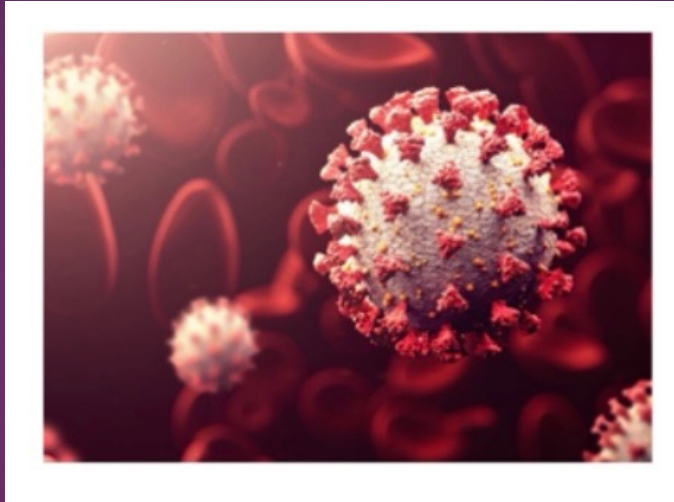


Cyclosporin H: A Novel Anti-Inflammatory Therapy with Applications for Covid-19 Patients



Salwa A. Elgebaly, Ph.D.

Founder & CEO, Nour Heart, Inc.

Vienna, Virginia, U.S.

Department of Surgery, Univ. of Connecticut

School of Medicine, Farmington, CT, U.S.

Cairo University - July 7, 2021



Publications



The Journal of Immunology

Nourexin-4 A Novel Anti-inflammatory Therapy for Influenza Flu (52.1)

Salwa Elgebaly, Daniel Perez, Kathleen Sullivan, Craig Whitaker, Stephanie Caspe, Qiao Yi and Donald Kreutzer

J Immunol April 1, 2010, 184 (1 Supplement) 52.1;

Journal of the Egyptian Society of Parasitology, Vol. 47, No. 1, April 2017
 J. Egypt. Soc. Parasitol. (JESP), 47(1), 2017: 25 - 33

CYCLOSPORIN H: A NOVEL ANTI-INFLAMMATORY THERAPY FOR INFLUENZA FLU PATIENTS

By

SALWA A. ELGEBALY^{1*}, TAMER ELBAYOUMI², and DONALD L. KREUTZER³

2020 Citations in Covid-19 Patients Papers

Repurposing Immunomodulatory Therapies against Coronavirus Disease 2019 (COVID-19) in the Era of Cardiac Vigilance: A Systematic Review.

Campbell CM, Guha A, Haque T, Neilan TG, Addison D

J Clin Med, 9(9), 11 Sep 2020

Cited by: 1 article | PMID: 32932930 | PMCID: PMC7565788

Cyclosporine therapy in cytokine storm due to coronavirus disease 2019 (COVID-19).

Cure E, Kucuk A, Cumhur Cure M

Rheumatol Int, 40(7):1177-1179, 15 May 2020

Cited by: 10 articles | PMID: 32415310 |

PMCID: PMC7227450

Cyclosporine: an old weapon in the fight against coronaviruses.

Molyvdas A, Matalon S

Eur Respir J, 56(5), 26 Nov 2020

Cited by: 2 articles | PMID: 32732332 |

PMCID: PMC7397953

Outline

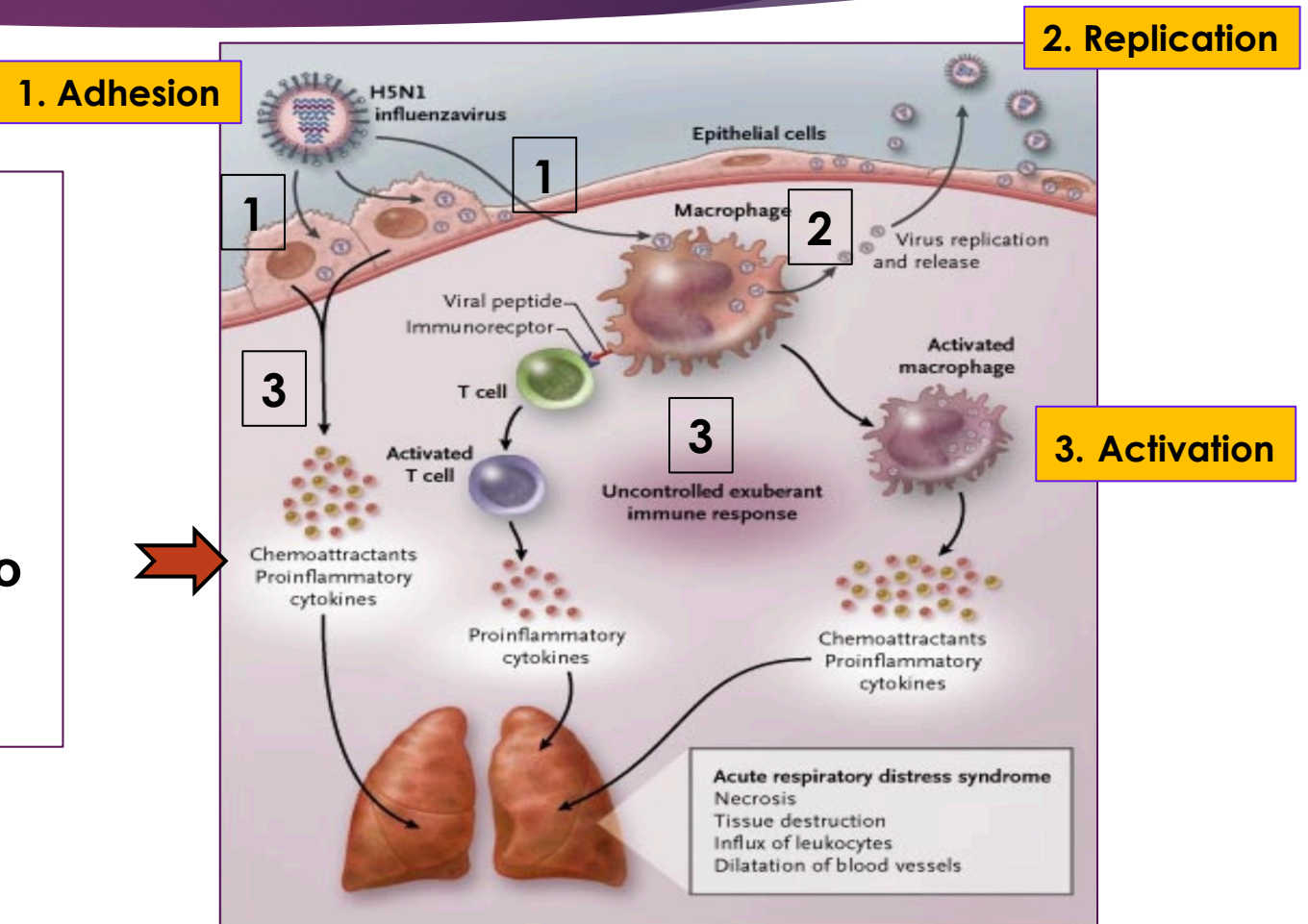
- ▶ **Cyclosporin H and Lethal H1N1 Swine Influenza Flu Virus**
 - Cytokine Storm
 - Nourin and Cytokine Storm
 - Cyclosporin H Inhibits Nourin and Lung Inflammation
- ▶ **Cyclosporin H and Covid-19 Patients**
 - Cyclosporin H and Cardiac Inflammation in Covid-19 Patients
- ▶ **Conclusions**

A Proposed Mechanism for Influenza Flu Virus Infection in Lungs (Corona Virus)

➤ Stages of Viral Infection:

1. Adhesion
2. Replication
3. Activation

➤ Virus-induced cytokine storm leads to acute respiratory distress syndrome (ARDS)



What is a Cytokine Storm?

“Role in Tissue Necrosis”

Viral and Bacterial Infection:

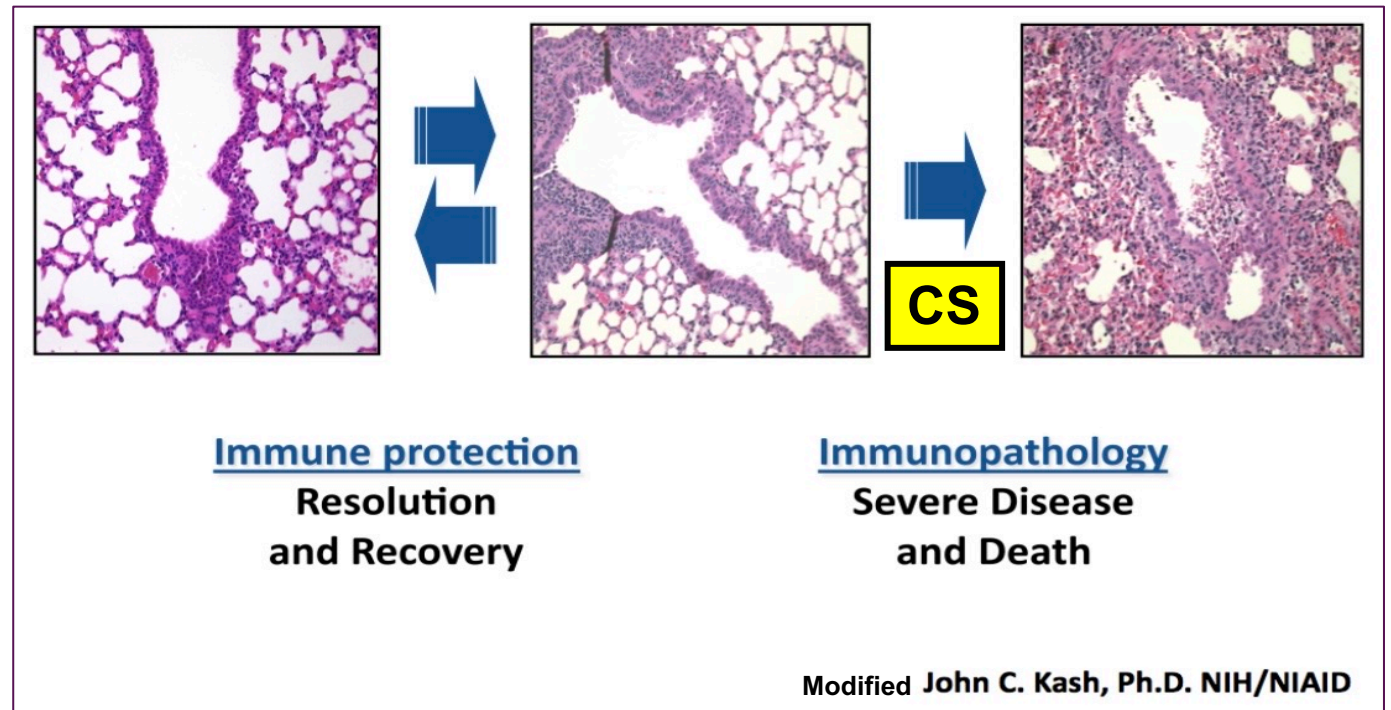
Immune Protection:

