WORK ON A SUBJECT CONCERNING THE CURRENT CORONAVIRUS PANDEMIA FOCUSING ON THERAPEUTIC APPROACHES FOR COVID-19 BASED ON THE DYNAMICS OF INTERFERON-MEDIATED IMMUNE REPONSES[1]

"This therapeutic approach does not provide medical advice. It is intended for informational purposes only. It is not a substitute for professional medical advice, diagnosis or treatment. Never ignore professional medical advice in seeking treatment because of something you have read on that paper. If you think you may have a medical emergency, immediately call your doctor or dial 15."

INTRODUCTION:

Coronavirus is a group of viruses with the largest RNA genome of any known virus today. COVID-19 is caused by the infectious agent SARS-CoV-2 as a result of SARS-CoV ('Severe Acute Respiratory Syndrome Coronavirus', 2002) and MERS-CoV ('Middle East Respiratory Syndrome Coronavirus', 2012). Indeed, all three have a close structure with a lipidic bilayer containing membrane proteins (respectively 89,6% and 96% of similarities for the envelope and the nucleocapsid) and similar genomic sequences.

Figure 1: Structure of SARS-Associated Coronavirus[2]

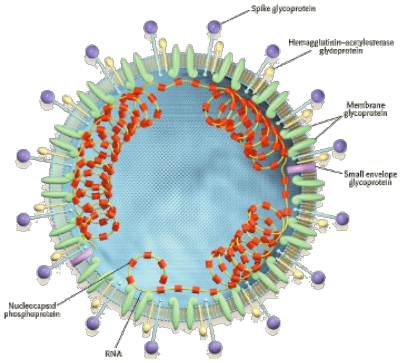
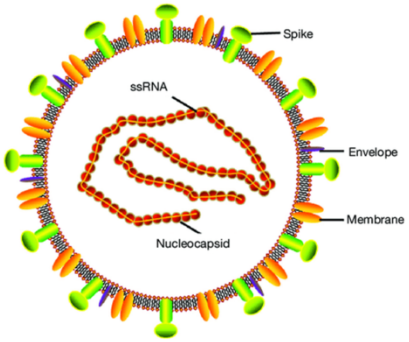


Figure 2: The MERS-CoV structure[3]



Coronaviruses replicate in the human body by complementarity of membrane proteins to target body cell receptors and by injection of the viral genome into target cells. Affected patients can have as symptoms: fever, cough, fatigue, gastro-intestinal infection, ARDS ('Acute Respiratory Distress Syndrome), cytokine storm, malaise, loss of taste or smell…

Moreover, an age-specific mortality pattern is to take into account. Indeed, elderly people would be the first to experience complications that can lead to death. Thanks to open data on "COVID-19 fatality rate by age" as stated by the Worldometers[4] website, Figure 3 was done.

Nearly 27% of the fatality rate is associated with patients between 60 and 80+ years old. Hence the problem arises:

Would something be responsible for the mortality rate linked to COVID-19? How to cure positive COVID-19 patients by therapeutic approaches?

All the stated theories have been possible based on the assumption that the immune response against COVID-19 is similar to other Coronaviruses (which should be validated through other insights on SARS-CoV-2).

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[1] Shahabi nezhad F, Mosaddeghi P, Negahdaripour M, Dehghani Z, Farahmandnejad M, Moghadami M, et al. Therapeutic Approaches for COVID-19 Based on the Dynamics of Interferon-mediated Immune Responses [Internet]. MEDICINE & PHARMACOLOGY; 2020 Mar [cited 2020 Mar 25]. Available from: https://www.preprints.org/manuscript/202003.0206/v2

[2] Holmes KV. SARS-Associated Coronavirus. N Engl J Med. 2003 May 15;348(20):1948–51.

[3] Wang Y, Sun J, Zhu A, Zhao J, Zhao J. Current understanding of middle east respiratory syndrome coronavirus infection in human and animal models. J Thorac Dis. 2018 Jul;10(S9):S2260–71.

