TLEMCEN N°D'ORDRE

République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

UNIVERSITE DE TLEMCEN- ABOU BEKR BELKAID
Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers

Département de biologie
Laboratoire de Biologie Moléculaire Appliquée et d'Immunologie – BIOMOLIM W0414100

MÉMOIRE

Présenté par

NEFTI ABIR

En vue de l'obtention du

Diplôme de MASTER

En Immunologie

Intitulé :

Modifications H3K4Me3 au sein du monocyte infecté par SARS-COV-2

Sous la direction du Professeur Mourad ARIBI

Soutenu le 15/ 07/2021

Jury :

Pr. SMAHI Mohammed ChemsEddine	Professeur	Université de Tlemcen, Algérie	**Président**
Pr. ARIBI Mourad	Professeur	Université de Tlemcen, Algérie	**Encadreur**
Dr. BRAHAMI Nabila	Maitre de conférences A	Université de Tlemcen, Algérie	**Examinatrice**

15 Juillet 2021

TLEMCEN N°D'ORDRE

République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

UNIVERSITE DE TLEMCEN-ABOU BEKR BELKAID
Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers

Département de biologie
Laboratoire de Biologie Moléculaire Appliquée et d'Immunologie – BIOMOLIM W0414100

MÉMOIRE

Présenté par

NEFTI ABIR

En vue de l'obtention du

Diplôme de MASTER

En Immunologie

Intitulé :

Modifications H3K4Me3 au sein du monocyte infecté par SARS-COV-2

Sous la direction du Professeur Mourad ARIBI

Soutenu le 15 / 07/2020

Jury :

Pr. SMAHI Mohammed ChemsEddine	Professeur	Université de Tlemcen, Algérie	**Président**
Pr. ARIBI Mourad	Professeur	Université de Tlemcen, Algérie	**Encadreur**
Dr. BRAHAMI Nabila	Maitre de conférences A	Université de Tlemcen, Algérie	**Examinatrice**

15 Juillet 2021

Résumé

Introduction : Le coronavirus 2 du syndrome respiratoire aigu sévère (SARS-CoV-2) de la maladie COVID-19 qui fait des ravages dans le monde entier est causé par le virus SARS-CoV-2. De même que d'autres virus,La réaction immunitaire innée montre un rôle important dans la défense contre COVID-19. Les cellules du système immunitaire inné affichent la capacité d'acquérir des caractéristiques de mémoire après une incitation transitoire, provoquant une réaction améliorée lors d'une exposition secondaire. Ce phénomène a été nommé immunité entraînée. L'immunité entraînée est conditionnée par différents programmes épigénétiques. Marques épigénétiques importantes impliquées dans l'immunité entraînée sont H3K4me3.

Objectif : nous avons évalué le niveau d'expression de H3k4me3 par monocytes suite à son infection par le SARS-CoV 2.

Matériels et méthodes : Les PBMC ont été isolées à partir de sang veineux frais provenant de donneurs de volontaires sains. Les MO ont été isolés à partir de PBMC sur la base d'une adhérence plastique différentielle. Le niveau de méthylation de l'histone H3 lysine 4 a été quantifié.

Conclusions : Les modifications épigénétiques de H3K4Me3 induisent une immunité entraînée dans le monocyte. L'immunité entraînée est une approche très prometteuse contre COVID-19 et son induction pourrait éventuellement améliorer la défense antivirale de l'hôte et mieux former les patients COVID-19 à combattre l'infection, il peut être utile de réduire la propagation du virus et les taux de mortalité.

Mots clé: COVID-19, SARS-CoV-2, Trained immunity, epigenetic, H3K4Me3

Abstract

Introduction: The severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) of the COVID-19 disease that is damaging all over the world is caused by the SARS-CoV-2 virus. Likewise other viruses,The innate immune reaction shows an important role in defence against COVID-19. The Cells of the innate immune system display the ability to gain memory features after transitory inducement,causing an improved reaction upon secondary exposition.This phenomenon has been named trained immunity.Trained immunity is conditioned on different epigenetic programs.Significant epigenetic marks involved in trained immunity are H3K4me3.

Objective: we evaluated the level of expression of H3k4me3 by monocyte following its infection with SARS-CoV 2.

Material and methods: PBMCs were isolated from fresh venous blood from healthy volunteers' donors.MOs were isolated from PBMCs based on differential plastic adherence.the level of histone H3 lysine 4 methylation were quantified.