Immune response results in resolution and recovery

&

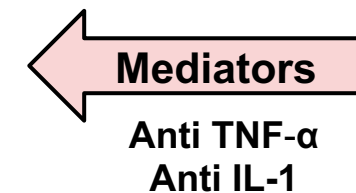
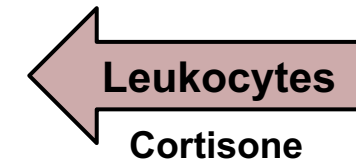
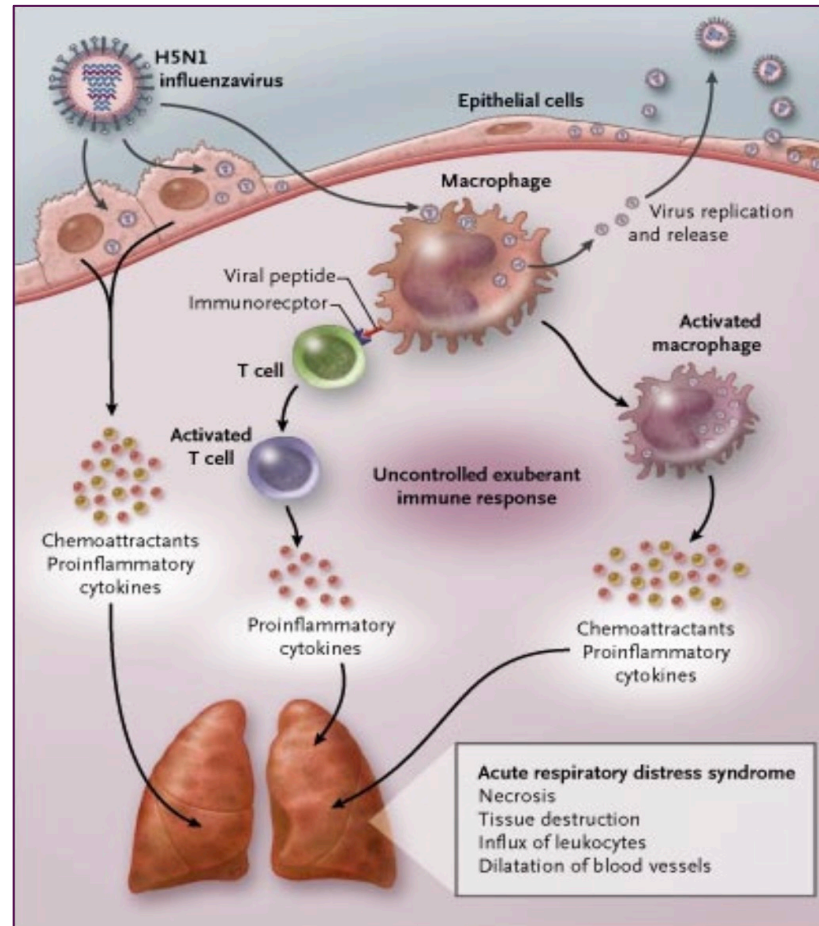
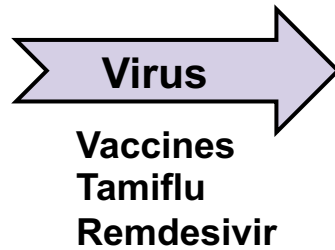
Immunopathology:

Viral and bacterial-Induced overactive inflammatory response, resulting in cytokine storm (**CS**)



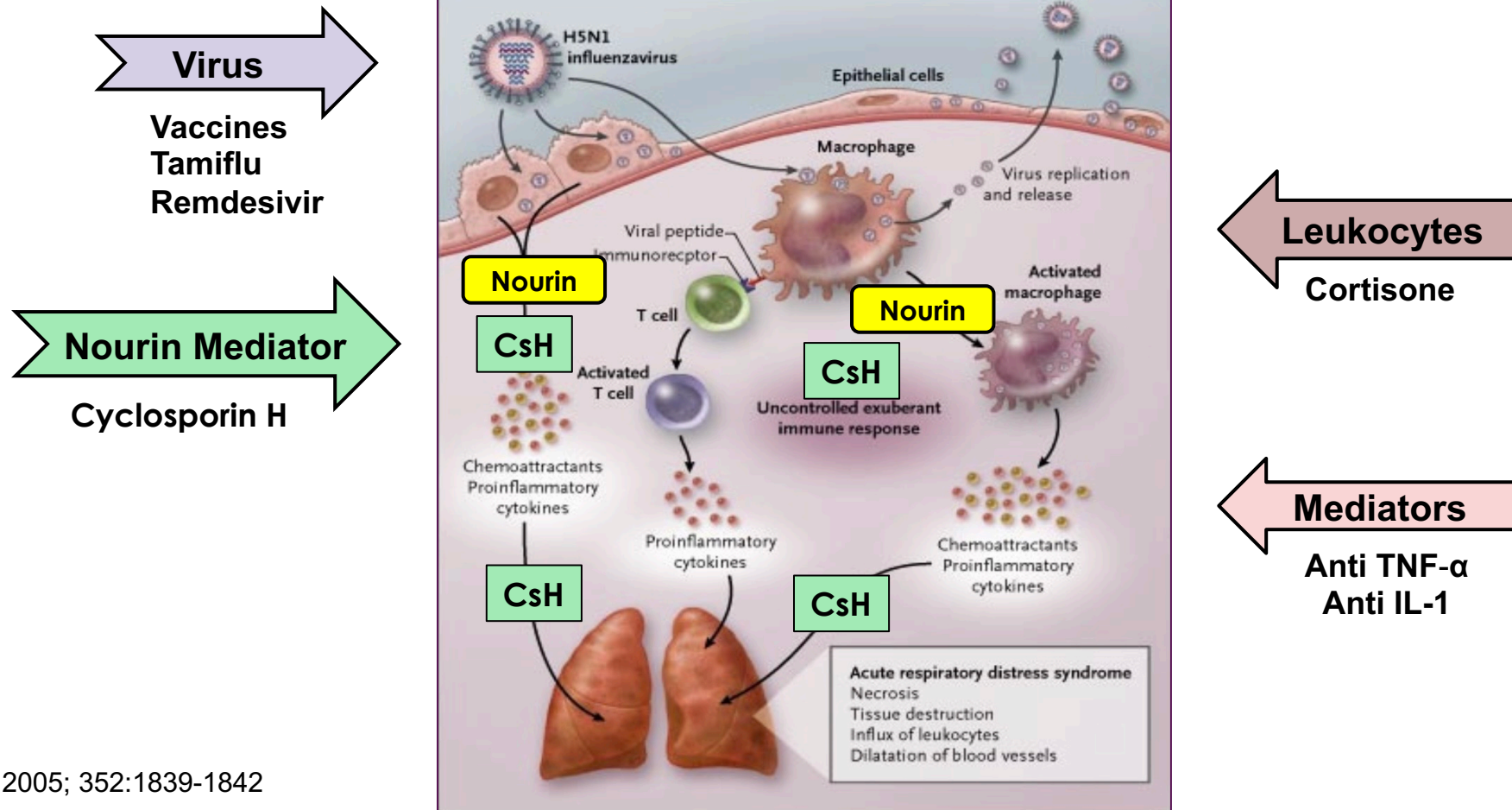
Current Therapeutic Approaches

“Targeting Virus, Leukocytes and Mediators”



Cyclosporin H (CsH): A Novel Anti-inflammatory

“Inhibitor of Inflammatory Mediator, Nourin”



Phases of Development of Cytokine Storm that Leads to Acute Respiratory Distress Syndrome (ARDS)

▶ Triphasic ARDS – 3 Weeks:

1. Viral Replication Phase – Week 1

- First 7 days
- Low levels of Cytokines & Leukocytes

2. Immune Hyperactive Phase – Week 2

- Second 7 to 14 days
- High Cytokine Storm + Inflammation

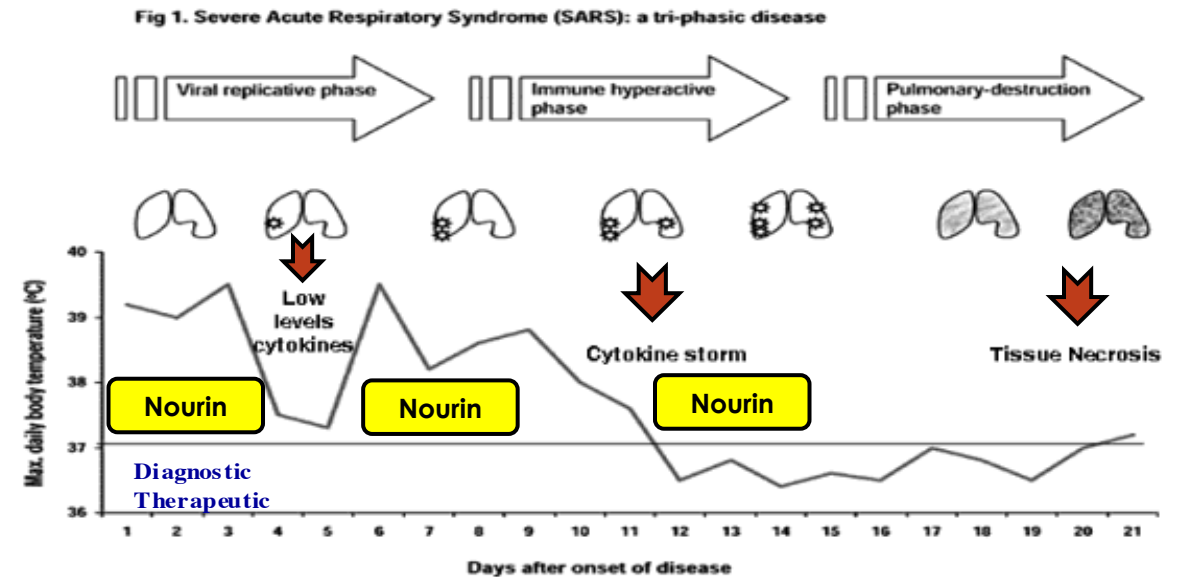
3. Pulmonary Destruction Phase – Week 3

- Third 14 to 21 days
- Tissue Necrosis

▶ Nourin & Cytokine Storm Mediators:

Leukocyte Chemotaxis, Adhesion & Activation

Nourin & Development of Cytokine Storm



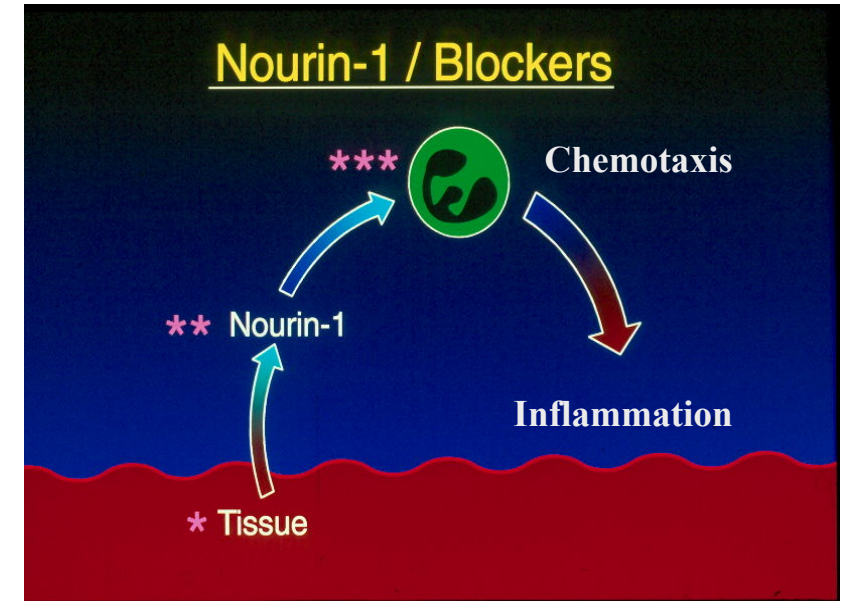
Modified from Lu PS, Science 312 (5772), 337, 2006

What is Nourin?