[4] Coronavirus Age, Sex, Demographics (COVID-19) – Worldometers – available from https://www.worldometers.info/coronavirus/coronavirus-age-sex- demographics/

Figure 3: Bar diagram of death rate because of COVID-19 as a function of age,
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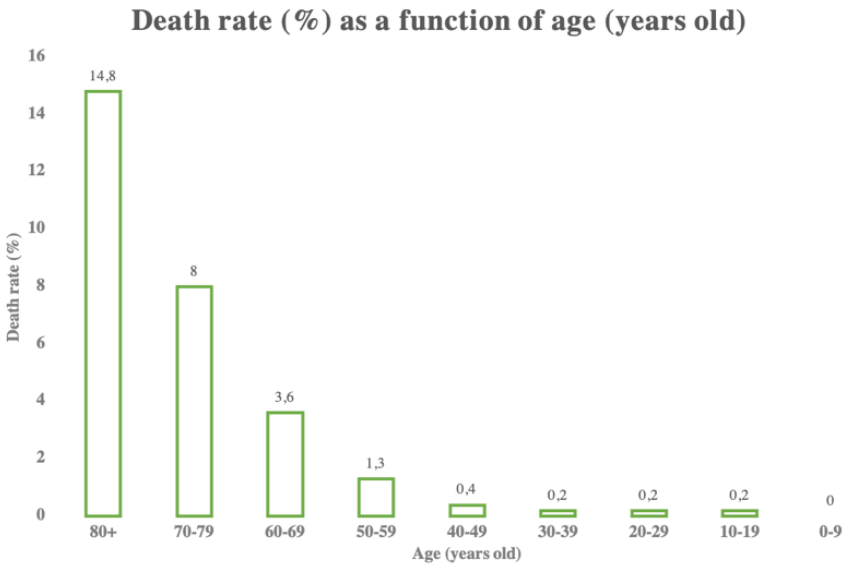


WHAT ARE THE DEFENSES ORCHESTRATED BY THE HUMAN BODY AGAINST COVID-19?

On one hand, the first lines of defense are Mannose-Binding Lectin[5] (MBL) molecules, pattern-recognition molecules (PRM) which recognize specific patterns found on the surface of the SARS-CoV-2. Thus, MBL provides the host before the adaptive immune system becomes operative and may be particularly important between 6 and 18 months of age when the adaptive system is still immature.

On the other hand, interleukin-12 seems to play a crucial role by activating interferons (IFNs) activation. Interferons are a cytokines group allowing the activation of Natural-Killer (NK) cells and macrophages.
Three types of interferons exist:

- Type 1 (IFN-I): IFN-α and IFN-β,
- Type 2 (IFN-II): IFN-ɣ, IFN-ǩ
- Type 3 (IFN-II).

However, the SARS-CoV-2 principally attacks IFN-I but how?

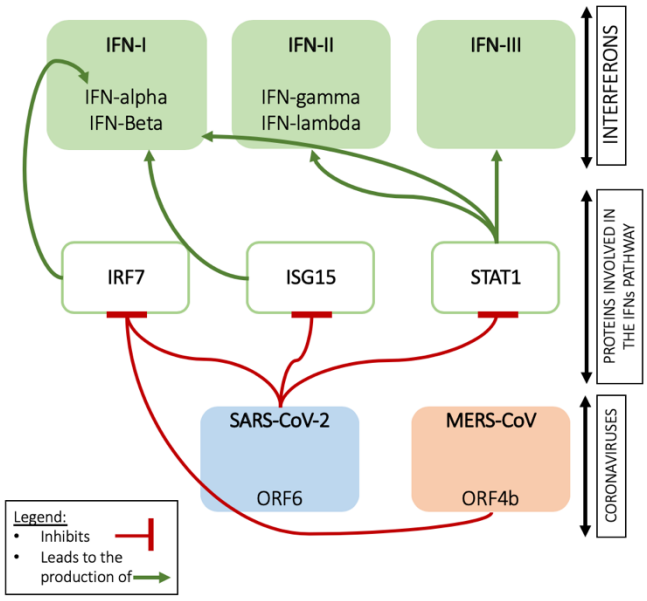
A WAY TO UNDERSTAND IFNs SIGNALING PATHWAYS UNDER CORONAVIRUS:

Host-pathogen interaction has a crucial role in the course of the disease, indeed, SARS-CoV-2 weakens important proteins needed for the defense.