Conclusions: Epigenetics modifications of H3K4Me3 induces trained immunity in the monocyte.trained immunity is a very promising approach against COVID-19 and its Induction could possibly improve antiviral-host defence and better train COVID-19 patients to fight the infection it may be useful to reduce the propagation of the virus and the mortality rates.

Key words: COVID-19, SARS-CoV-2, Trained immunity, epigenetic, H3K4Me3

ملخص

مقدمة: إن فيروس كورونا المتلازمة التنفسية الحادة الوخيمة 2 (SARS-CoV-2) لمرض COVID-19 الذي يعيث فساداً في جميع أنحاء العالم ناجم عن فيروس .SARS-CoV-2 مثل الفيروسات الأخرى ، تُظهر الاستجابة المناعية الفطرية دورًا مهمًا في الدفاع ضد .COVID-19 تُظهر خلايا الجهاز المناعي الفطري القدرة على اكتساب خصائص الذاكرة بعد تحفيز عابر ، مما يتسبب في استجابة محسنة عند التعرض الثانوي. هذه الظاهرة تسمى المناعة المدربة. تكون المناعة الناتجة مشروطة ببرامج الوراثة اللاجينية المختلفة. العلامات التجارية اللاجينية العامة المشاركة في المناعة المستحثة هي.H3K4me3

الهدف: اختبار النشاط والتعديلات اللاجينية لـ H3K4Me3 على البلاعم / وحيدات في سياق.COVID 19

المواد والطرق: تم عزل PBMCs من الدم الوريدي الجديد الذي تم الحصول عليه من متبرعين لمتطوعين أصحاء. تم عزل MOs من PBMC على أساس التصاق البلاستيك التفاضلي. تم قياس مستوى مثيلة هيستون H3ليسين 4.

الاستنتاجات: التعديلات اللاجينية لـ H3K4Me3 تحفز المناعة في الخلية الوحيدة. تعد المناعة المدربة نهجًا واعدًا للغاية ضد COVID-19 ويمكن أن يؤدي تحريضها إلى تحسين دفاع المضيف المضاد للفيروسات وتدريب مرضى COVID-19 بشكل أفضل على مكافحة العدوى ، وقد يكون من المفيد تقليل انتشار الفيروس ومعدلات الوفاة.

الكلمات المفتاحية: COVID-19، SARS-CoV-2 ، مناعة مدربة ، التخلق المتوالي ، H3K4Me3

Avant-propos

Ce travail a été réalisé au niveau du Laboratoire de Biologie Moléculaire Appliquée et Immunologie (BIOMOLIM), Université de Tlemcen, sous la direction du Professeur Mourad ARIBI.

A l'issue de ce travail, je tiens à adresser mes plus sincères remerciements au Pr. Mourad ARIBI, mon encadreur. Merci vivement pour votre aide, votre clémence, votre volonté votre rigueur scientifique et vos précieux conseils, ainsi que pour vos qualités humaines qui étaient toujours une source de motivation. Je suis très enchantée de vous avoir comme encadreur de Thèse et d'avoir effectué mes travaux au sein de votre prestigieux Laboratoire.

Je voudrais également remercier les membres du jury qui m'ont fait l'honneur d'ausculter ma Thèse à savoir le Pr. SMAHI Mohammed ChemsEddine (Université de Tlemcen), Dr. BRAHAMI Nabila (Université de Tlemcen).

Un grand merci à tous les membres du Laboratoire de BIOMOLIM enseignants, doctorant, ingénieures de laboratoire pour leurs compétences.

Je tiens également à remercier mes grand parents, ma grande tante ainsi que mon cousin Olivier et ma sœur Doha et surtout Mr Drissi l'enseignant qui a marqué mon parcours universitaire vous m'avez toujours aidé, soutenu. C'est grâce à vous si aujourd'hui j'en suis arrivée là, merci à ma meilleure amie Maëva qui m'a toujours inspirée, et à ma petite Sarata, Romaissa et Latifa,Hicham, merci à vous tous.