A Novel “Injury Response” Molecule!

NOURIN:

- ▶ Rapidly released by local tissues in response to injury
- ▶ Potent inflammatory mediator (*Review: Elgebaly 2017 & 2019*)
- ▶ Shares 3 KDa formyl peptide, but differs in isoelectric point
- ▶ Binds to formyl peptide receptor (FPR) on leukocytes and vascular endothelial cells (VECs)
- ▶ Stimulates leukocyte chemotaxis, adhesion and activation
- ▶ It is associated with acute and chronic tissue inflammation
- ▶ **Activates human leukocytes and VECs to express:**
 - Cytokine storm mediators
 - Digestive enzymes
 - Free radicals



Elgebaly SA, et al. Expert Review of Cardiovascular Therapy. 2019 ,Sep 2;17(9):683-97, REVIEW

Elgebaly SA, et al. J Eg Soc Parasitol. 2017;47(1):27–35

Elgebaly SA, et al. Amer. Assoc. of Immunologists. 2010; May 97, 52

Elgebaly SA, et al. *Circulation*. 2020; 142, A13051-A13051

Elgebaly SA, et al. *Circulation*. 2020; 142, A13103-A13103

Elgebaly SA, et al. *Biomolecules*. 2021; 11, 368

Elgebaly SA, et al. *Diagnostics*. 2021; 11, 703

Elgebaly SA, et al. *Int. J. Mol. Sci*. 2021; 22, 3575

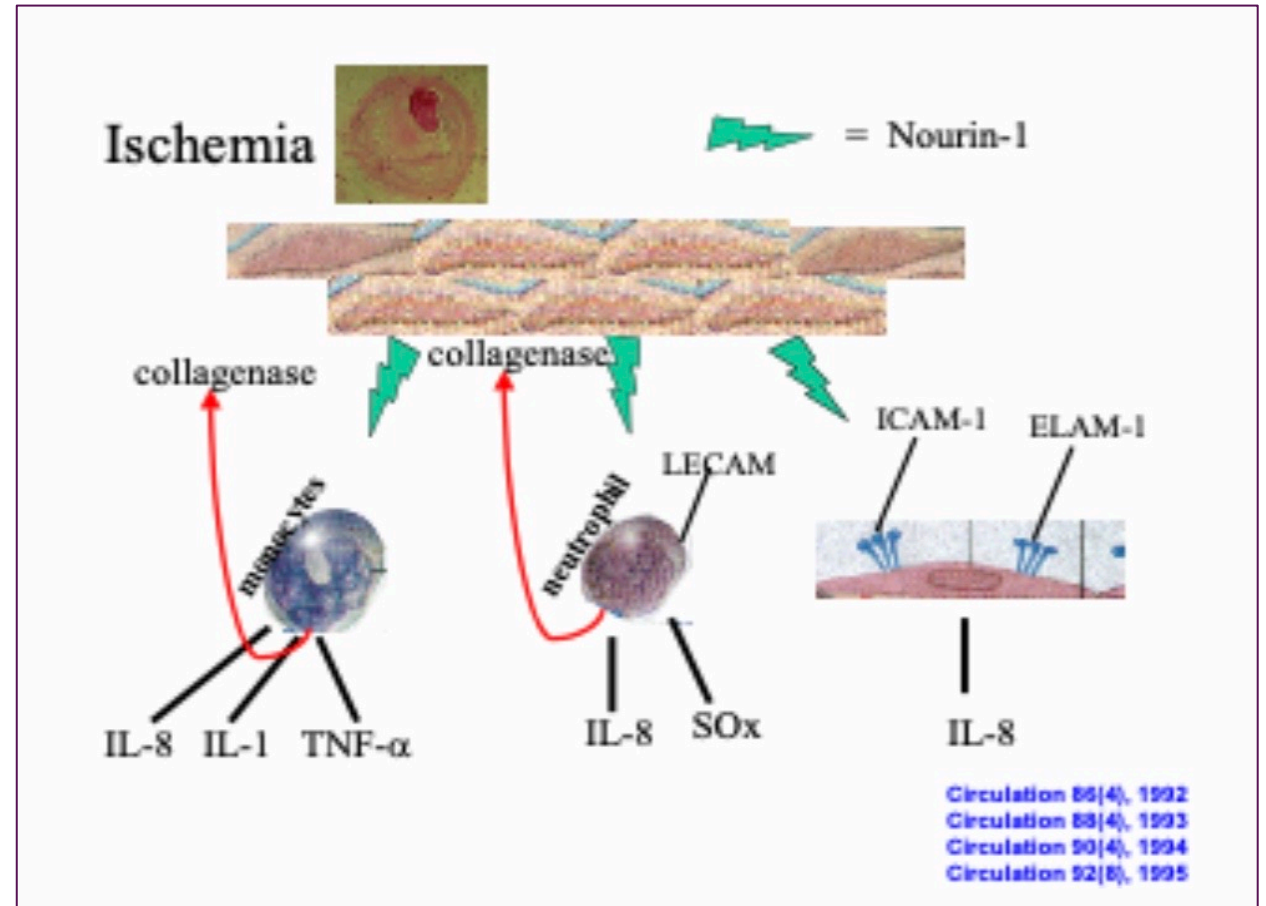
Nourin Stimulates Expression of Cytokine Storm Mediators by “Neutrophils” & “VECs”

Neutrophils (24 hours):

- Chemotactic factor, IL-8
- Adhesion molecule, LECAM-1
- Free radicals, superoxide anion
- Digestive enzymes:
 - N-acetyl-B-glucosaminidase
 - Collagenase
 - Gelatinases (matrix remodelling)

Aortic Vascular Endothelial Cells (24 hours):

- Chemotactic factor, IL-8
- Adhesion molecule, ICAM-1
- Adhesion molecule, ELAM-1



Nourin Stimulates Expression of Cytokine Storm Mediators by Human “Monocytes”

Monocytes (4 hours)

Inflammatory Mediator	Treatment	
	Nourin	Control Media
Interleukin-8 (ng/mL)	12,000	2,000
Interleukin-1 β (pg/mL)	400	10
TNF- α (pg/mL)	400	<10

IL-8, IL-1 β and TNF- α : Key mediators in cytokine storm

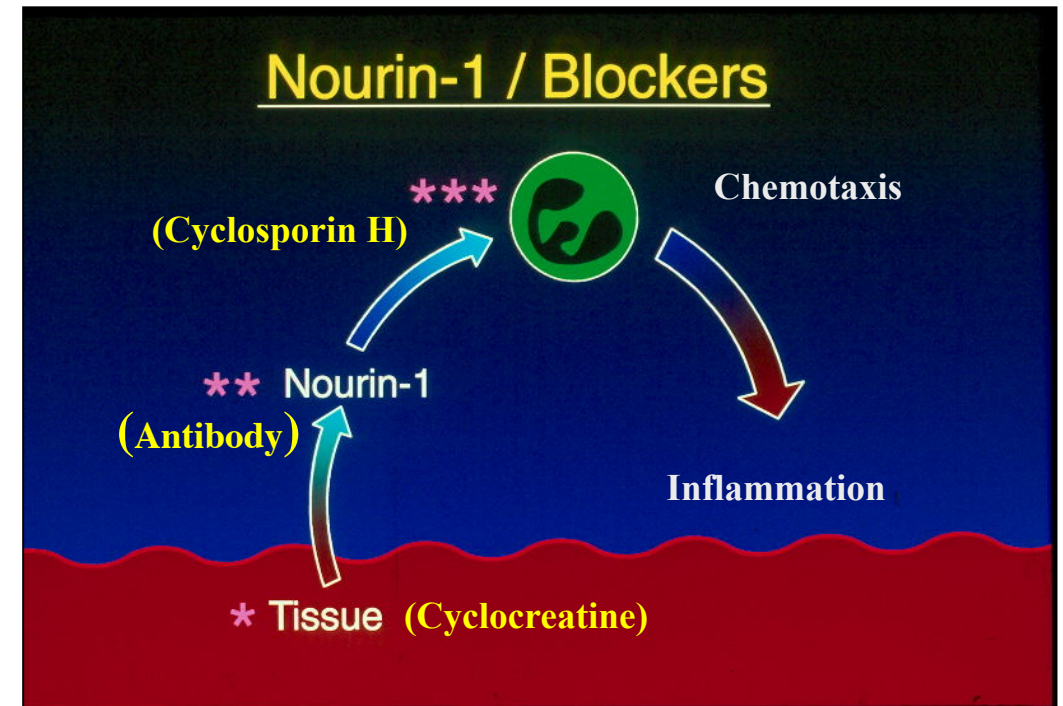
TNF- α : Key stimulant of apoptosis

TNF- α concentration in **excess of 1 ng/ml** is frequently predictive of a **lethal outcome**

What Are Nourin Antagonists?

- ▶ **Competitive antagonists** inhibit Nourin chemotactic activity and reduce tissue inflammation:
 - Cyclosporin H
 - Spinorphin
 - Soluble FPR fragment 17 aa loop peptide

- ▶ **A bioenergetic compound**, Cyclocreatine Phosphate (CCrP) prevents tissue injury, reduces Nourin intracellular formation and circulating levels, resulting in reduction of tissue inflammation



Elgebaly SA, et al. Expert Review of Cardiovascular Therapy. 2019 ,Sep 2;17(9):683-97, REVIEW

Elgebaly SA, et al. J Eg Soc Parasitol. 2017;47(1):27-35

Elgebaly SA, et al. Amer. Assoc. of Immunologists. 2010; May 97, 52

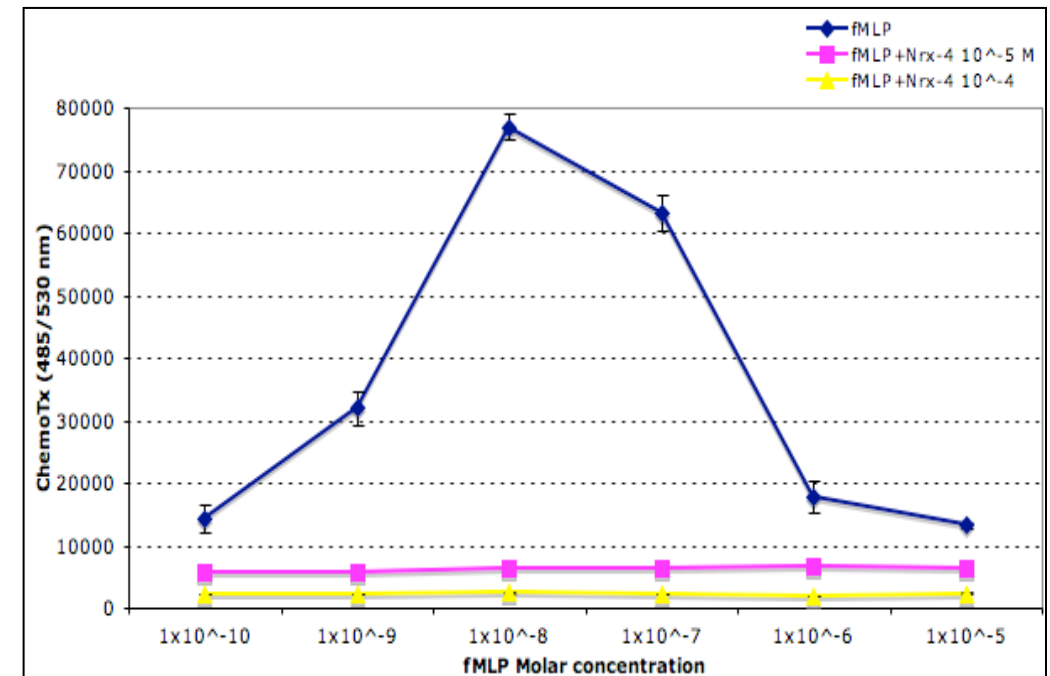
Elgebaly SA, et al. Int. J. Mol. Sci. 2021; 22, 3575

What is Cyclosporin H?

