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

Grand Rounds Presentation by
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08 April 2020

University of Colorado
Anschutz Medical Campus

The New York Times | https://nyti.ms/3bOln0J

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-1α and IL-1β with anakinra

Blocking IL-6R with tocilizumab

Blocking Upstream early with oral NLRP3 inhibitor

The first reports from China established the cytokine storm in CVID-19 pneumonia

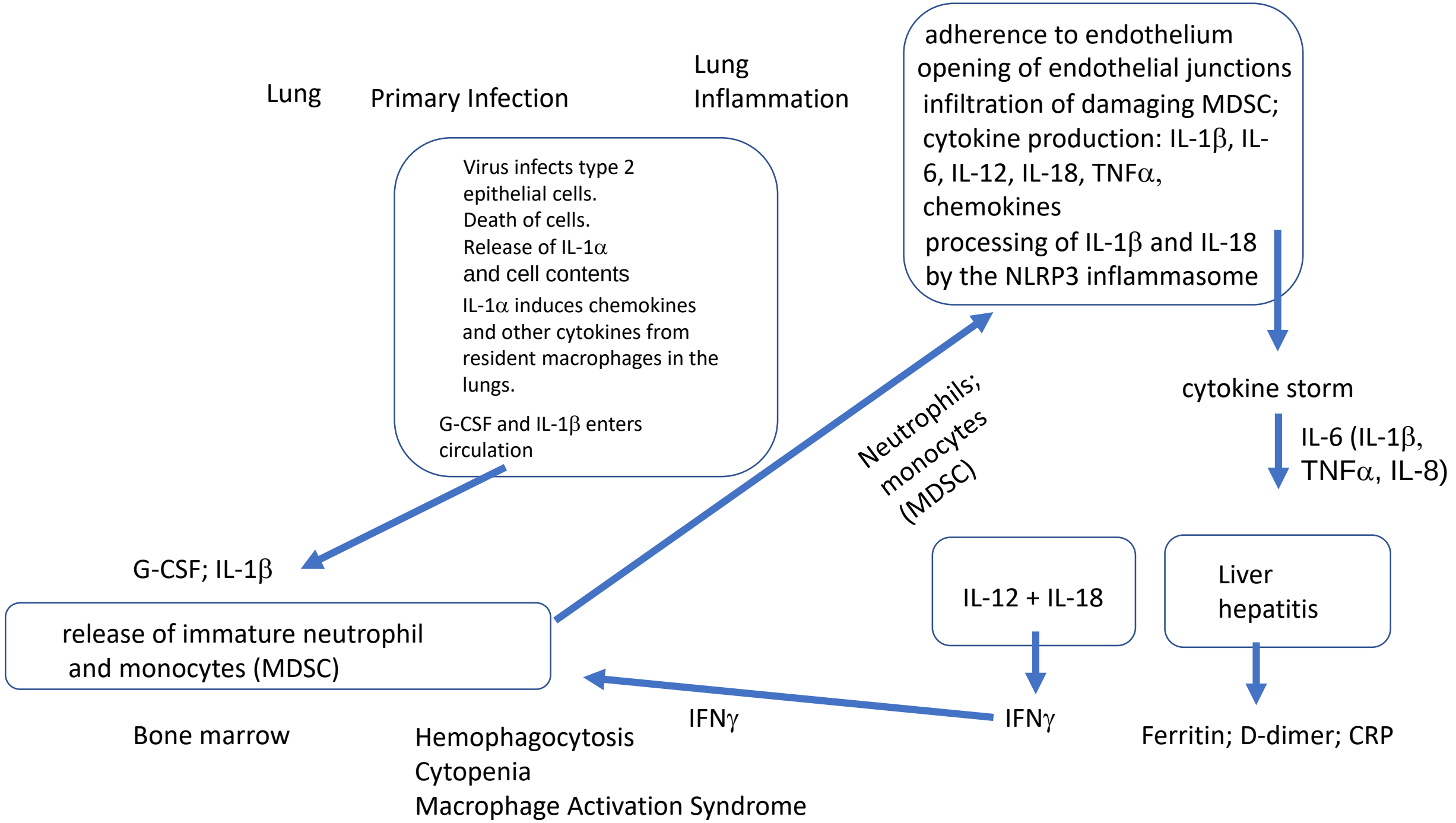
Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients

Yong Xiong[a*], Yuan Liu[b*], Liu Cao[c*], Dehe Wang[b*], Ming Guo[b], Ao Jiang[b], Dong Guo[b], Wenjia Hu[a], Jiayi Yang[b], Zhidong Tang[b], Honglong Wu[d], Yongquan Lin[d], Meiyuan Zhang[d], Qi Zhang[b], Mang Shi[c], Yingle Liu[b], Yu Zhou[b], Ke Lan[b] and Yu Chen[b]

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


Lung
Primary Infection
Lung
Inflammation
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
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
cytokine storm
IL-6 (IL-1β, TNFα, IL-8)
Neutrophils; monocytes (MDSC)
G-CSF; IL-1β
release of immature neutrophil and monocytes (MDSC)
IL-12 + IL-18
Liver hepatitis
IFNγ
IFNγ
Bone marrow
Hemophagocytosis
Cytopenia
Macrophage Activation Syndrome
Ferritin; D-dimer; CRP

