-1β enter circulation. Neutrophils and monocytes (MDSC) adhere to endothelium opening of endothelial junctions increased infiltration of mononuclear cells; cytokine production: IL-1β, IL-6, IL-12, IL-18, TNFα, chemokines. Processing of IL-1β and IL-18 by the NLRP3 inflammasome. Cytokine storm IL-6, IL-1β. Liver hepatitis. Hemophagocytosis. Cytopenia. IFNγ. IL-12 + IL-18. Ferritin; D-dimer; CRP. NLRP3i anakinra anti-IL-1α anti-IL-1β anti-IL-6R IL-18BP anti-IFNγ JAKi.
Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC

SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes

Cheng-Shan Shi1, Neel R. Nabar1, Ning-Na Huang1 and John H. Kehrl1

Severe Acute Respiratory Syndrome Coronavirus Viroporin 3a Activates the NLRP3 Inflammasome

I-Yin Chen1, Miyu Moriyama1, Ming-Fu Chang1 and Takashi Ichinohe1*
What is available to specifically inhibit NLRP3 in humans?

At present, there is only one clinically used specific NLRP3 inhibitor in Phase 2

OLT1177 (generic dapansutrile) is a small, synthetic compound, orally active, safe in 3 completed clinical trials

One Phase 2 trial has been completed in acute gout flares and another Phase 1b/2a trial has been completed in heart failure
Dapansutril, an oral selective NLRP3 inflammasome inhibitor, for treatment of gout flares: an open-label, dose-adaptive, proof-of-concept, phase 2a trial

Fall in peripheral neutrophils

Fall in circulating IL-6
Effect of 14 days oral dapansutrile on patients with chronic systolic heart failure (many of whom obese and with Type 2 Diabetes)(double-blinded, dose-escalating, randomized), Antonio Abbate, et al

Significant improvement in high-dose dapansutrile cohort in:

- Left ventricular ejection fraction
- Treadmill exercise time
- Fasting plasma glucose values
- IL-1β and IL-18 plasma values
Treatment for ARDS

Blocking the IL-1 Receptor with anakinra (approved)

Neutralization of IL-6 Receptor with tocilizumab
Sites using anakinra for ARDS

Italy
Greece
Canada
New Zealand
UK
REMED CAPS

Sloan-Kettering
Brigham Hospital (Boston)
Mt. Sinai
University of Alabama
All cells express the two IL-1 Receptors: IL-1R1 binds IL-1 and IL-1R3 is the co-receptor. Both are needed to transmit the IL-1 signal.
IL-1Ra binds IL-1R1 and IL-1R3 is not recruited. The TIR domains do not approximate and there is no signal in the presence of IL-1
Domain-Specific Appendix:
COVID-19 Immune Modulation Therapy

In this domain of the REMAP-CAP trial, participants meeting the platform entry criteria for REMAP-CAP admitted to participating intensive care units with suspected or microbiological testing-confirmed COVID-19 infection will be randomized to receive one of up to three interventions depending on availability and acceptability:

- No immune modulation for COVID-19 (no placebo)
- interferon-beta-1a (IFN-β1a)
- anakinra i.e. interleukin-1 receptor antagonist (IL1Ra)
What is the rationale for Interferon-β?

RESULTS

IFN-β Suppresses Both Pro-IL-1β Availability and IL-1β Maturation
Hydroxychloroquine treatment for primary Sjögren’s syndrome: its effect on salivary and serum inflammatory markers

Hydroxychloroquine Inhibits IL-1β Production From Amyloid-Stimulated Human Neutrophils

Hydroxychloroquine decreases Th17-related cytokines in systemic lupus erythematosus and rheumatoid arthritis patients
JAK inhibitors

Tofacitinib inhibits granulocyte–macrophage colony-stimulating factor-induced NLRP3 inflammasome activation in human neutrophils
Special thanks to Dr. Antonio Abbate of Virginia Commonwealth University for sharing his data on dapansutrile in patients with heart failure, high BMI’s and Type 2 diabetes

Also, thanks to Olatec, LLC for supporting trials of dapansutrile in acute gout and in the Abbate trial in heart failure.

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