Protein-protein interactions have been studied and three proteins have been identified as targeted by the virus:

- ISG15[6]: Interferon-Stimulated Gene 15 located on human chromosome 1 which provides to the human body an induced anti-viral activity in response to IFN-I,
- STAT1[7]: Signaling Transducer and Activator of Transcription 1 located on human chromosome 2, a protein activated by IFN-I, IFN-II and IFN-III and which, in return, stimulates interferons. However, a malfunction of IFN-I will lead to a malfunction of STAT1 and so no stimulation of interferons,
- IRF7[8]: Interferon-Regulatory Factor 7 located on human chromosome 11 which has an important role by activated IFN-I (notably IFN-α). But when that protein is 'silent', the immune response will be not efficient.

[5] Turner MW. The role of mannose-binding lectin in health and disease. Molecular Immunology. 2003 Nov;40(7):423–9.
[6] « P05161 (ISG15_HUMAN) »; Uniprot; https://www.uniprot.org/uniprot/P05161
[7] « STAT1 »; Wikipedia; https://en.wikipedia.org/wiki/STAT1
[8] « IRF7 »; Wikipedia; https://en.wikipedia.org/wiki/IRF7



SARS-CoV-2 has the ability to weaken IFNs signaling pathway and escapes from the immune response by hiding his double-stranded RNA inside his vesicles. Indeed, ORF6 SARS-CoV-2 proteins block STAT1 and so inhibit IFNs. Moreover, IRF7 and ISG15 are defective under the virus.

Previously, MERS-CoV by his ORF4b proteins had the ability to weaken IFN-β by inhibiting IRF7 and the SARS-CoV had the capacity to escape the immune response thanks to Papain-Like Proteases (PLPs) that are able to impede the immune response function.

Figure 4: Diagram of the interferons signaling pathway and the influence of SARS-CoV-2 and MERS-CoV ©Ilona BUSSOD

The body, despite the virus action, must defend himself and the immune responses shift toward immunopathogenic over-reactions and cytokine storm[9] which might indicate a need for tempering the immune system activity. The cytokine storm is related to an immune system gone awry and an inflammatory response flaring out of control due to the interferons pathway dysfunction. It occurs when large number of white blood cells are activated and release inflammatory cytokines which, in turn, activate yet more white blood cells. Because that system is deregulated by COVID-19, it leads to a big 'cytokine storm' with organ failure, hypotensive shock, even death.

HOW TO UNDERSTAND THE AGE-LINKED SEVERITY OF THE DISEASE:

1) Why children have a near to zero mortality?

   Children operate earlier induction of IFNs, and their less developed immune system could contribute to that fact. Even if the induction of IFN-ɣ by NK cells is higher in adults, the induction threshold is lower in children and so easier to reach. Children have this capacity to immune answer quickly during the incubation period while avoiding replication of the virus.

2) Adults case?

   Even if adults have a more complete adaptive immunity, the COVID-19 has the ability to inhibit the IFNs signaling pathway and pushes back the immune answer of 48 hours. The innate immunity is totally attacked by the virus which can impede IFN induction and IFN mediated responses may be suppressed in severe cases of the infection. Indeed, the lack of proper antiviral immune responses in affected patients can be due to antagonization at several days post-infection. A delayed IFN-related antiviral response is responsible for the rapid virus replication at the early stages of infection.

   It has been shown that T cells number, in infected patients of more than 60 years old or patients in intensive care, is really low and their efficacity is lesser because IFN-I are crucial in the survival of T lymphocytes cells. Indeed, STAT1 and STAT5 phosphorylation increase under the influence of the Coronavirus and lead to a T lymphocytes failure.

