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The Pathogenesis Factors and Mechanisms of COVID-19, Methods and Small molecules as COVID-19 Immunotherapy and modulatory

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Abstract

Background:

Cytokines are critical players in the activation, regulation of immune responses. With the help of these soluble factors, immune cells undergo proliferation, activation, differentiation, and inactivation or even cell death. Among various types of cells involved in immune responses, CD4 T cells play central roles in immune response and regulation.

Objective:

To determine the effective potential of IFNY +ZINC in combination with 9 CIS retinoic acid, TGF beta, Recilisib, Astaxanthin, Selenium on killing COVID -19, activation of immune response and maintaining tolerance in B cell receptors (IgM, IgG), APCs, CD4+T cell subsets (Th1, Th2, Th17 and Treg), and CD8+ T cells response and gene expression in animal model

Methods:

After Mice induction with new antigen, in the presence of IFNY +ZINC in combination with 9 CIS retinoic acid, TGF beta, Recilisib, Astaxanthin, Selenium on the 10 weeks old female C57BL/6 mice, we will be analyze the spleenocyte levels to determining Tbet, RORγt, FOXP3, IFN γ, STAT-3, SOCS, STAT-5,IL-17, IL-4, IL-10, IL-1β, TNFα, IL12, IL-6 and HPRT1, gene expressions using Real time PCR method. Will perform according to the protocol of the manufacturer

We will be analyze the culture supernatant or serum levels to determining the level of IFN-γ, IL-6, IL-1β, IL-4, IL-12, IL-10, IL-17, TGFβ, IgG and IgM using ELISA method and will perform according to the manufacturer's instructions.

Flow Cytometer Analyses: FITC-, PE-, and PE-Cy5- conjugated mAbs against Th1 cells, Th17 cells, Tregs, B cell receptors, APCs and CD8 T cells (BD Bioscience), will be used to stain cells from spleen. Cells will incubated with antibodies for 20 min on ice, will wash twice in FACS media (FM; HBSS [BD Bioscience] plus 1% BSA [Sigma Chemical Co.] plus 1% azide [wt/vol; Sigma Chemical Co.]) and then will suspend in 0.3 ml of FM. Stained cells will be analyzed by FACScan™ (Becton Dickinson).

Results/ Conclusions:

IFNY +ZINC in combination with 9 CIS retinoic acid, TGF beta, Recilisib, Astaxanthin, Selenium exerts a dual effect (inhibition, modulatory versus enhancement effects in in B cell receptors (IgM, IgG), APCs, CD4+T cell subsets (Th1, Th-2, Th17 and Treg), and CD8+ T cells. Which are implicated in the immunepathogenesis, molecular mechanism, and cytokine pathways, and gene expression However, while exciting discoveries have been made, further work is required to understand the diverse roles of IFNY +ZINC in combination with 9 CIS retinoic acid, TGF beta, Recilisib, Astaxanthin, Selenium in COVID-19 Immunotherapy and modulatory

Key Word: Immunotherapy; Modulatory; C57BL/6 mice; B cell receptors (IgM, IgG); APCs; CD4+ T cell Subsets (Th1, Th17, and Treg); and CD8+ T cell

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Background

Definitions of the virus:

The family Coronaviridae comprises two genera. One, the genus Coronavirus, contains a substantial number of pathogens of mammals and birds that individually cause a variety of diseases, including pneumonia, reproductive disease, enteritis, polyserositis, sialodacryoadenitis, hepatitis, encephalomyelitis, nephritis, and other disorders.

The second genus, Torovirus, contains two viruses of animals: Berne virus, which was first isolated from a horse with diarrhea, and Breda virus, which was first isolated from neonatal calves with diarrhea.

A nidovirus from fish—white bream virus, which is related to the toroviruses—recently was Bafinivirus.

The genus Coronavirus can be subdivided into three cluster groups on the genetic and serologic properties, with two subgroups.

Group 1a includes transmissible gastroenteritis virus, porcine respiratory coronavirus, canine coronavirus, and feline infectious peritonitis virus.Group 1b includes porcine epidemic diarrhea virus, and bat coronavirus.Group 2a includes mouse hepatitis virus, porcine hemagglutinating encephalomyelitis virus, and canine respiratory coronavirus. Group 2b includes human SARS coronavirus. Group 3 includes avian infectious bronchitis virus.

Member viruses of the family *Coronaviridae* are enveloped, 80–220 nm in size, pleomorphic although often spherical (coronaviruses), or 120–140 nm in size and disc, kidney, or rod shaped (toroviruses). Coronaviruses have large (20 nm long) club-shaped spikes (peplomers) enclosing what appears to be an icosahedral internal core structure within which is a helical nucleocapsid. Some coronaviruses have a second fringe of shorter (5 nm long) spikes (hemagglutinin).