Pro-inflammatory properties of immature neutrophils
Infiltrating the lung (also called Myeloid Derived Suppressor Cells)

Nitric Oxide (immunosuppressive)

PGE_2 (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

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

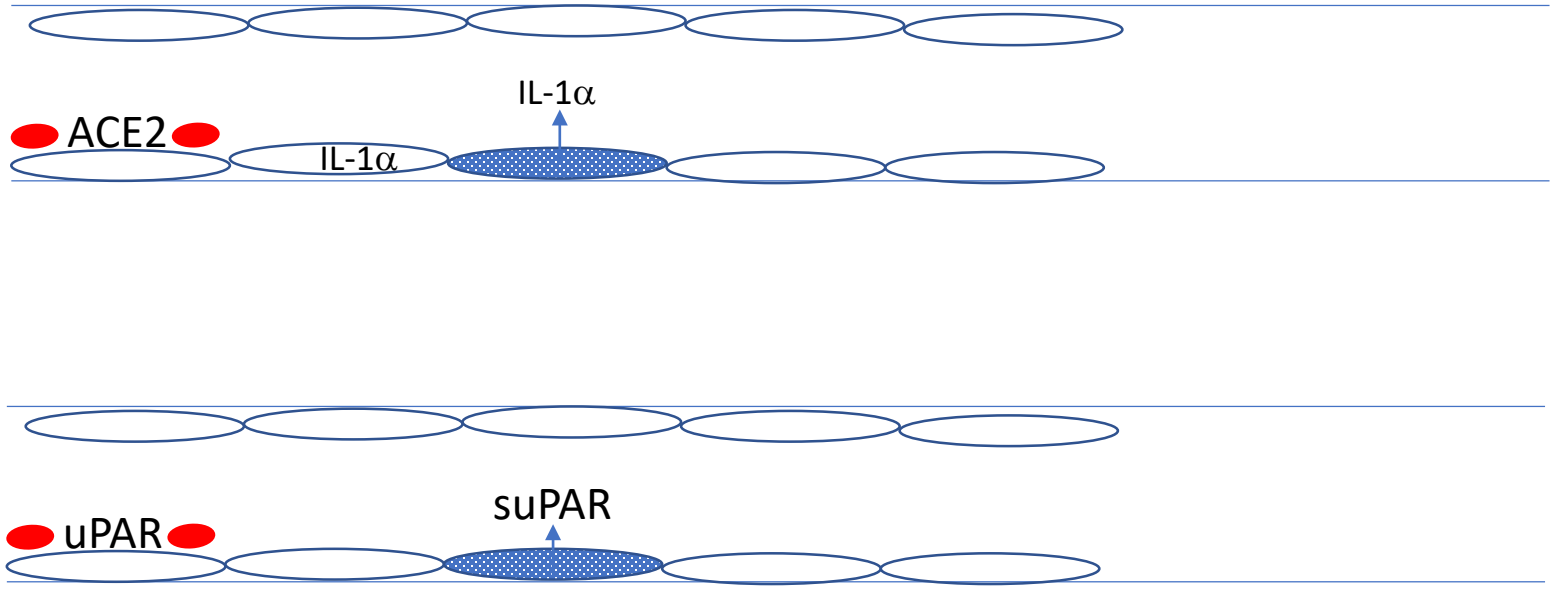
↓ IL-1β

Induction of chemokines from lung epithelium

↓

Immature neutrophils damage lungs

Activation of endothelium by COVID-19 binding

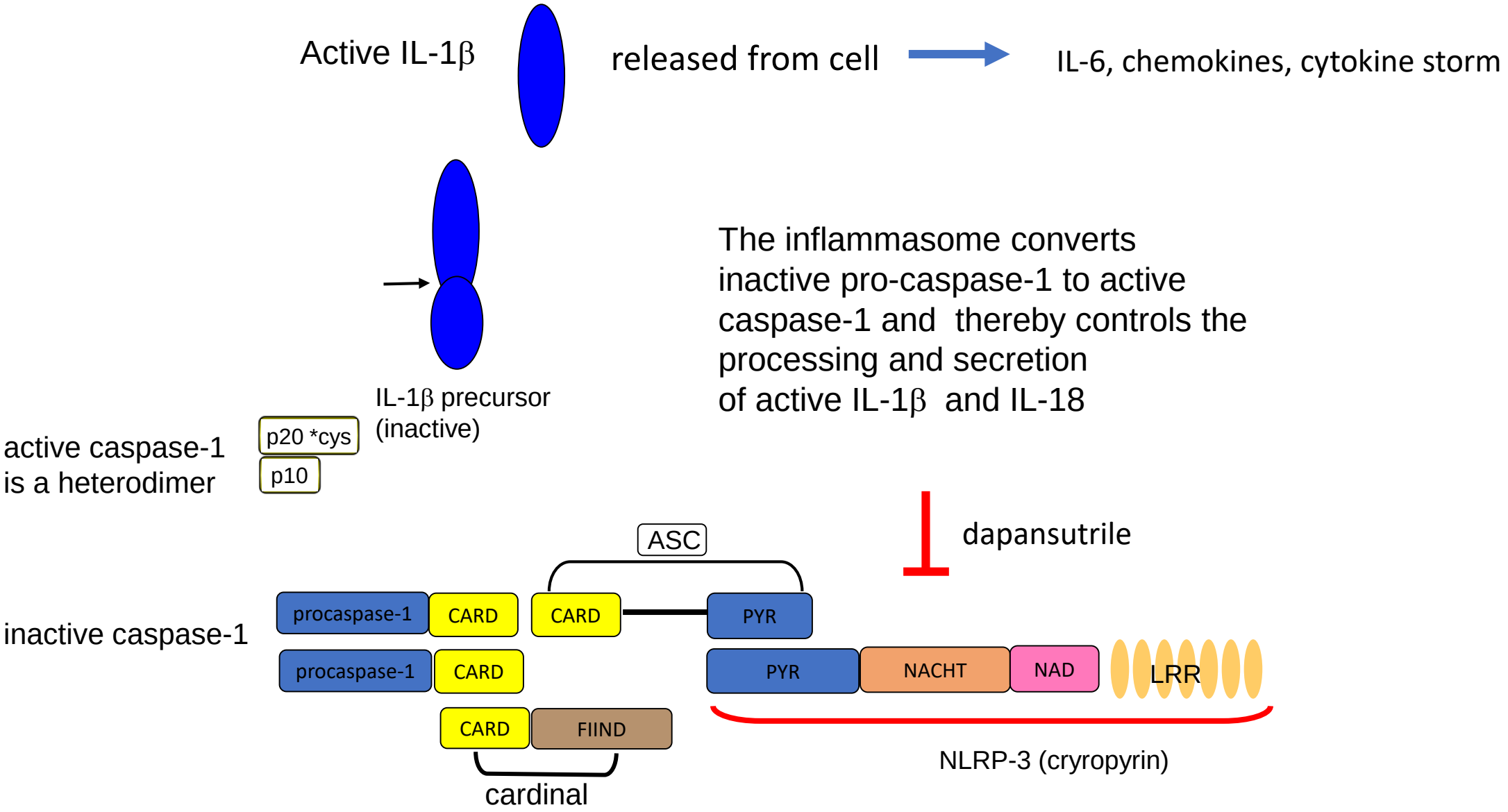


Urokinase plasminogen activator receptor

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

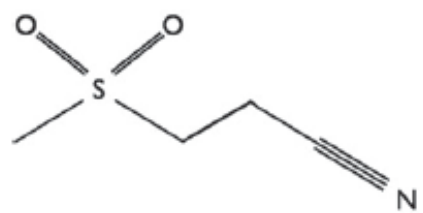
NLRP3 (Nucleotide-binding domain and Leucine-rich Repeat Pyrin containing 3)