TABLE DES MATIÈRES

LISTE DES FIGURES

LISTE DES ABRÉVIATIONS

ACE2: Angiotensin-Converting Enzyme 2

ADAM17: a disintegrin and metalloprotease17

Ang 1-7: angiotensin 1-7

BCG : bacillus Calmette-Guérin

CCR2: C-C chemokine receptor type 2

CD11c^{+} cluster of differentiation 11c^{+}

CD4 : cluster of differentiation 4

CD74: cluster differentiation 74

CD8: cluster differentiation 8

CT: computed tomography

DNA: deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

FCS: fetal calf serum

GM-CSF: Granulocyte-macrophage colony-stimulating factor

H1N1: hemagglutinin Type 1 and Neuraminidase Type 1

H3K27: histone H3 lysine 27

H3K4me1: Mono-methylation of histone H3 lysine 4

H3K4me3: Tri-methylation of histone H3 lysine 4

HLA-DPB1: Major Histocompatibility Complex, Class II, DP Beta 1

HLA-DRA : Major Histocompatibility Complex, Class II, DR Alpha

HSPCs : Hematopoietic stem/progenitor cells

IFI30 : interferon gamma inducible protein 30

IL10 : interleukin-10

IL-18 :interleukine**-**18

IL-1β : interleukin**-**1

IL-6 : Interleukin 6

LacNAc : N**-**Acetyl**-**D**-**lactosamine

LYz: lysozyme

MOs: monocytes

No: nitric oxide

nsp1: nonstructural polyproteins

ORFs: open reading frames

Pbmcs : Peripheral blood mononuclear cells

PBS: Phosphate-buffered saline

Plpro : Papain-like protease

Pp1a: Protein Phosphatase 1 a

Pp1ab: Protein Phosphatase 1 ab

RAAS: renin-angiotensin-aldosterone system

RBD: receptor-binding domain

RPMI: Roswell Park Memorial Institute medium

SARS-CoV-2 : severe acute respiratory syndrome

TBET: trypan blue exclusion test

Introduction

SARS-CoV-2 is causing a health problem in several countries and has altered day-to-day life world wild. This disease is caused by the SARS-CoV-2 virus, and is depending on age,sex and health status of the patient, it may manifest with different symptoms like mild contamination, a severe form or even asymptomatic development of the infection. Likewise other viruses, innate immune reaction shows an important role in defense against COVID-19 (*Sohrabi et al.,* 2020).

The Cells of the innate immune system display the ability to gain memory features after transitory inducement,causing an improved reaction upon secondary exposition.This phenomenon has been named trained immunity.Trained immunity is characterized by nonspecific amplified receptiveness,facilitated through wide metabolic and epigenetic reprogramming (*Adams et* al*., 2020*).

Trained immunity is conditioned on different epigenetic programs,leading to a first contact with a stimulus, monocytes are activated and upregulate gene target transcription,which is related to a speedy acquisition of activating histone modifications.Once the elimination of the stimulus,though some of these epigenetic characters persevere,which leads to earlier and stronger activation of gene transcription upon next exposition.Significant epigenetic marks involved in trained immunity are H3K4me3 (Netea *et* al*., 2020*).

Trained immunity is mediated by modifications in chromatin accessibility to differentially methylated DNA and alterations in histone tail modifications,mutually result in chromatin remodeling.Chromatin remodeling through these changes modulates the augmentation or decrease of immune cytokine release (*Adams et al., 2020)*. .

Chapter 1. literature review

1. Coronavirus (2019 -nCOV)

1.1 History and origin

SARS-CoV-2 of the COVID-19 disease,that is damaging all over the world,has been reported to be active well before this year,the infectious potential of this pathology outbreak destructively in Wuhan,causing the onset of the epidemic in China and possibly elsewhere as well,which failed in extending the epidemic potential.The origin of its zoonosis can be attributed to changes in the ecosystem that have reduced biodiversity,considerably simplify the contacts between humans and the animal holder that transfer pathogens,as well as SARS-CoV-2.These reservoirs are the bats.The progression of the epidemic that had manifested until 2019 and its explosion of December–January was made possible by the great amplification of the general negative conditions that had caused the preceding small outbreaks.Now,the eruption was expected,and could have occurred anywhere the environments that had allowed it, could be reproduced. What could not have been expected was the second alteration,from epidemic to pandemic.Research has now discovered that the globalization of the contamination gives the idea to have been initiated by a mutation in the spike protein of the SARS-CoV-2, that has intensely amplified its spreading (*Platto* et *al., 2021*).

Coronavirus Disease 2019 (COVID-19) was confirmed as pandemic by the World Health Organization on March 11th,2020 primarily because of the rapidity and scale of the diffusion of the infection.Previously,it emerged as an epidemic in mainland China with the attention being primarily informed in the city of Wuhan,Hubei province in February 26th.The etiologic mediator of COVID-19 was isolated and recognised as a new coronavirus,firstly designated as 2019-nCoV.After that, the virus genome was sequenced and for its similarity to the coronavirus eruption responsible for the SARS outbreak of 2003,the virus was called as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee for Taxonomy of Viruses (dos *Santos*, *2020*).