The genome of the family *Coronaviridae* consists of a single molecule of linear positive-sense, single-stranded RNA, 27.6–31 kb in size for coronaviruses and 25–30 kb for toroviruses, the largest known non-segmented RNA viral genomes. The genomic RNA is 5’-capped and 3’-polyadenylated, and is infectious.

The major virion proteins include a nucleocapsid protein (N, 50–60 kDa, 19 kDa for toroviruses) and several envelope/spike proteins: (1) the major spike glycoprotein (S, 180–220 kDa); (2) a triple-spanning transmembrane protein (M, 23–35 kDa); (3) a minor transmembrane protein (E, 9–12 kDa), which together with the M protein is essential for coronavirus virion assembly.

The secondary, smaller spikes, in group 2 coronaviruses and in toroviruses, consist of a dimer of a class I membrane protein, a hemagglutinin esterase (HE) that shares 30% sequence identity with the N-terminal subunit of the HE fusion protein of influenza C virus.

coronaviruses are unique among nidoviruses because their genomes encode accessory proteins. The accessory proteins encoded by the SARS virus open reading frames 3b and 6 are antagonists of innate immune responses, specifically interfering with the development of type I interferon responses. The host spectrum of individual coronaviruses is determined by the S protein, portions of which mediate receptor binding and virus cell fusion that occur at the plasma membrane or within endosomes of cells. Coronaviruses utilize a variety of cellular proteins as receptors.

The functional receptor for group 3 coronaviruses such as infectious bronchitis virus is undefined, although heparan sulfate and sialic acid residues may serve as non-specific attachment factors.The strategy of expression of the coronavirus genome is complex. First, the viral RNA serves as messenger RNA (mRNA) for synthesis of the RNA-dependent RNA polymerase. The two large open reading frames encoding the units of the polymerase are translated—the larger via ribosomal frameshifting—as a single polyprotein that is then cleaved.

These proteins assemble to form the active RNA polymerase. This enzyme transcribe full-length complementary (negative- sense) RNA, from which in turn are transcribed, not only full-length genomic RNA, but also a 3’ co-terminal nested set of subgenomic mRNAs.

The nested set comprises up to 10 overlapping mRNAs that extend for different lengths from common 3’ ends and share a common 5’ leader sequence. They are generated by a leader-primed mechanism of discontinuous transcription: the polymerase first transcribes the noncoding leader sequence from the 3’ end of the complementary (negative-sense) RNA.

The capped leader RNA then dissociates from the template and reassociates with a complementary sequence at the start of any one of the genes, to continue copying the template right through to its 5’ end. Only the unique sequence that is not shared with the next smallest mRNA in the nested set is translated; this strategy yields the various viral proteins in regulated amounts. Intergenic sequences serve as promoters and attenuators of transcription.