IL-1β "inflammasome"



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

Carlo Marchetti[a], Benjamin Swartzwelter[a], Fabia Gamboni[a], Charles P. Neff[a], Katrin Richter[b], Tania Azam[a], Sonia Carta[c], Isak Tengesdal[a], Travis Nemkov[d], Angelo D'Alessandro[d], Curtis Henry[e], Gerald S. Jones[f], Scott A. Goodrich[f], Joseph P. St. Laurent[f], Terry M. Jones[g], Curtis L. Scribner[h], Robert B. Barrow[h], Roy D. Altman[i], Damaris B. Skouras[h], Marco Gattorno[j], Veronika Grau[b], Sabina Janciauskiene[k], Anna Rubartelli[c], Leo A. B. Joosten[l], and Charles A. Dinarello[a,l,1]

A



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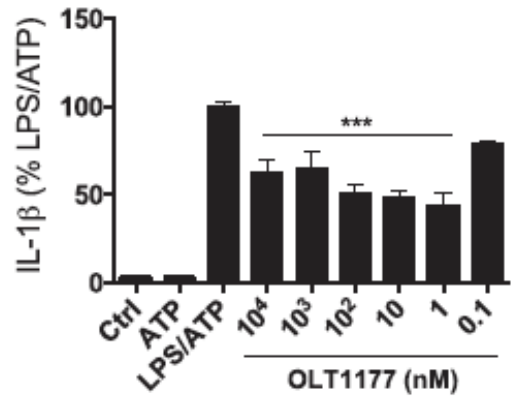