“Inhibitor of Bacterial Product 1 – FMLP”

- ▶ Cyclosporin H (CsH) is L isomer of Cyclosporin A
- ▶ Cyclosporin H is a potent **anti-inflammatory**
- ▶ Cyclosporin A is an **immunosuppressant**. In mice infected with influenza flu virus, CsA treatment:
 - Increased virus load in lungs
 - Delayed the rate of viral elimination
 - Increased mortality
- ▶ CsH and FMLP; a Nourin-related bacterial product
 - CsH is a competitive antagonist of formyl peptides (FP) on leukocyte FPR
 - Inhibits FMLP-induced neutrophil chemotaxis

CsH (Nourexin-4) (5×10^{-4} M - 5×10^{-5} M) Inhibition of fMLP Chemotactic Activity in vitro

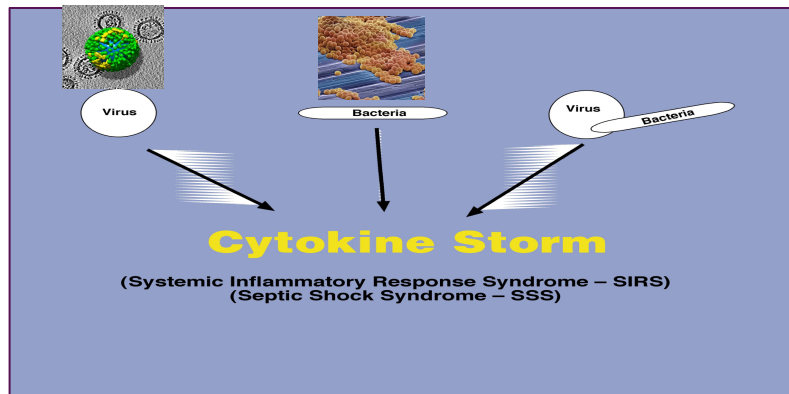


Elgebaly SA, et al. J Eg Soc Parasitol. 2017;47(1):27–35

Elgebaly SA, et al. Amer. Assoc. of Immunologists. 2010; May 97, 52

Other Anti-inflammatory Activity of CsH

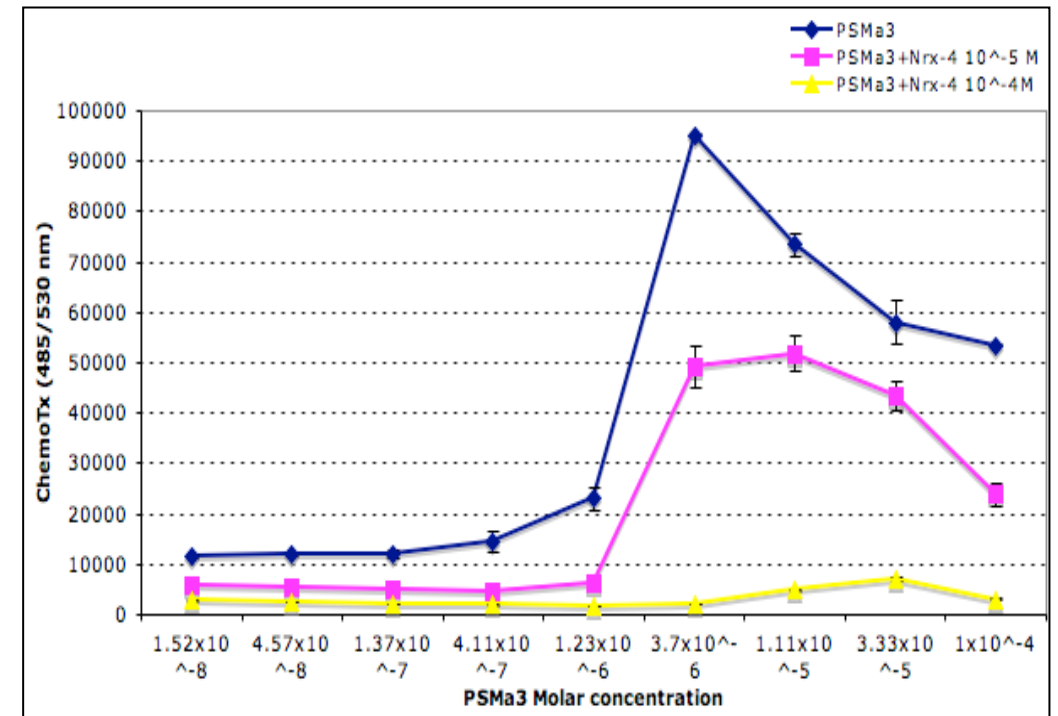
“Inhibitor of Bacterial Product 2 - Staph. aureus Toxin”



Sepsis

- ▶ **MRSA: Methicillin Resistant *Staph. aureus***
 - ▶ Toxins released by *S. aureus* are formyl peptides: **PSMa3** (*J. Nature Medicine Vol 11, 1-5, 2007*)
 - ▶ *S. aureus* formyl peptides are *potent* inflammatory mediators
 - ▶ Removal of *S. aureus* formyl peptide toxins increased survival
- ▶ **CsH inhibits neutrophil chemotaxis by *S. aureus* toxin, PSMa3**

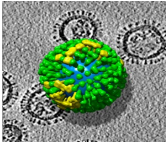
CsH (Nourexin-4) (5×10^{-4} M - 5×10^{-5} M) Inhibition of Chemotactic Activity Induced In-vitro by *S. aureus*-derived PSMa3



Elgebaly SA, et al. J Eg Soc Parasitol. 2017;47(1):27–35

Elgebaly SA, et al. Amer. Assoc. of Immunologists. 2010; May 97, 52

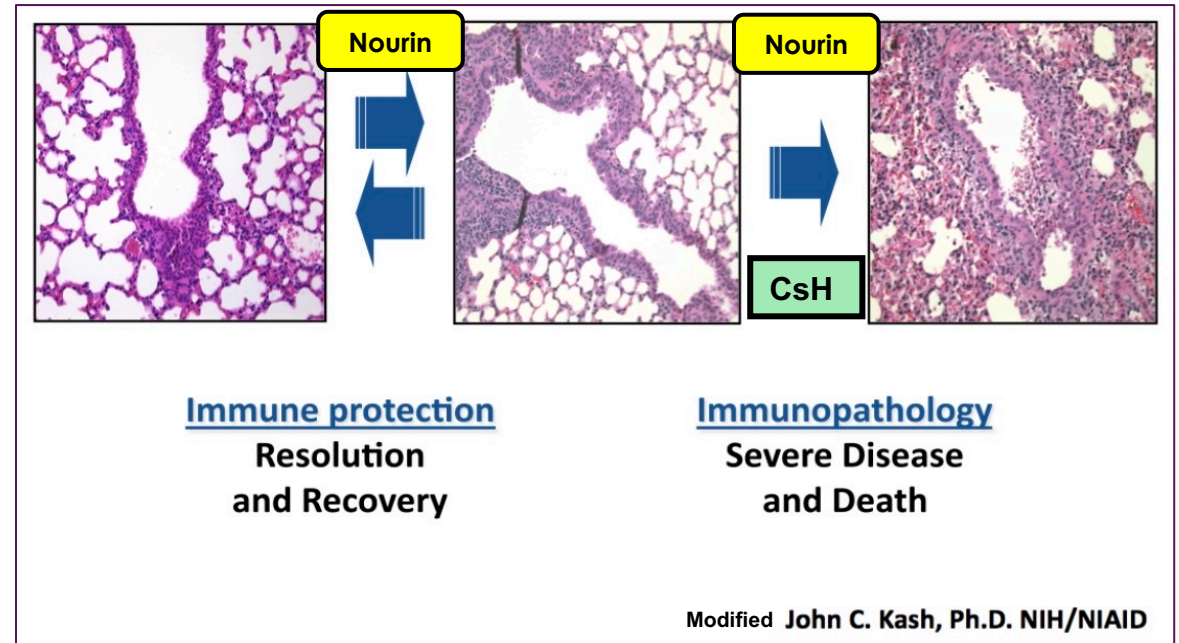
Cyclosporin H and Viral Infection



Lethal H1N1 Swine
Influenza Flu Virus

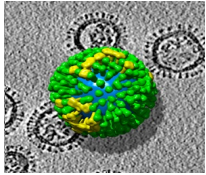
Hypothesis

1. Influenza flu virus infection of airway epithelial cells will trigger cell injury and the release of cell-derived pro-inflammatory mediators, including the formyl peptide, Nourin.
2. The Nourin antagonist, CsH will inhibit Nourin activity and initial lung inflammation in mice infected with the lethal H1N1 Swine influenza flu virus for 5 days (CS 3 to 8 days).