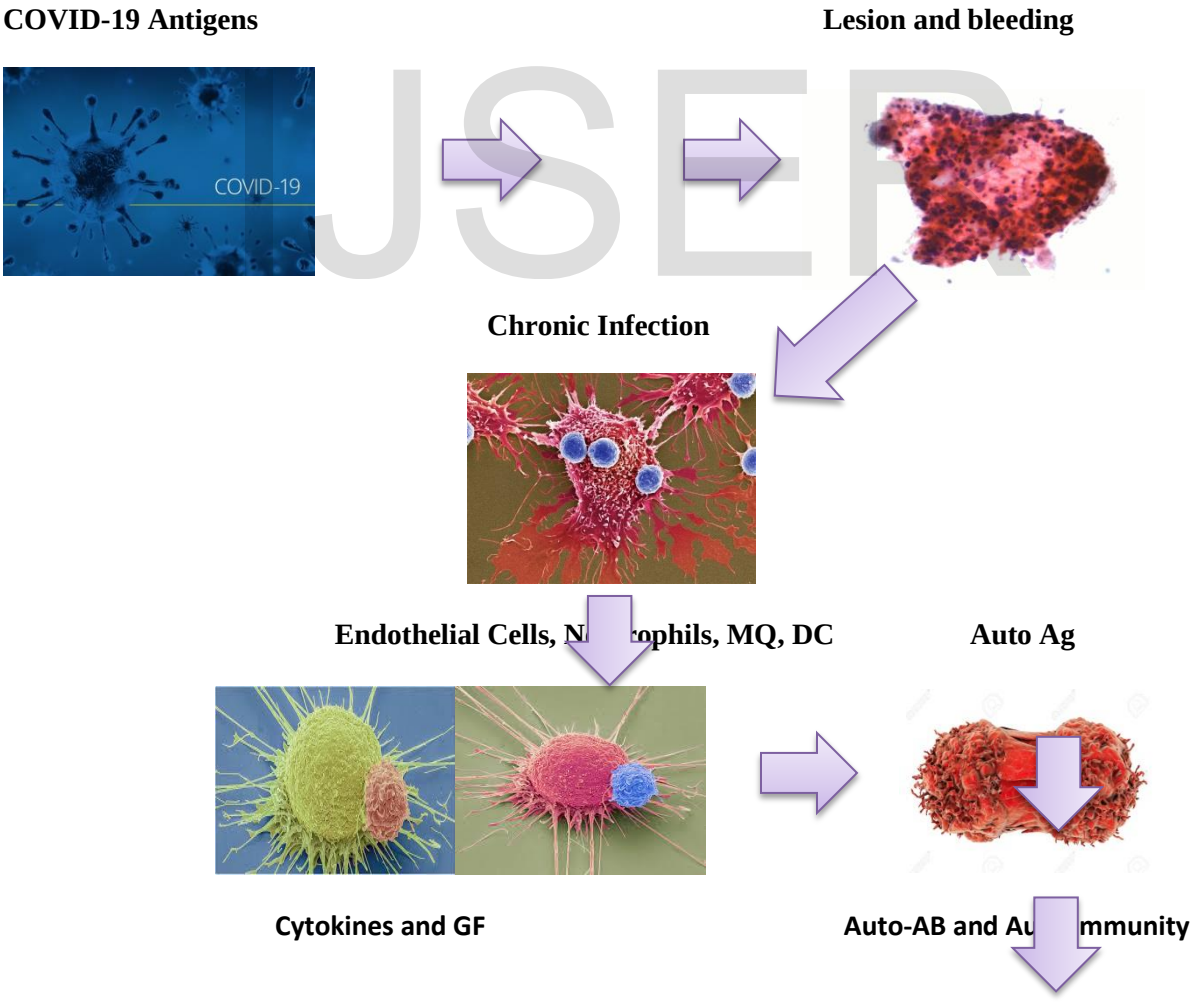
The synthesis, processing, oligomerization, and transport of the several envelope glycoproteins of coronaviruses display unusual features. The envelope protein M, which contains O-linked rather than N-linked glycans, is directed exclusively to cisternae of the endoplasmic reticulum and other pre-Golgi membranes. Thus, virions bud only there and not from the plasma membrane. Virions are transported in vesicles to the plasma membrane and released by exocytosis. After release, many enveloped virions remain adherent to the outside of the cell.

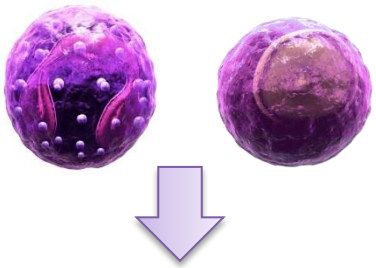
In this article discuss the Pathogenesis Factors and Mechanisms of Corona Virus “COVID-19”, Material, Study Design and Methods

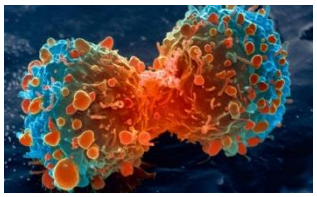
The Pathogenesis Factors and Mechanisms of Corona Virus "COVID-19"

a. COVID-19 Antigens,
b. Lesions and bleeding
c. Chronic Infections,
d. Endothelial Cells, Neutrophils, Macrophage, DCs,
e. Cytokines and GF,
f. NK cells, NKT cells,
g. CD4+Tcells **(Th1,Th17, and Treg)**
h. MQ/DCs
i. MHC II
j. CD8+ T cells,
k. High Production of -inflammatory Cytokine
l. Immune Activation
m. Antibodies production against COVID-19 **(IgG)**

The Pathogenesis Factors and Mechanisms of Corona Virus "COVID-19"