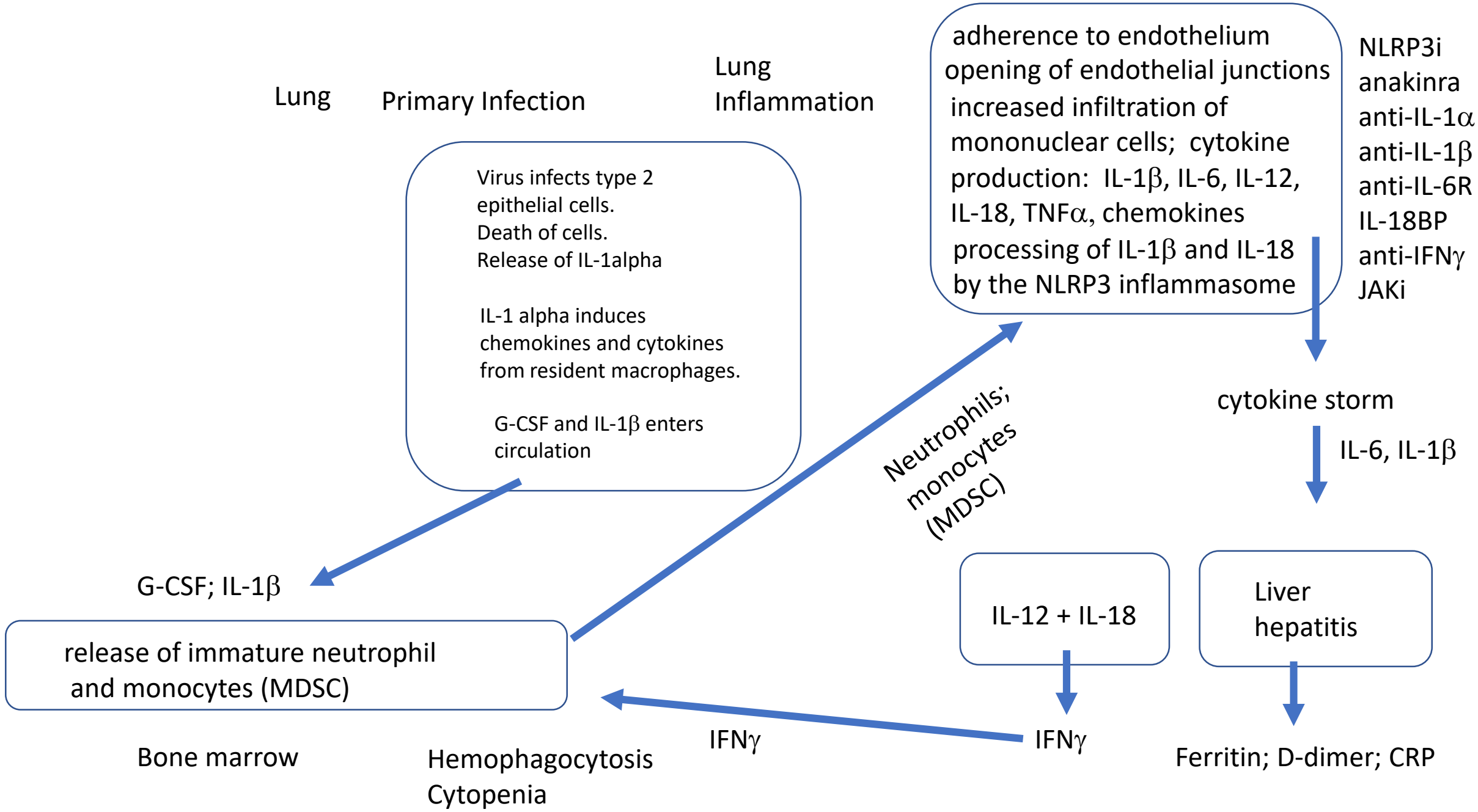
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from resident macrophages.
G-CSF and IL-1β enters
circulation
adherence to endothelium
opening of endothelial junctions
increased infiltration of
mononuclear cells; cytokine
production: IL-1β, IL-6, IL-12,
IL-18, TNFα, chemokines
processing of IL-1β and IL-18
by the NLRP3 inflammasome
NLRP3i
anakinra
anti-IL-1α
anti-IL-1β
anti-IL-6R
IL-18BP
anti-IFNγ
JAKi
cytokine storm
IL-6, IL-1β
Neutrophils;
monocytes
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release of immature neutrophil
and monocytes (MDSC)
IL-12 + IL-18
Liver
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IFNγ
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Hemophagocytosis
Cytopenia
Ferritin; D-dimer; CRP

Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC

Shi et al. *Cell Death Discovery* (2019)5:101
https://doi.org/10.1038/s41420-019-0181-7

Cell Death Discovery

ARTICLE Open Access

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

Chong-Shan Shi[1], Neel R. Nabar[1], Ning-Na Huang[1] and John H. Kehrl[1]

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

*I-Yin Chen[1], Miyu Moriyama[1], Ming-Fu Chang[2] and Takeshi Ichinohe[1]**

What is available to specifically inhibit NLRP3 in humans?

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

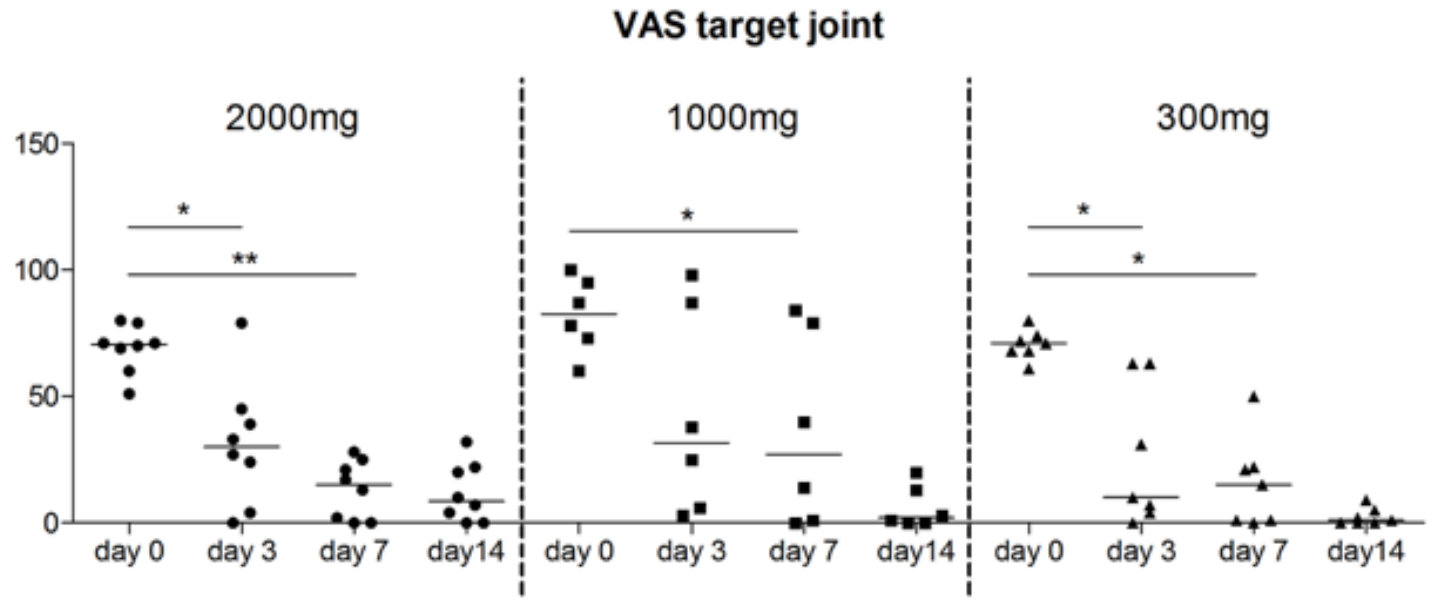
OLT1177 (generic dapansutrile) is a small, synthetic compound, orally active, safe in 3 completed clinical trials