Experimental Plan 1:

- To confirm Nourin Release in vitro and in vivo
- Inhibition of Nourin Activity by CsH



Lethal H1N1 Swine Influenza Flu Virus

- ▶ Release of Nourin by cultured epithelial cells infected with laboratory influenza virus (PR8) for 24 h; *inhibition by CsH*
- ▶ Detection of Nourin in serum of mice infected with lethal H1N1 Swine influenza flu virus for 6 h; *inhibition by CsH*
- ▶ Detection of Nourin in plasmas of patients with *severe, moderate* H1N1 Swine influenza flu virus and *mild* RSV; *inhibition by CsH*



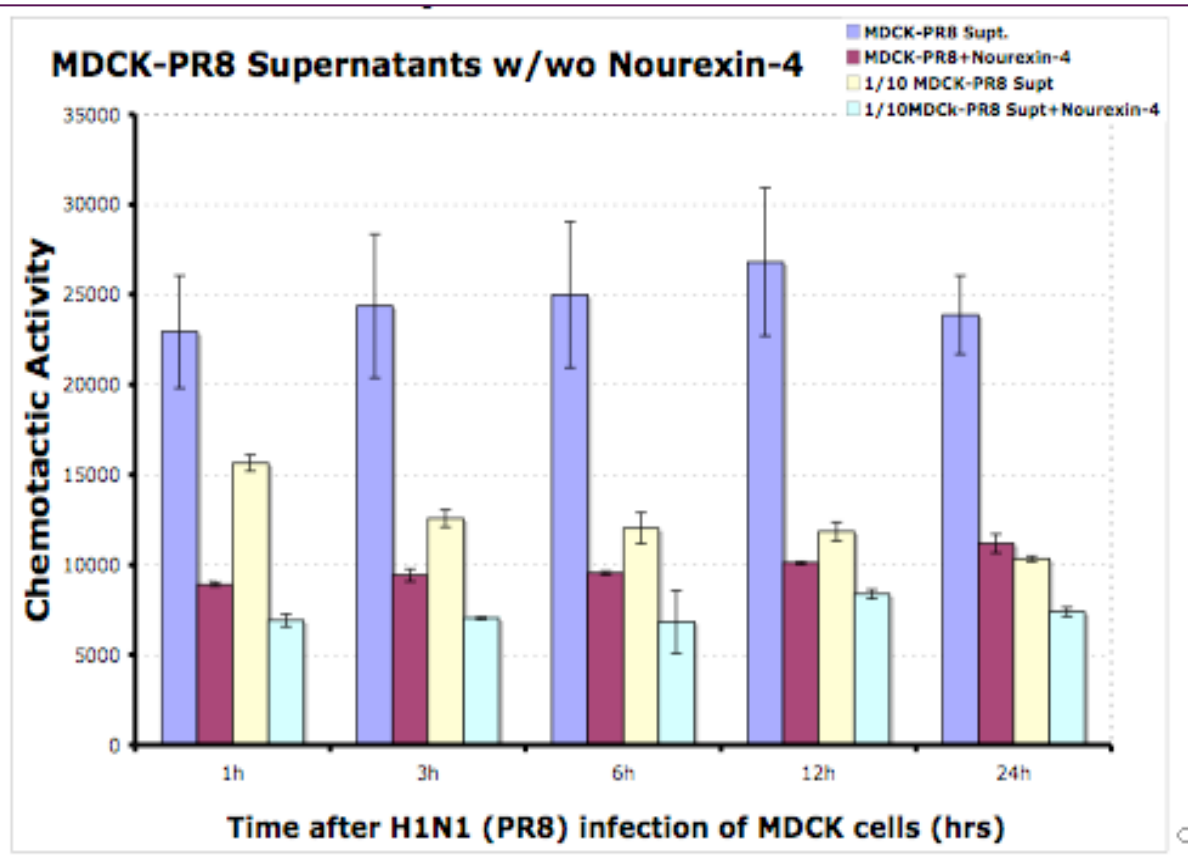
Results:

1. Cultured Epithelial Cells Infected with PR8 Influenza Virus

Cell Culture



- ▶ Nourin is rapidly released by 1 h after PR8 virus-infected epithelial cells
- ▶ Nourin release continued for 24 h
- ▶ Cyclosporin H (Nourexin-4) (5×10^{-6} M) inhibited Nourin-induced neutrophil chemotactic activity to control value



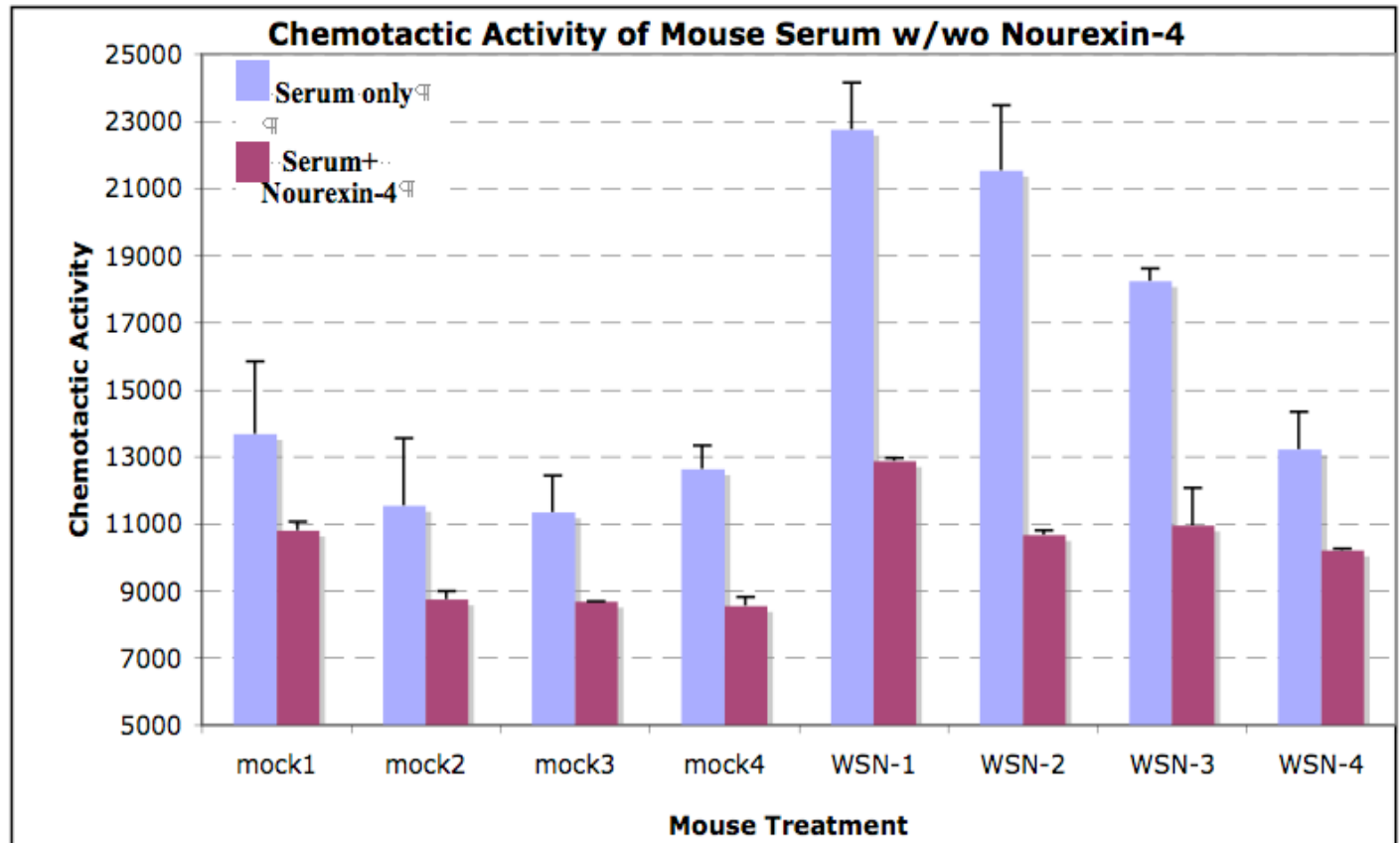
Results:

2. Mice Infected with H1N1 Swine Influenza Flu Virus for 6 hours

Mice



- ▶ **Nourin detected in serum 6 h after infection with H1N1 Swine influenza flu virus (nasal inoculation)**
- ▶ **CsH (5×10^{-6} M) inhibited Nourin-stimulated neutrophil chemotaxis to control level in mice infected for 6 h**



Results:

3. Nourin Levels in Patients with H1N1 Swine Influenza Flu Virus

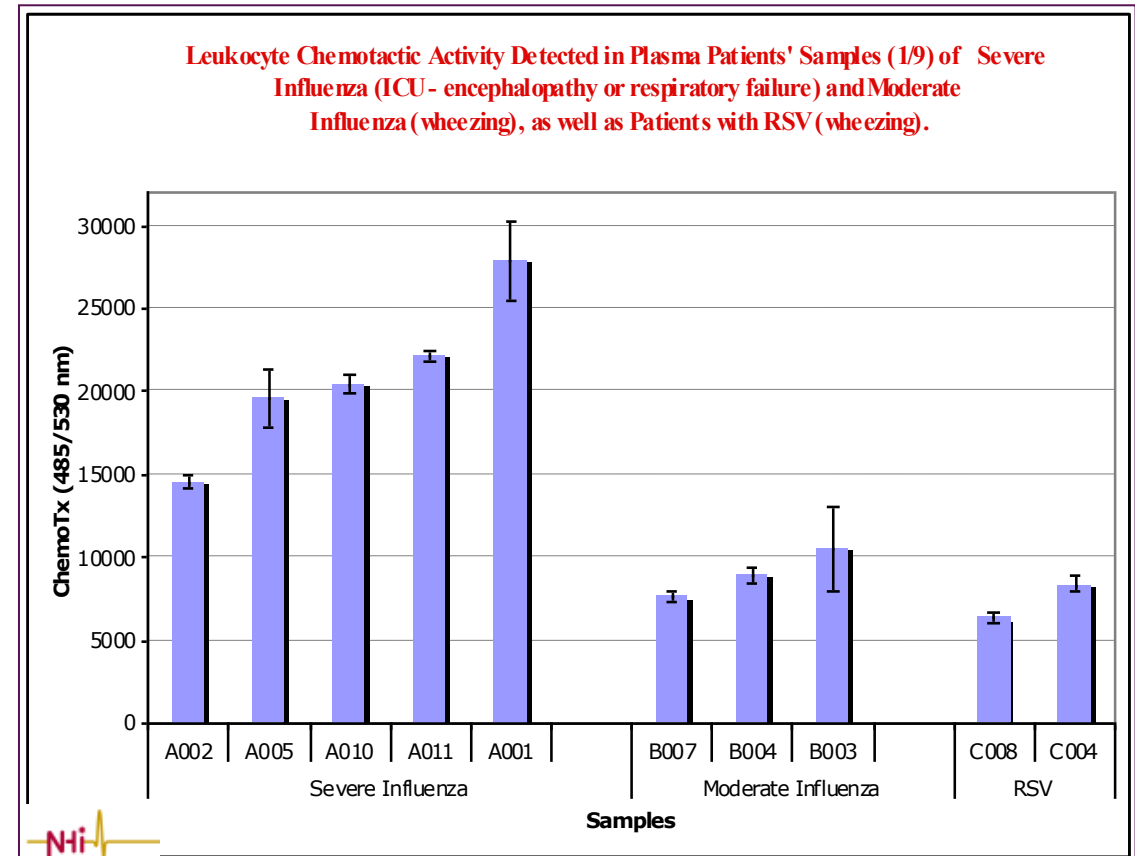
Patients



Dr. Kathleen Sullivan, PA

High level of Nourin was detected in plasma samples collected from “severely” ill patients infected with H1N1 Swine influenza flu virus (ICU patients)

- ▶ To a lesser extent, Nourin level in hospitalized “moderate” influenza patients
- ▶ Low level of Nourin was detected in plasma samples collected from patients with “low” respiratory syncytial virus (RSV) infection