1.2 Characteristics of Coronavirus (2019-ncov)

1.2.1 SARS-CoV-2 Virus Structure and Integration

SARS-CoV-2 is one of the Betacoronavirus genus and a member of the Coronavirinae family.The virus elements are spherical or pleomorphic in form,with a diameter of about 60–140 nm.Coronaviruses have one of the biggest single-strand RNA genomes with 27–32 kilobases.Approximately the coronaviruses encode for the hemagglutinin-esterase protein, 3a/b protein and 4a/b protein on their surface.The genome organization of SARS-CoV-2 is comparable to other coronaviruses,which is made from the open reading frames ORFs (ORFs). Approximately 67% of the genome encodes by the ORF1a/b and it encodes for 16 nonstructural polyproteins (nsp1-16),even though the lasting 33% encodes for accessory proteins and structural proteins.ORF1a and ORF1b have a frameshift which synthesis two polypeptides,pp1a and pp1ab.Papain-like protease (PLpro) or chymotrypsin-like protease (3CLpro),proceeding these two polypeptides into 16 nsps.SARS-CoV-2 encodes at a minimum four major structural proteins that includes spike protein (S),membrane protein (M),an envelope protein (E), and nucleocapsid protein (N).These structural proteins are encoded by S, M, E, N genes at ORFs 10 and 11 on the one-third of the genome near the 30.These mature structural proteins are responsible for viral conservation and replication.Most of the probes and primers used to detect the SARS-CoV-2 are made against the genetic targets of ORF1ab and the N gene region (Shah et *al., 2020*).