One Phase 2 trial has been completed in acute gout flares and another Phase 1b/2a trial has been completed in heart failure

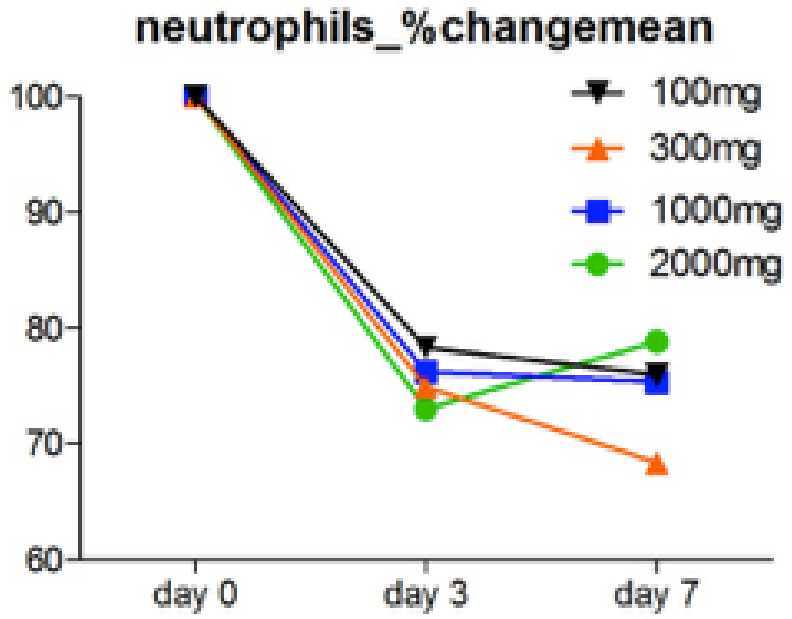
Lancet Rheumatology April 7, 2020)

Dapansutrile, an oral selective NLRP3 inflammasome inhibitor, for treatment of gout flares: an open-label, dose-adaptive, proof-of-concept, phase 2a trial

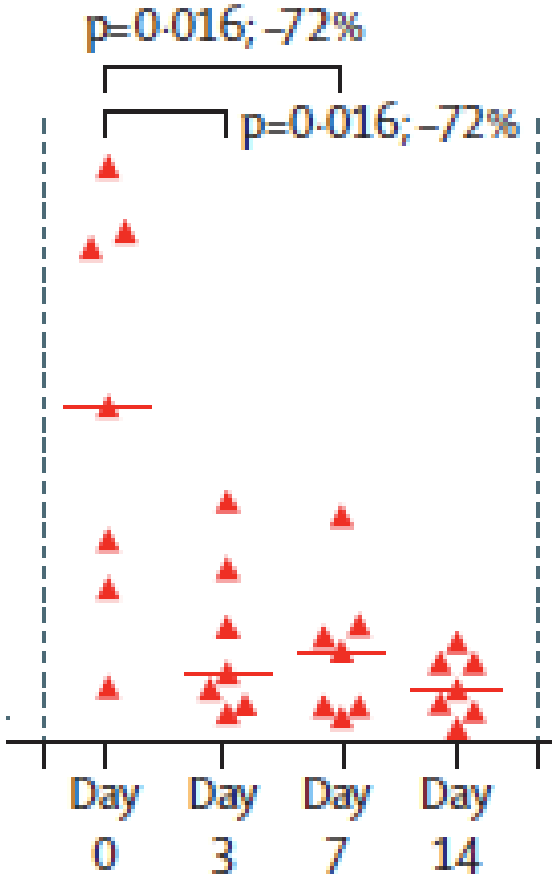
Viola Klück, Tim L Th A Jansen*, Matthijs Janssen, Antoaneta Comarniceanu, Monique Efdé, Isak W Tengesdal, Kiki Schraa, Maartje C P Cleophas, Curtis L Scribner, Damaris B Skouras, Carlo Marchetti, Charles A Dinarello, Leo A B Joosten*