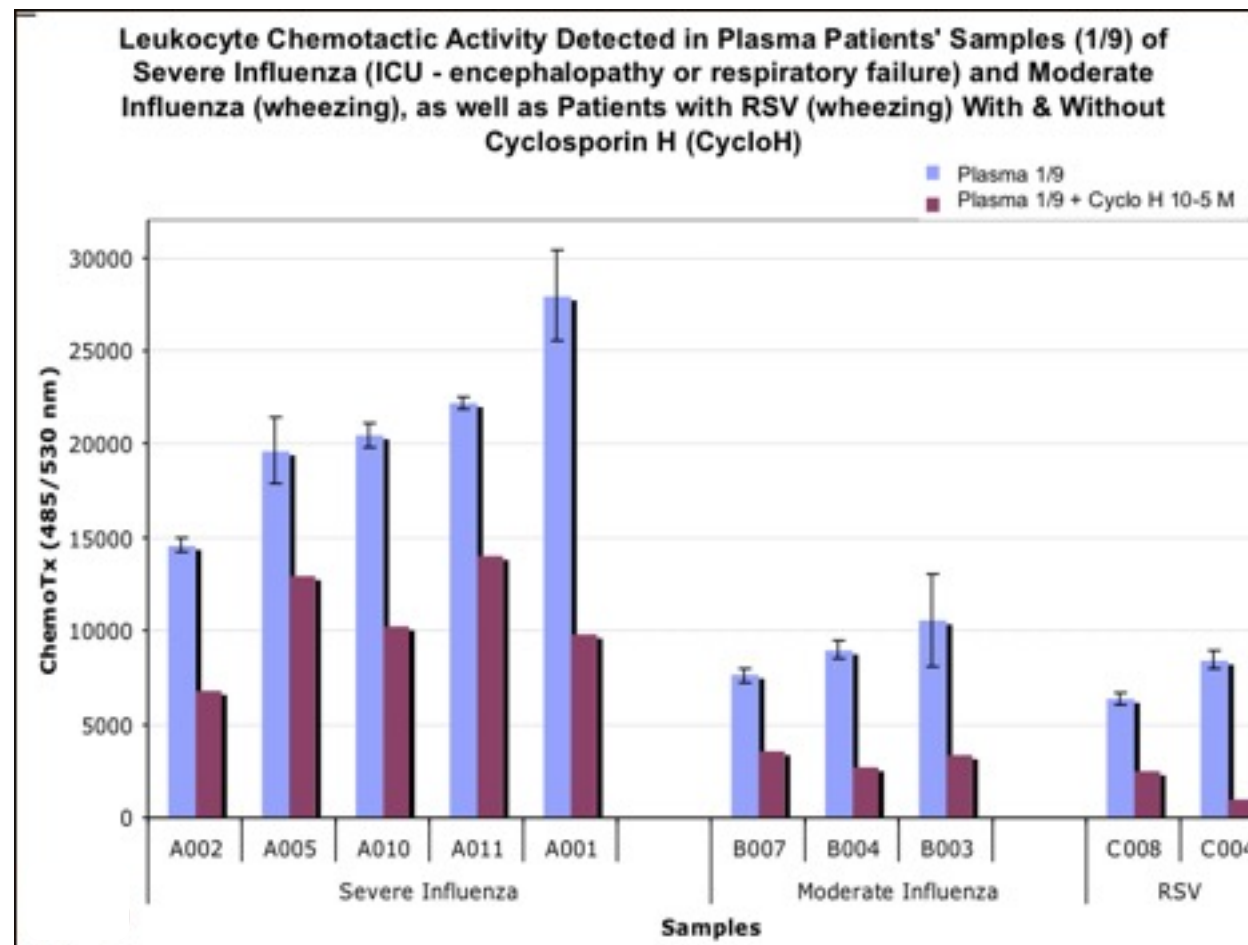
Results:

4. CsH Inhibits Nourin in Patients' Plasmas

Patients



The Nourin competitive antagonist, Cyclosporin H at 10^{-5} M significantly inhibited neutrophil chemotaxis induced by host-derived Nourin as detected in all influenza patients' samples



Experimental Plan 2:

- Effect of CsH on Lung Inflammation

Mice

- ▶ **Mice infected with H1N1 Swine influenza flu virus were treated for 5 days with:**
 - **H1N1 + CsH (n=5)**
 - **H1N1 + CsH vehicle (n=4)**
 - **H1N1 + Saline (n=5)**

- ▶ **Healthy mice were treated with saline (n=4) for 5 days**

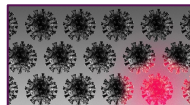
- ▶ **Analysis at day 5:**
 - **Viral titer in lungs**
 - **Body weight**
 - **Lung inflammation (histology)**



Results:

5. CsH Did Not Increase Viral Titer in Lungs

Mice

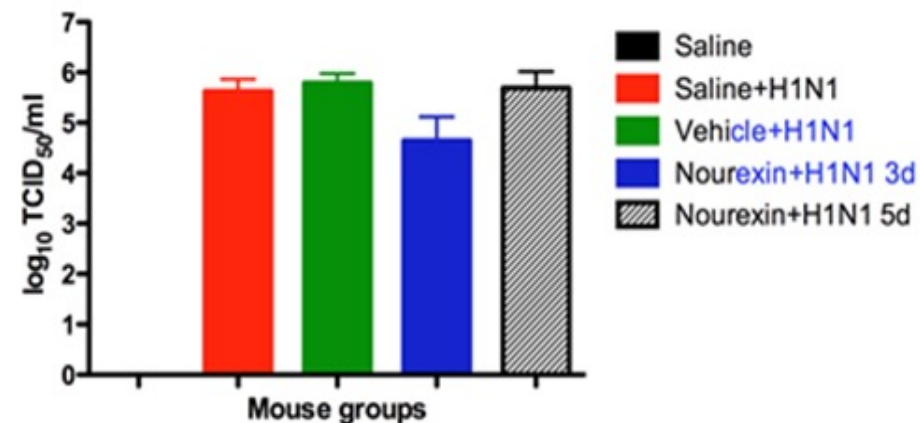


- ▶ Unlike Cyclosporin A, CsH did not increase viral load in the infected mice at day 5, when compared to H1N1 control
- ▶ CsH is not an immunosuppressant
- ▶ CsH does not target the influenza virus
- ▶ CsH did not change body weight compared to H1N1 saline control mice

Pilot study to test the *in vivo* anti-inflammatory activity of **Nourexin-4**

RESULTS:

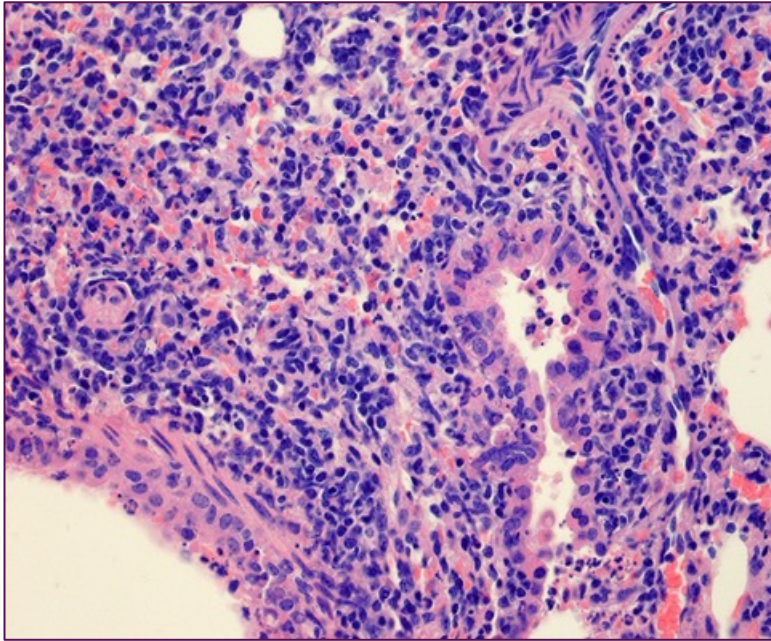
Lung titers 5 days post-challenge



Results:

6. CsH Reduces Mouse Lung Inflammation at Day 5

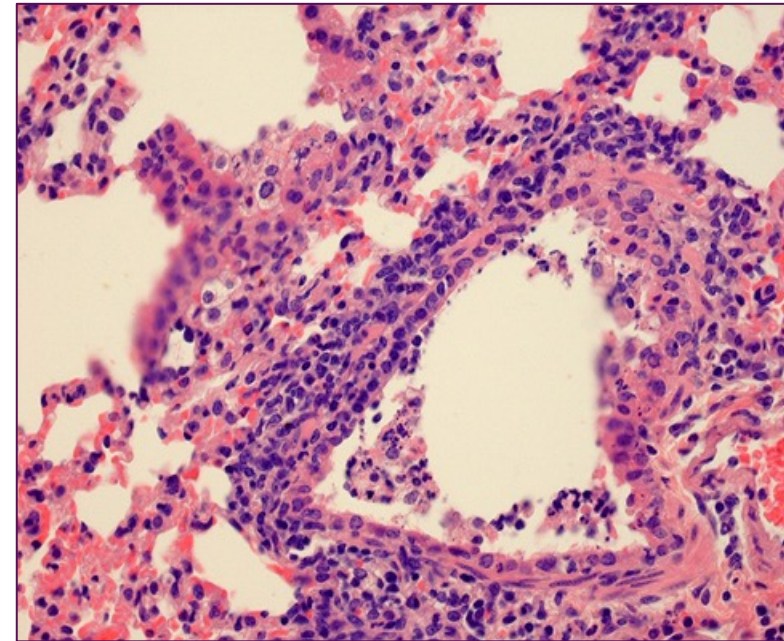
Saline



Saline
H1N1/Saline treated lung mouse – **HIGH** inflammation



CsH



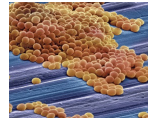
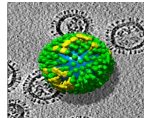
Cyclosporin H
H1N1/CsH treated lung mouse – **REDUCED** inflammation

Summary

Nourin

- ▶ Released “early” after viral infection and is critical for development of cytokine storm
- ▶ Plays a key role in viral-induced lung inflammation
- ▶ Plasma level correlates with disease severity in patients infected with H1N1 Swine influenza flu virus

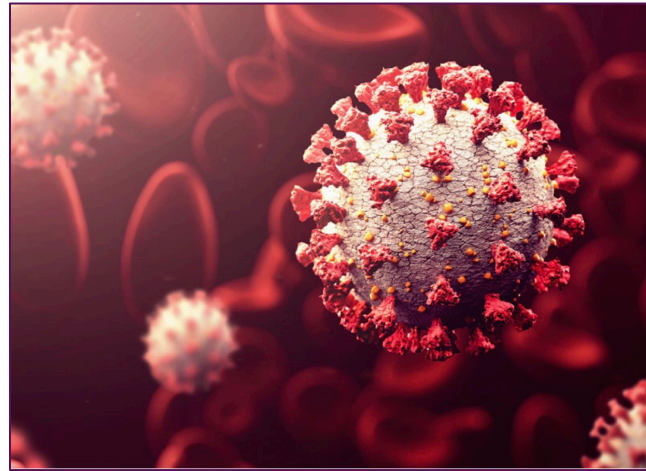
Cyclosporin H



- ▶ Significantly reduced Nourin activity and lung inflammation induced by H1N1 Swine influenza flu virus
- ▶ Not an immunosuppressant (did not reduce mice immunity – thus, no increase in viral replication)
- ▶ Does not target the influenza virus, but targets Nourin
- ▶ Completely blocked chemotactic activity induced by the lethal bacterial *S. aureus* toxin, PSM α 3

Cyclosporin H and Covid-19 Patients

Cyclosporin H and Lung Inflammation



SARS-CoV-2 Virus

Cyclosporin H and Heart Inflammation



Cardiac Inflammation in 60% of COVID-19 Patients

(JAMA Cardiology 2020)

Outcomes of Cardiovascular Magnetic Resonance Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19)

[Valentina O. Puntmann, MD, PhD¹](#); [M. Ludovica Carerj, MD^{1,2}](#); et al.