[9] Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. The Lancet. 2020 Mar;395(10229):1033–4.

COVID-19 leads to abnormalities in the health of patients that are the result of a poorly balanced and malfunctioning immune system associated with the lack of time for IFN-specific immune responses to do their role which gives extraordinary inflammatory reactions, lethal inflammations in the lungs, vascular leakage, cytokine storm[10]…

THERAPEUTIC APPROACHES AGAINST THE CORONAVIRUS:

Several studies have been done in order to understand how the induction of IFNs plays a key role in the body defense against SARS-CoV-2 infections:

- The first idea will be to inject type I interferons which will inhibit the replication of the virus *in vitro.* Larkin & al.[11] showed that the IFN-α and IFN-ɣ combination *in vitro* leads to a huge antiviral activity which leads to uncontrolled secondary effects.
- Then, Nagata & al.[12] showed that the cytokine storm can have a really destructive effect, however, by administrating intraperitoneal IFN-ɣ injection, the results were positive. Another study showed that the IFN-α and IFN-β coupling causes hyperactivation of IRF7 and STAT1 which allows a better antiviral reaction against the replication of the virus.
- Clinatl & al.[13] reported an *in vitro* superiority of IFN-β over IFN-ɣ and IFN-α
- Bellomi & al.[14] reported synergistic effects of IFN-ɣ and IFN-β
- Another study showed that IFN-ɣ combination with another IFN-I might induce synergistic effects and so maximize the benefits.

The first conclusion is that combinational IFNs therapy could inhibit virus replication and overcome the increased response threshold of IFNs, especially in the elderies. The second conclusion is that such interventions can act as a 'double-edged sword' by aiding the imbalance of the immune reactions which may occur at the later stages of the disease.

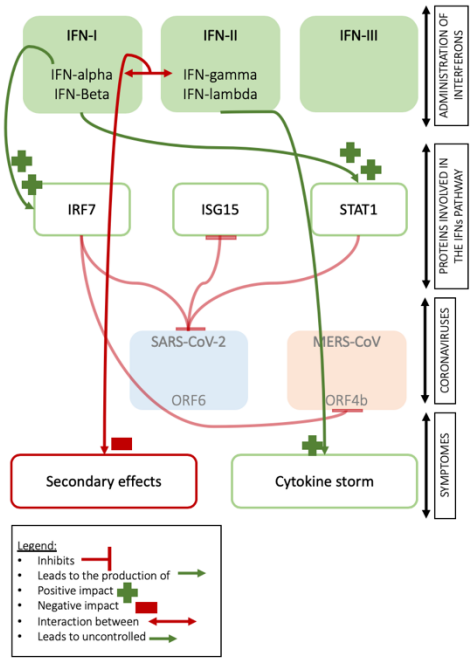


Figure 5: Diagram of the administration of interferons and the effects on SARS-CoV-2, MERS-CoV and on the symptoms. ©Ilona BUSSOD

[10] Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the Eye of the Cytokine Storm. Microbiology and Molecular Biology Reviews. 2012 Mar 1;76(1):16–32.

[11] Larkin J, Jin L, Farmen M, Venable D, Huang Y, Tan SL, et al. Synergistic antiviral activity of human interferon combinations in the hepatitis C virus replicon system. J Interf Cytokine Res [Internet]. 2003 May 1 [cited 2020 Mar 4];23(5):247–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12804067

[12] Nagata N, Iwata N, Hasegawa H, Fukushi S, Harashima A, Sato Y, et al. Mouse-passaged severe acute respiratory syndrome-associated coronavirus leads to lethal pulmonary edema and diffuse alveolar damage in adult but not young mice. Am J Pathol. 2008 Jun 1;172(6):1625–37.

[13] Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. Lancet [Internet]. 2003 Jul 26 [cited 2020 Mar 4];362(9380):293– 4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12892961

[14] Scagnolari C, Vicenzi E, Bellomi F, Stillitano MG, Pinna D, Poli G, et al. Increased sensitivity of SARS-coronavirus to a combination of human type I and type II interferons. Antivir Ther. 2004;9(6):1003–11.