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 lL-18 plasma values

Treatment for ARDS

Blocking the IL-1 Receptor with anakinra (approved)

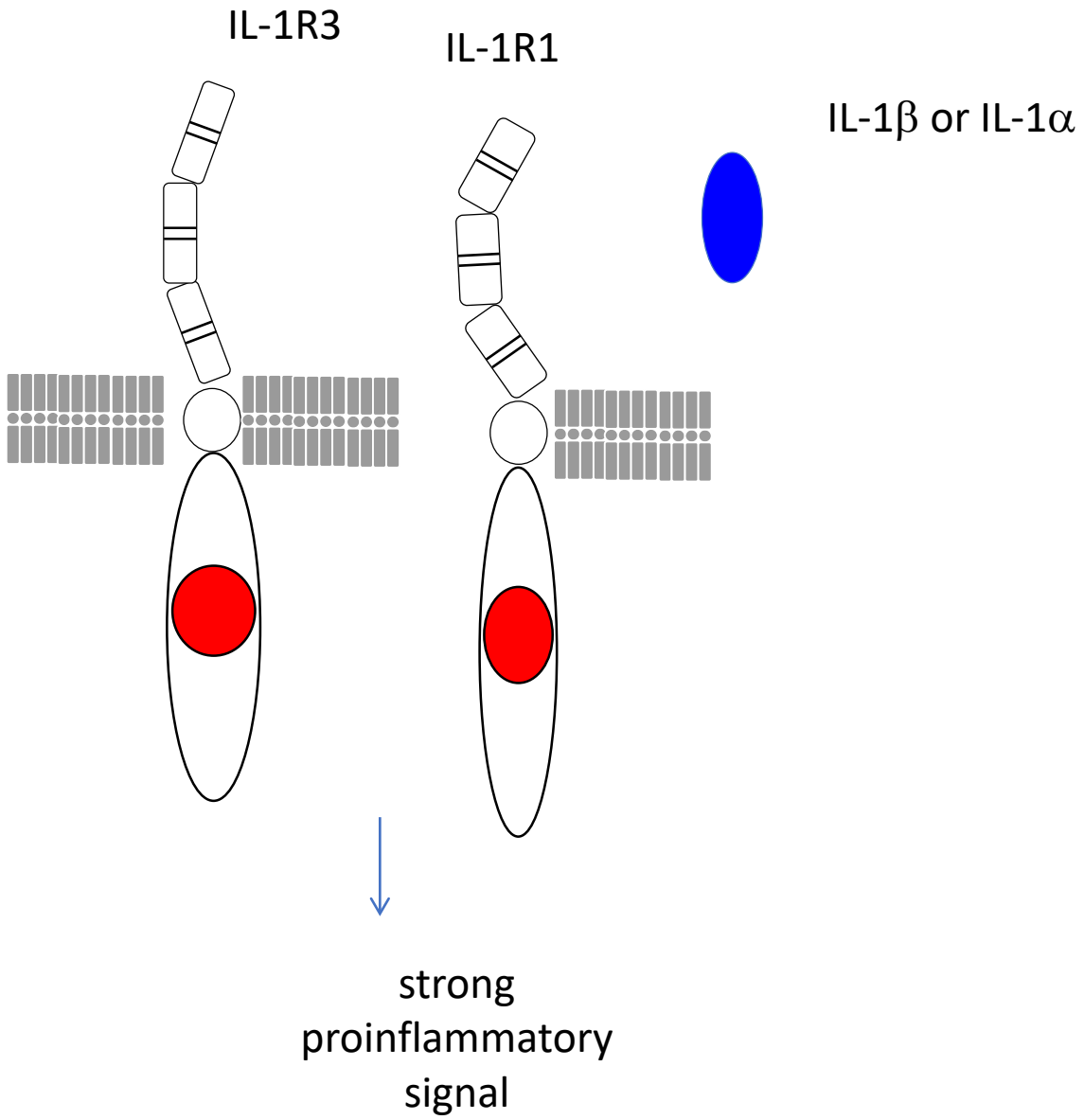
Neutralization of IL-6 Receptor with tocilizumab