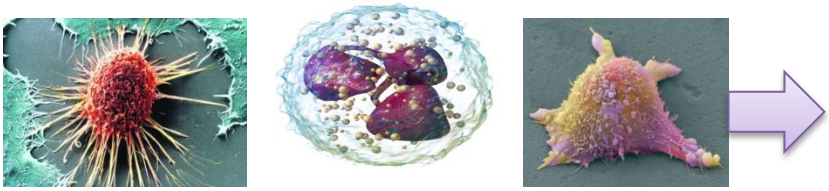




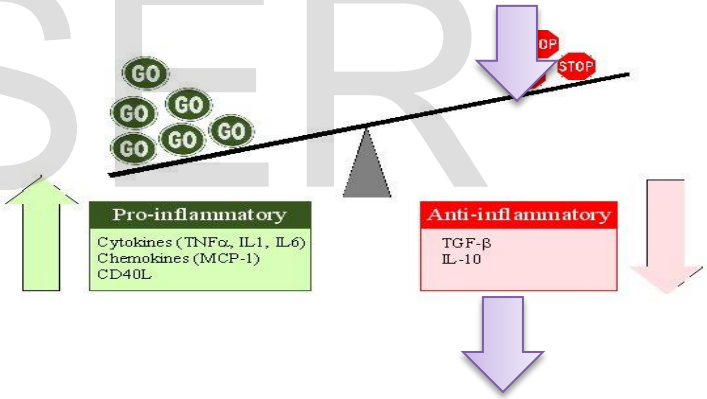


NK Cell, Neutrophil and NKT Cell

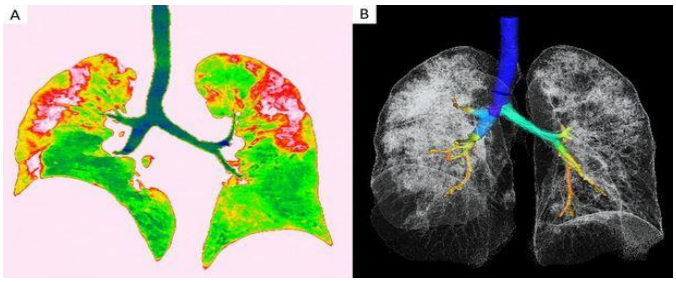
CD4+Tcells, CD8+ T cells,



High Production of Pro-inflammatory Cytokine



Inflammation, Tissues Distractions,
Loss of Functions, and Organ failure



Original and Drawn by Zelalem Kiros Bitsue (Ph.D.) "US-AHO"

Material and Study Design

Study period and area

The study will be conducted in **……from May, 2020 to August, 2020**

Study design

It is an experimental study, using Experimental Animal model, to determine the effect ofImmunotherapy, and modulatory recombinant proteins after induction using mice as compared to healthy and control group

Animals will house in specific pathogen-free conditions. Animals will housed in a temperature-controlled room under illumination with a 12 h light: 12 h dark cycle (lights on from 06h00 to 18h00) and both food and water will available.

Group	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6	Group-7
The Type of peptides	IFNY +ZINC in combination with 9 CIS retinoic acid, TGF beta, Recilisib, Astaxanthin	IFNY +ZINC in combination with 9 CIS retinoic acid,	IFNY +ZINC in combination with acid, TGF beta, Recilisib, Astaxanthin	IFNY +ZINC	IFNY +ZINC in combination with 9 CIS retinoic acid, TGF beta,	Health Group	Control Group
Number of mice	10 mice	10 mice	10 mice	10 mice	10 mice	10 mice	10 mice
						Total	**80 Mice**

Note: In several experimental model the number of mice 5-6 per group, because its conventional and the mice has good health and in good condition. But in some case the number of mice increases up to 8-10 this due to the reagents, kits we are using and manufacture instructions

I have stated 40 mice b/se 70 of them for 7 group and the ten extra mice will the replacement of any mice died from the group

Group 6 is a healthy group without any recombinant protein and hormone group 7 is a control group without any recombinant proteins and a hormones but with a fetal bovine serum(for stimulation of cytokines)

Inclusion Criteria

Age 8-10 weeks
Type of mice C57BL/6, BALB/6
Sex Female

Exclusion Criteria

Under and above age of 8-10 weeks
Different type of strain mice
Any severe clinical signs

Methods

After Mice induction with new antigen, in the presence of IFNY +ZINC in combination with 9 CIS retinoic acid, TGF beta, Recilisib, Astaxanthin, Selenium on the 10 weeks old female C57BL/6 mice, we will be analyze the spleenocyte levels to determining Tbet, RORγt, FOXP3, IFN γ, STAT-3, SOCS, STAT-5,IL-17, IL-4, IL-10, IL-1β, TNFα, IL12, IL-6 and HPRT1, gene expressions using Real time PCR method. Will perform according to the protocol of the manufacturer

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Molecular biology, Result and Discussion and Conclusion

Not yet, after analysis soon

Note: United States of African Health Organization **US-AHO** needs Your Support to develop Immunotherapy and modulatory for COVID-19

Support US-AHO, prevent and protect your community

Note: on development of Immunotherapy and modulatory on COVID-19; please contact for consultation and further discussions via bitsue.zelalem29@gmail.com and or +251991130307