But, the IFN-I injections should be given before the virus reaches its maximum, i.e. one day or two after infection. A late injection does not change anything because monocytes, macrophages and neutrophils are accumulated & activated in the lungs causing pro-inflammation by induction of cytokines & therefore severe pneumonia. In such circumstances, therapeutic approaches to reduce possible lungs inflammations may be needed (maybe via immunosuppression).

Feasibility has to be taken into account:

- That method works only at an early-stage disease (the exact duration of the administration has yet to be determined in order to have proper information on the timing)
- The dosing has also to be clarified and which combinational IFNs have to be administrated
- Secondary effects have to be checked, indeed, IFNs are available as medicinal products and it has been shown that some secondary effects such as flu-like syndrome and bone marrow suppression should be considered for their direct indication
- Clinical trials have to be done in order to conclude on the efficacity and feasibility of that therapeutic approach

As another idea, the Toll-Like Receptor 3 (TLR3) approach has been tackled. Indeed, those TLR3 could naturally induce IFNs. Two studies demonstrated that the pretreatment of mice with TLR3 agonists protected them from SARS-CoV infected mice. For instance, polyinosinic: polycytidylic acid (poly (I:C), a double-stranded RNA immunostimulant), a TLR3 agonist could augment the production of IFN-α, IFN-β, and IFN-γ, which consequently inhibited CoV replication and compensated for the inhibitory effects of CoV on IFN signaling pathways. Nevertheless, some TLR can over-stimulate the immune system and lead to unwanted or even toxic reactions.

In addition, PHA[15] (Phytohemagglutinin) at low dose could be beneficial at the early stages of the disease or incubation period to stimulate IFNs production. Another risk is that PHA is a natural compound found in high concentrations in red kidney beans and induce gastrointestinal toxicity and mitogenicity at a high concentration, a crucial information to take into account to avoid complications.

CONCLUSION:

Thanks to the previous Coronaviruses (SARS-CoV & MERS-CoV), many assumptions on SARS-CoV-2 have been done. The immunopathogenic over-reactions, cytokine storm and imbalanced immune reactions should be calm down through interferons administration. Indeed, thanks to the observation of differences between dynamics of IFN-related innate immune responses in children, adults and elderly explain the fatality rates. Thus, the key for success is to reduce fatality by stimulate the innate immune system notably through administration of IFNs or agents that are able to augment IFNs production. Finally, despite the evidences for the efficacy of IFNs, dosing and ideal timing have to be verified in clinical trials.

One clinical trial is ongoing at Frédéric Rieux-Laucat's Laboratoire at the Imagine Institute[16] in Paris, France. Indeed, since March 19th, 2020, the researcher team received the 1st sample from a patient with COVID-19 to assess the antiviral response to interferon type I.

Finally, the Cuban Center for Genetic Engineering and Biotechnology (CIGB)[17] has received more than 45 requests from different countries to acquire the interferon Alpha 2b, created by scientists at the Nico Maury translation center. Indeed, due to its proven effectiveness against different viruses with similar characteristics and its potential to improve respiratory conditions, the Cuban drug has reached the interest of several countries to counter the effects and spread of Covid-19.

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[15] Lawlorjr G, Stiehm E, Kaplan M, Sengar D, Terasaki P. Phytohemagglutinin (PHA) skin test in the diagnosis of cellular immunodeficiency. Journal of Allergy and Clinical Immunology. 1973 Jul;52(1):31–7.

[16] 'COVID-19 : plusieurs équipes d'Imagine se mobilisent'; IMAGINE; available from https://www.institutimagine.org/fr/covid-19-plusieurs-equipes-dimagine-se-mobilisent-693

[17] 'Coronavirus : 45 pays souhaitent acquérir le médicament cubain' ; Témoignages ; available from : https://www.temoignages.re/politique/sante/coronavirus-45-pays-souhaitent-acquerir-le-medicament-cubain,97551