Sites using anakinra for ARDS

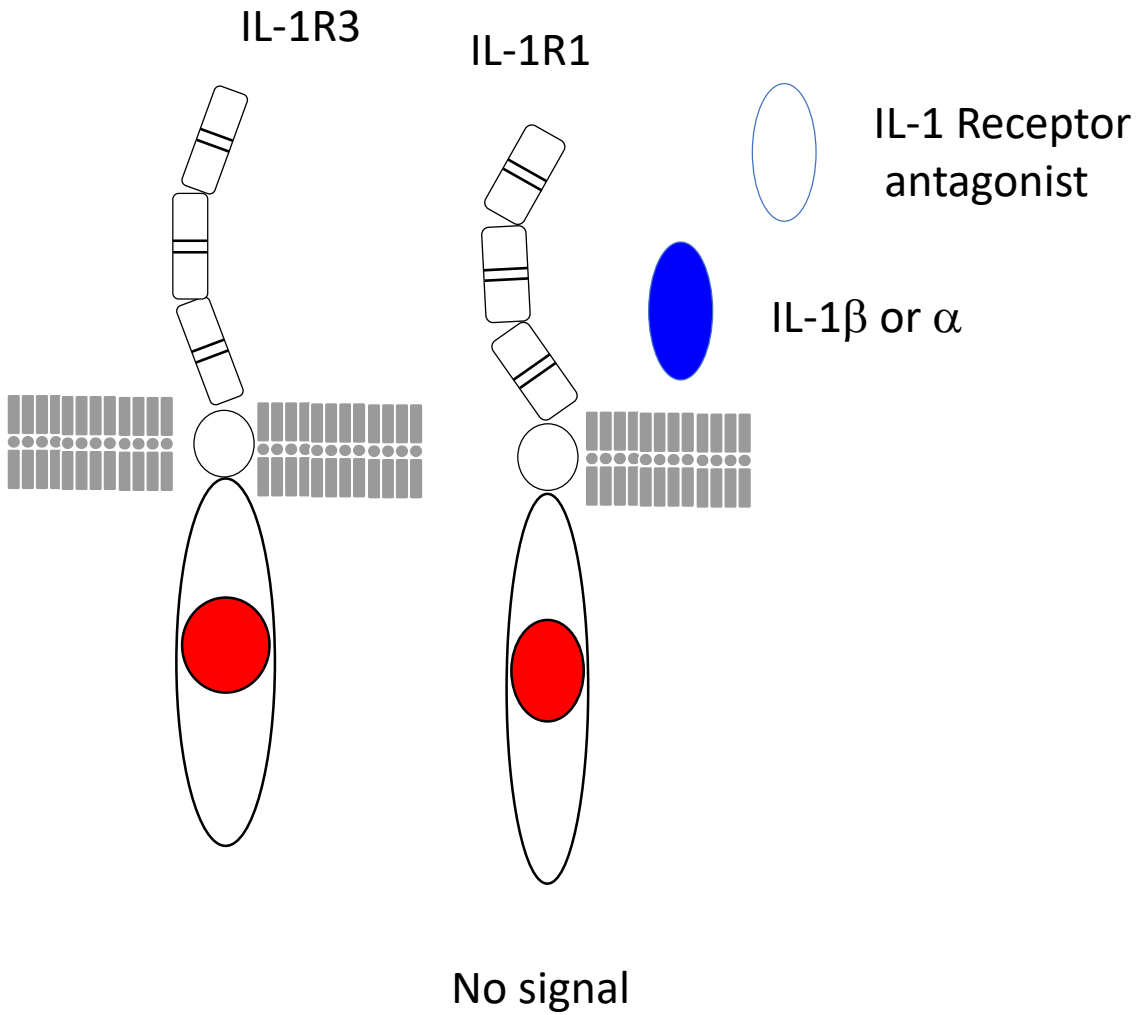
Italy
Greece
Canada
New Zealand
UK
REMED CAPS

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



REMAP-CAP
Randomized, Embedded, Multifactorial Adaptive Platform trial for Community-Acquired Pneumonia

Domain-Specific Appendix:
COVID-19 Immune Modulation Therapy

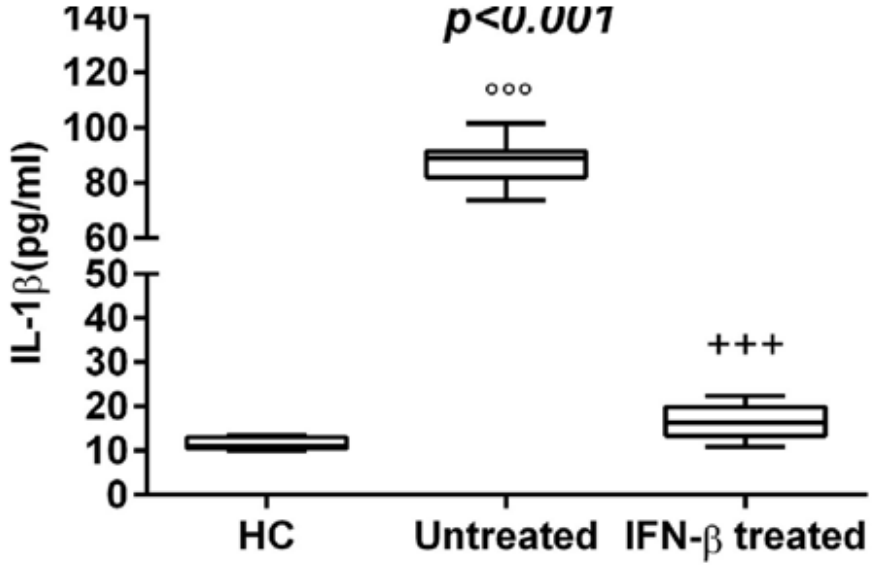
In this domain of the REMAP-CAP trial, participants meeting the platform entry criteria for REMAP-CAP admitted to participating intensive care units with suspected or microbiological testing-confirmed COVID-19 infection will be randomized to receive one of up to three interventions depending on availability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