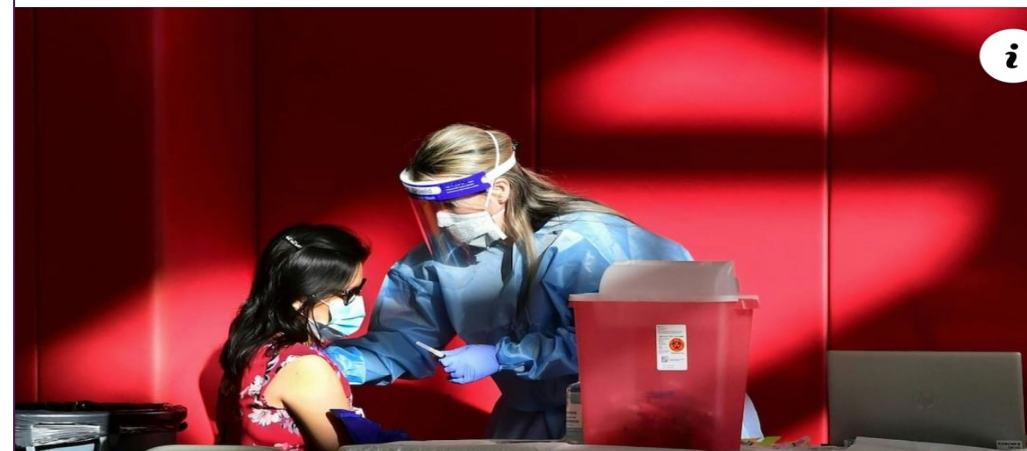
JAMA Cardiol. 2020;5(11):1265-1273. doi:10.1001/jamacardio.2020.3557



National Geographic ✓

2d · 🌐

The CDC has convened an emergency meeting of its advisory committee on June 18 to discuss rare reports of heart inflammation among people who have received the Pfizer and Moderna vaccines.

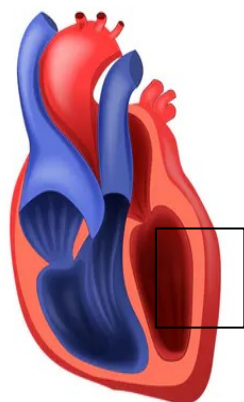


NATIONALGEOGRAPHIC.COM

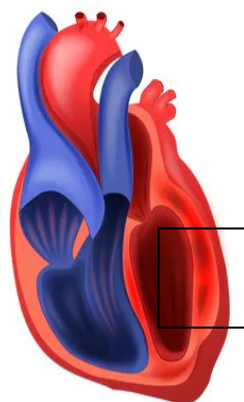
Latest: CDC to hold emergency meeting over rare cases of heart inflammation after vaccination

Cardiac Inflammation

MYOCARDITIS



healthy heart



myocarditis

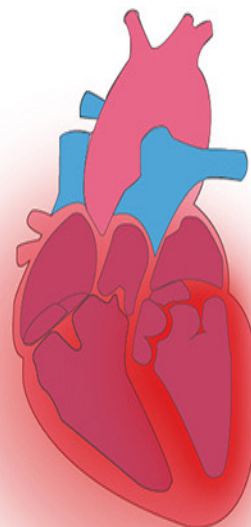
Myocardial Inflammation

Causes

Viruses
eg. Coxsackie B-Virus

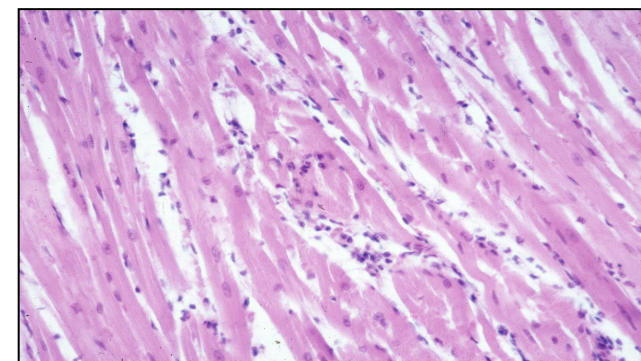


Bacteria



Symptoms

Weakness
Shortness of breath
Cardiac arrhythmias



Cardiac-derived Nourin is released by injured myocardium and associated with cardiac inflammation

Cyclosporin H will potentially inhibit cardiac inflammation in Covid-19 patients

Elgebaly SA, et al. *Expert Review of Cardiovascular Therapy*. 2019, Sep 2;17(9):683-97 - REVIEW

Elgebaly SA, et al. *Circulation*. 2020; 142, A13051-A13051

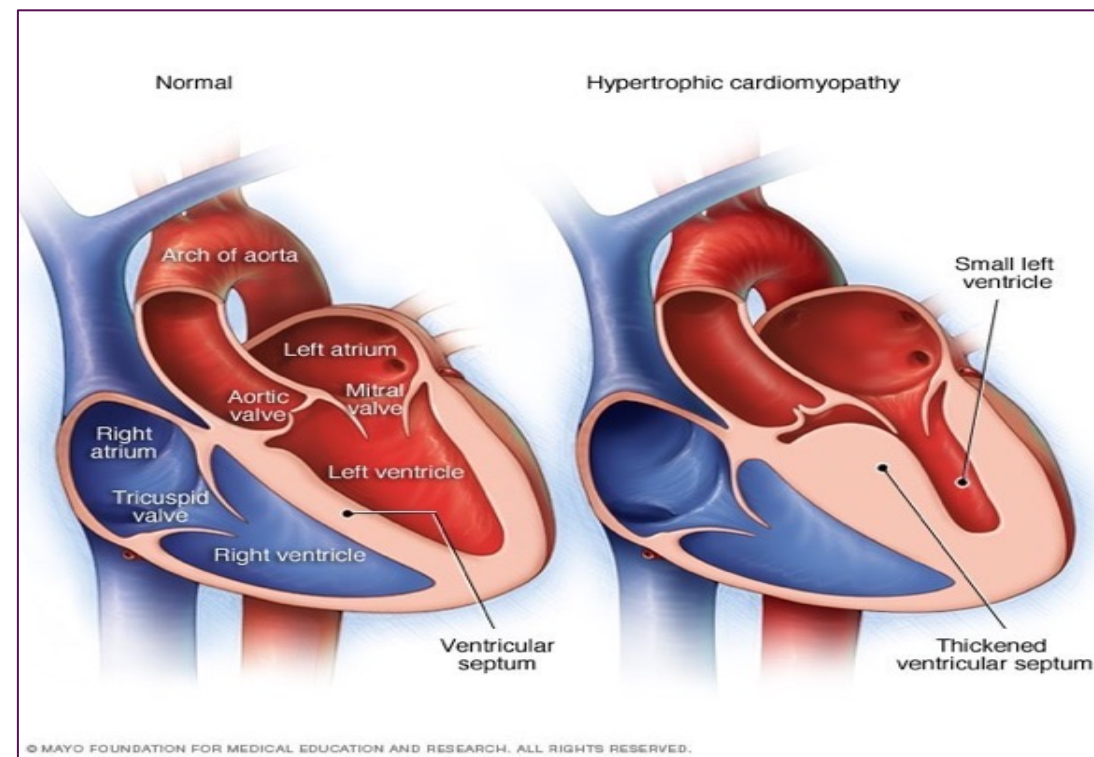
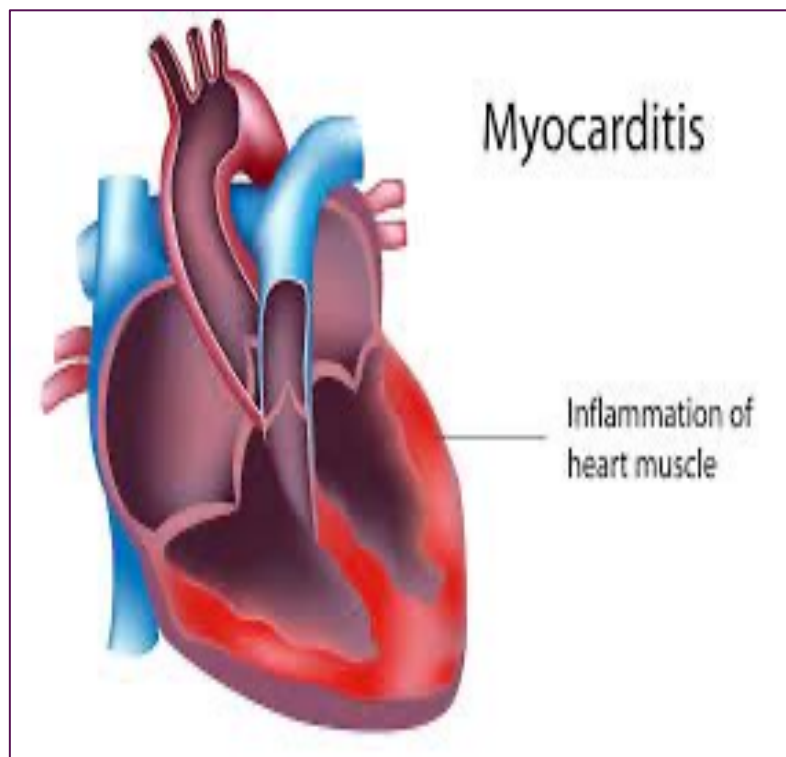
Elgebaly SA, et al. *Circulation*. 2020; 142, A13103-A13103

Elgebaly SA, et al. *Biomolecules*. 2021; 11, 368

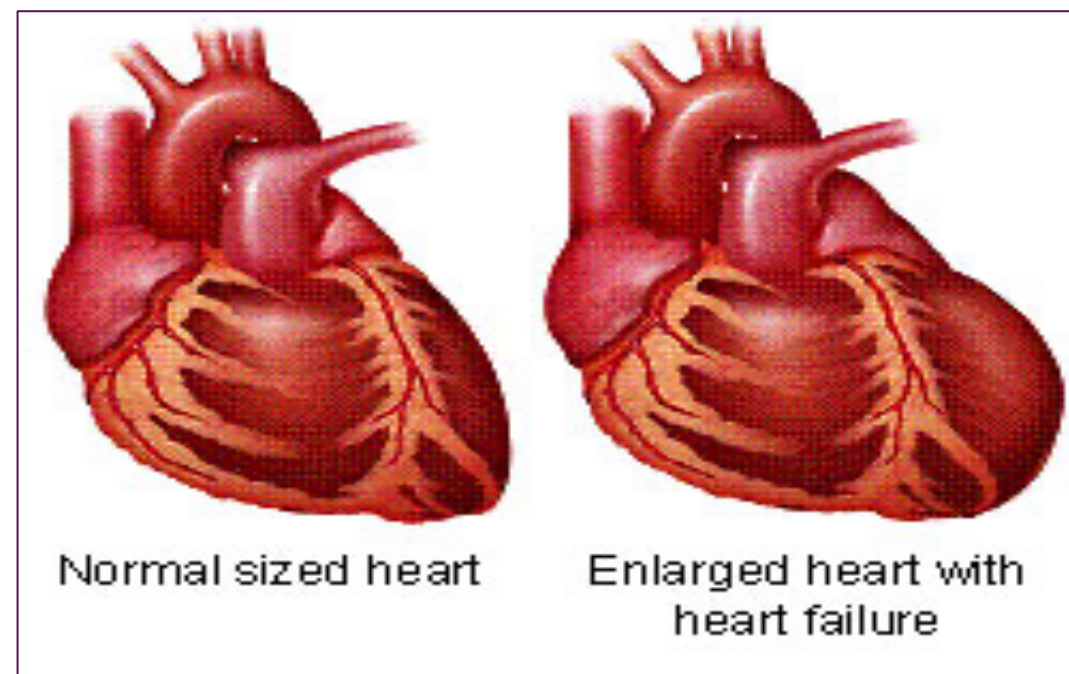
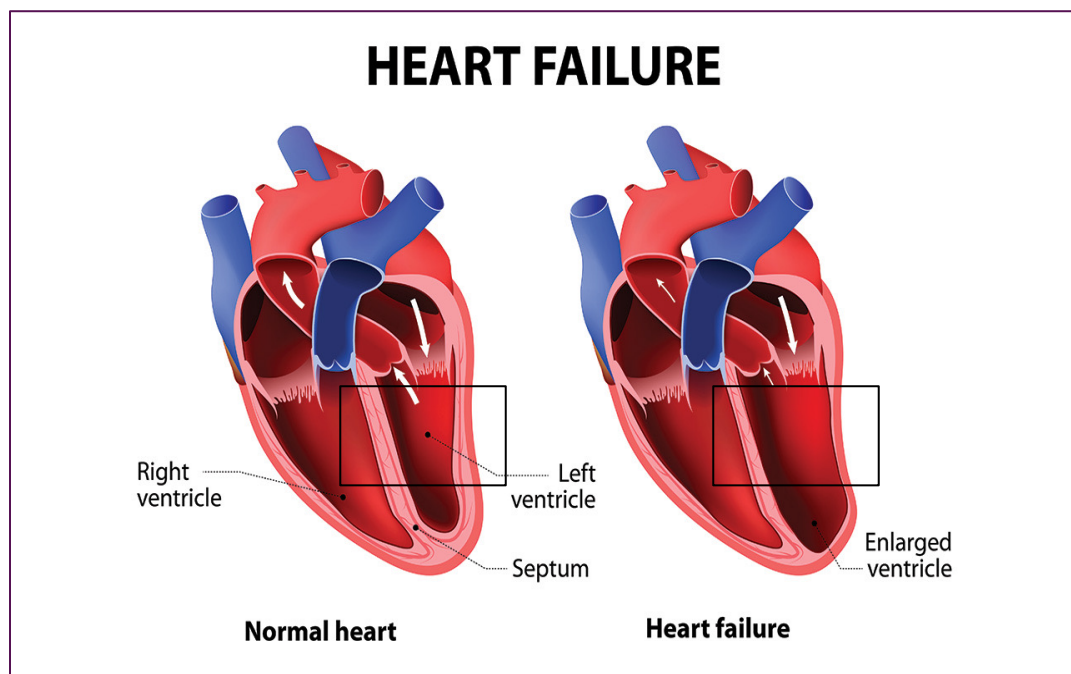
Elgebaly SA, et al. *Diagnostics*. 2021; 11, 703

Elgebaly SA, et al. *Int. J. Mol. Sci.* 2021; 22, 3575

Cardiac Inflammation Associated with Hypertrophic Cardiomyopathy

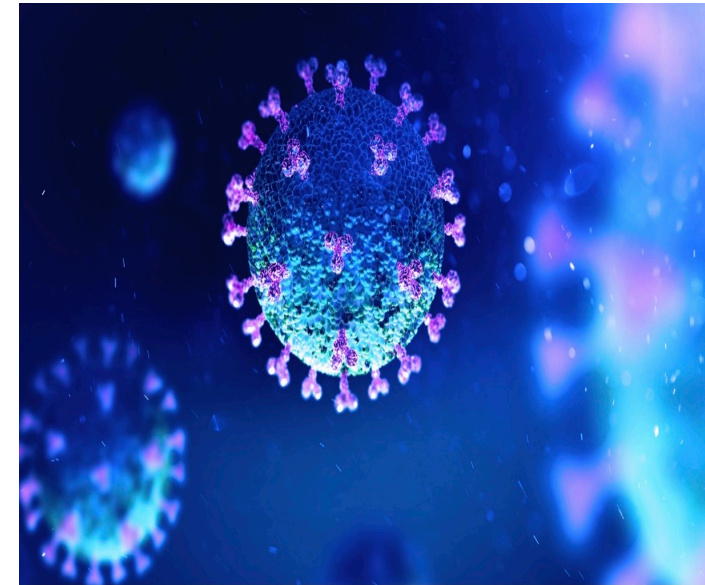


Cardiac Inflammation Associated with Heart Failure



Conclusions

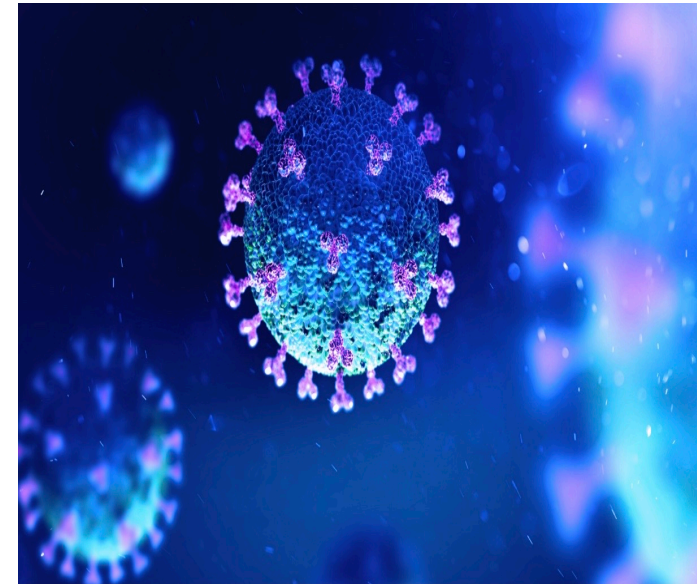
- ▶ **Nourin is an novel “early” lung-derived inflammatory biomarker:**
 - Can diagnose and monitor patients with influenza flu virus infection
 - Can stratify “severity” of infection with higher expression in ICU severely ill patients compared to moderate hospitalized and mild
- ▶ **Cyclosporin H is a potential novel anti-inflammatory therapy for influenza flu virus patients, including COVID-19 to:**
 - Reduce lung and heart inflammation
 - “Early” protect against cytokine storm
- ▶ **By controlling excessive host inflammatory responses, Cyclosporin H will potentially increase survival**



SARS-CoV-2 Virus

Conclusions

- ▶ Cyclosporin H targets Nourin and not the virus, thus, it will not:
 - Have time restriction like Tamiflu and Remdesivir (first one to two days of infection)
 - Develop vaccine resistance to “new” strains of flu viruses and “existing” viruses with mutations
- ▶ Cyclosporin H is not an immunosuppressant, thus, will not:
 - Affect the host defense immune system
 - Subject patients to additional infections and cancer



SARS-CoV-2 Virus

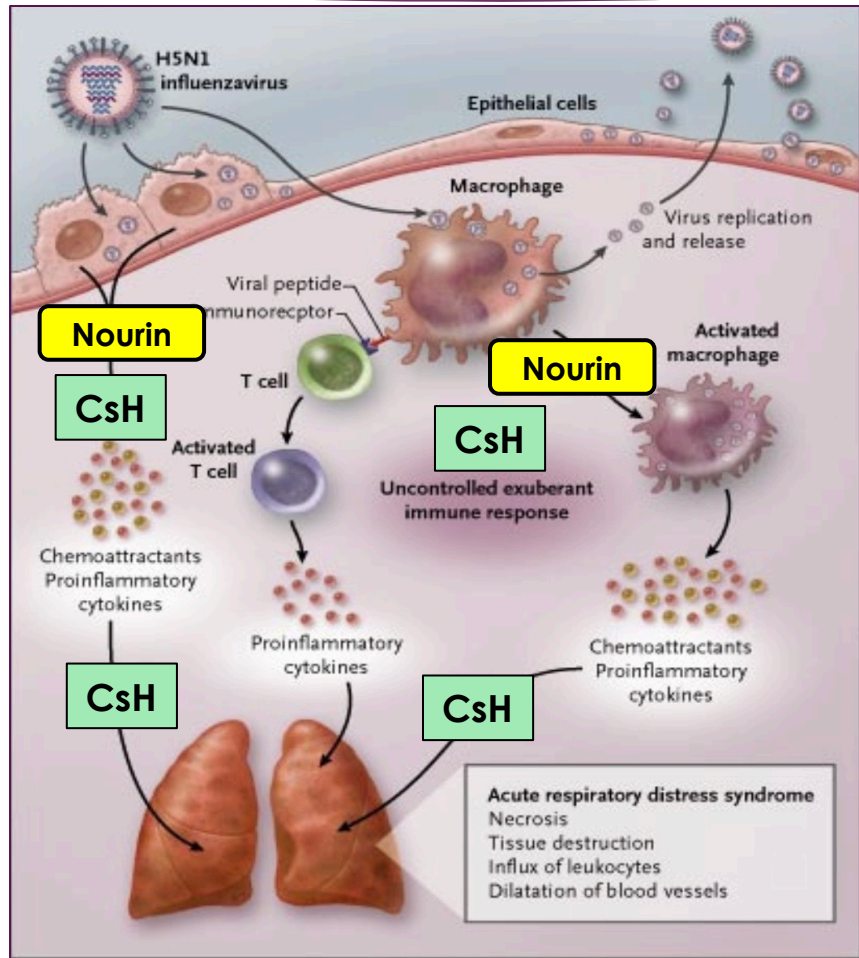
Cyclosporin H: A Novel Anti-inflammatory

“Inhibitor of Inflammatory Mediator, Nourin”

NO

Virus
Vaccines
Tamiflu
Remdesivir

Nourin Mediator
Cyclosporin H



Leukocytes
Cortisone

NO

Mediators
Anti TNF- α
Anti IL-1

Nourin Inflammatory Biomarker to “Early” Diagnose and Monitor Disease Severity

Cyclosporin H for “Early” Treatment of Lung Inflammation to Limit Tissue Necrosis

Thank You.



Prof. Salwa Ahmed Elgebaly
selgebaly@nourheart.com