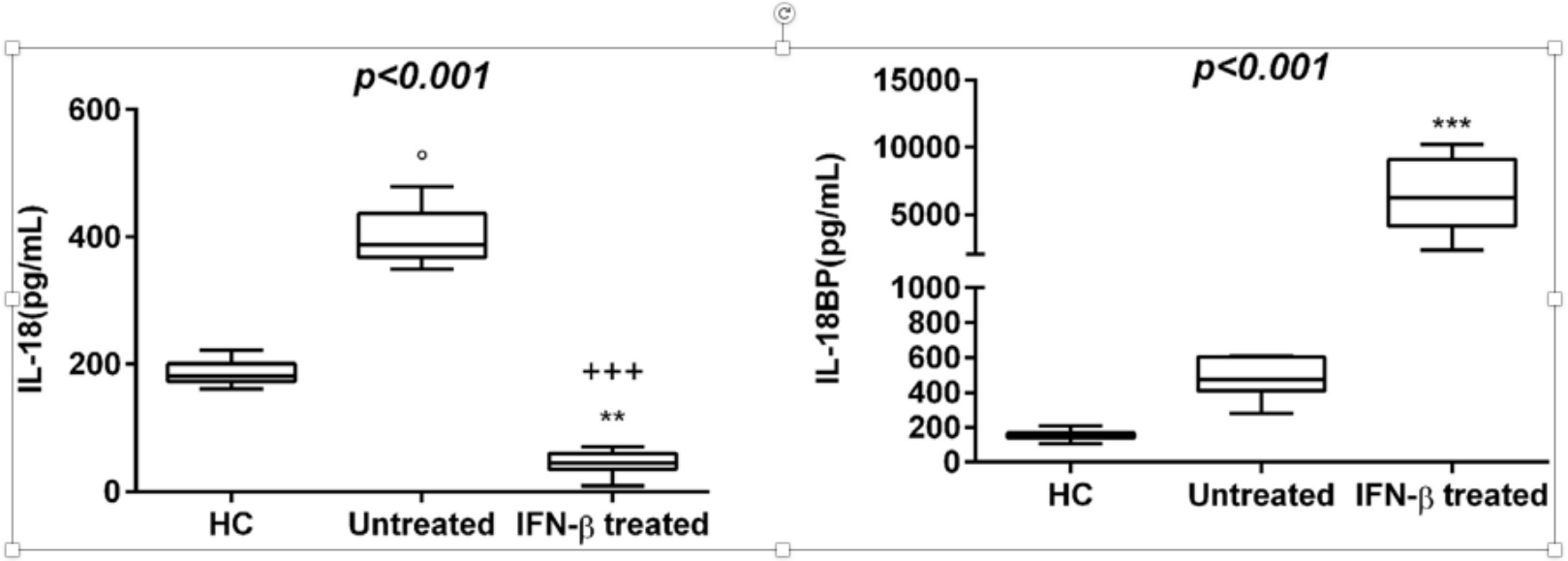
- No immune modulation for COVID-19 (no placebo)
- interferon-beta-1a (IFN-β1a)
- anakinra i.e. interleukin-1 receptor antagonist (IL1Ra)

What is the rationale for Interferon-β?

RESULTS

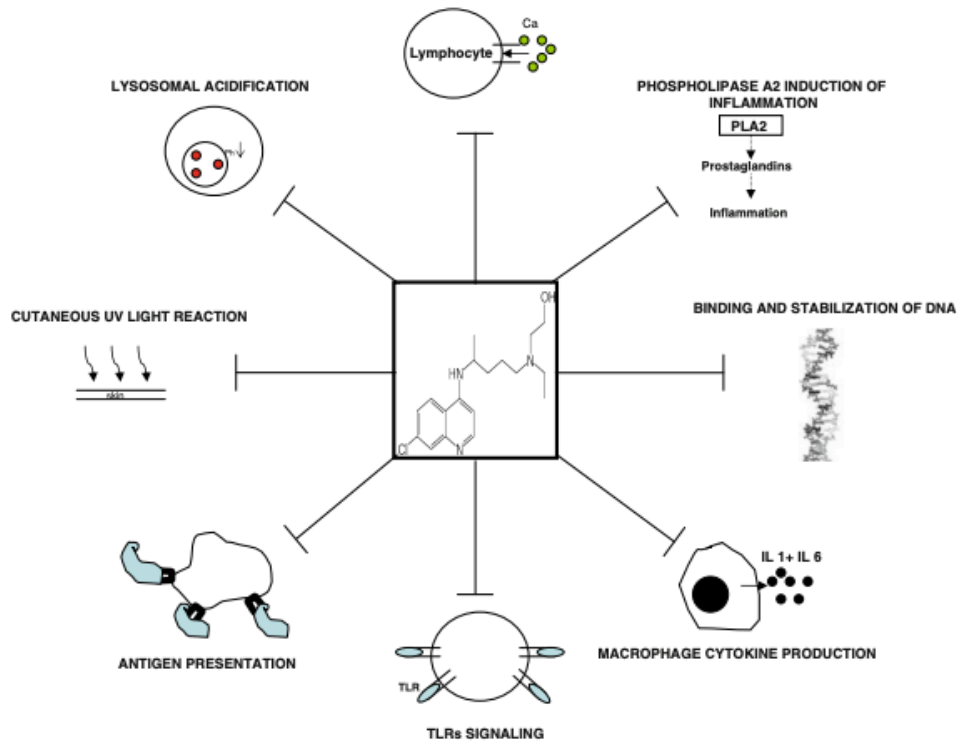
IFN-β Suppresses Both Pro-IL-1β Availability and IL-1β Maturation





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.

Disclosures: CAD serves as the Chairperson of Olatec's SAB. The Dinarello Laboratory at the University of Colorado receives support from Olatec.