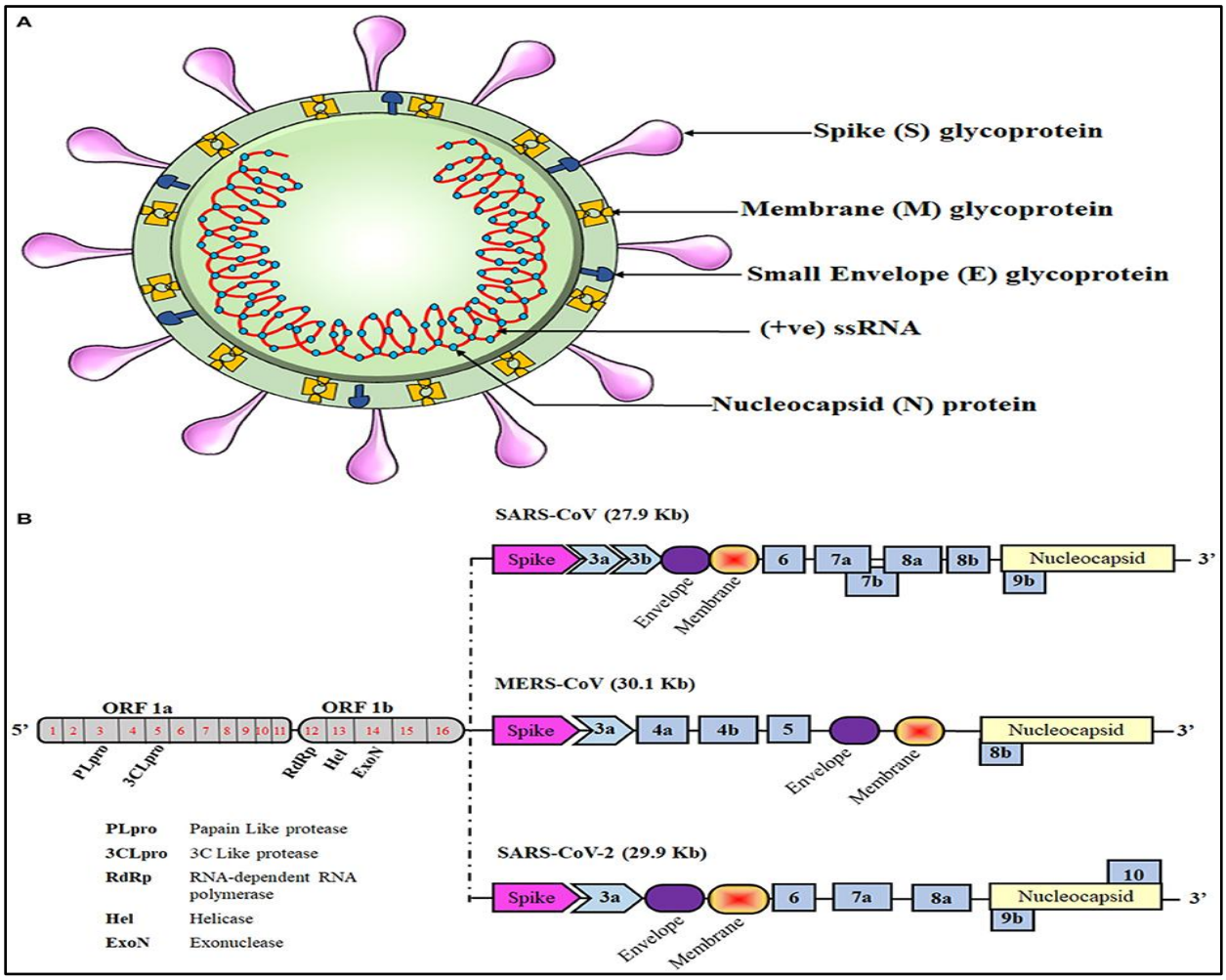

Figure 1 : Schematic representation of the coronavirus structure and genomic comparison of coronaviruse *(Shah et al., 2020).*

1.2.2 Pathogenesis of the 2019-nCoV

2019-nCoV contaminations are almost like seasonal influenza with the notable symptoms of fever,headache,shortness of breath,cough,muscle aches,and tiredness.The brutality of the pathology in infected persons is mild to moderate,and they can manage their symptoms at home without the hospitalization.Although patients with severe symptoms for example effort breathing,chest ache or pressure,and loss of speech or movement required urgent medical care.Additional syndromes seen in acute conditions comprise hemoptysis, diarrhea,dyspnea,acute heart injuries,and ground-glass opacities (*Esakandari*, *2020*).

The lungs are the main site of 2019-nCoV infection.The chest CT of the infected patients habitually displays bilateral ground-glass opacity lesions in the posterior and peripheral lungs that are reported as the characteristic of 2019-nCoV pneumonia.Studies on biopsy samples of lung,liver,and heart found from death COVID-19 patients have shown that the lung is the central affected tissue with pathological alterations as well as hyperplasia of

type II pneumocytes,destruction to the alveolar epithelial cells,the development of the hyaline membrane and diffuse alveolar destruction.Thrombotic microangiopathy,important accumulations of CD4+ mononuclear cells around small thrombotic vessels and important hemorrhage seem to be significant causes of death in these persons.Triggered local megakaryocytes in the lung, platelet aggregation,fibrin deposition,and mass formation are intricate with the declared procedure.In addition,the profusion of viral RNA in neutrophils inside the alveoli and the existence of deteriorated neutrophils show the viral infection in these cells .Megakaryocyte reaction and platelet production have been reported in H1N1 virus disease. Multifocal hepatic necrosis,mild lymphocytic infiltration,sinusoidal dilation and steatosis are pathologic alterations detected in the liver of COVID-19 patients with moderate to severe disease.Minor myocardial hypertrophy modifications and focal fibrosis are tissue changes seen in the heart biopsies of death COVID-19 patients.Thus,the scientists consider that operational treatment for COVID-19 must not be restricted only to the virus-related pathogen as a target, but also the microangiopathic and thrombotic properties of the virus and the immune response to viral contamination need to be measured in the infection management (*Esakandari*, *2020*).

1.2.3 The ACE2 receptor

The angiotensin-converting enzyme 2 (ACE2) is the main controller of the renin-angiotensin system and was just recognized as a receptor for the SARS virus.The ACE2 sequence is like (sequence characteristics 43% and 35%, and resemblances 61% and 55%, correspondingly to those of the testis-specific form of ACE (tACE) and the Drosophila homolog of ACE (AnCE).The great level of sequence likeness permitted us to construct a strong homology exemplary of the ACE2 structure with a root-mean-square deviation from the aligned crystal structures of tACE and AnCE less than 0.5A.A prominent feature of the model is a deep channel on the top of the molecule that contains the catalytic site.Negatively charged ridges surrounding the channel may provide a possible binding site for the positively charged receptor-binding domain (RBD) of the S-glycoprotein,which we recently identified [Biochem. Biophys. Res. Commun. 312 (2003) 1159]. Several distinct patches of hydrophobic residues at the ACE2 surface were noted at close proximity to the charged ridges that could contribute to binding. These results suggest a possible binding region for the SARS-CoV S-glycoprotein on ACE2 and could help in the design of experiments to further elucidate the structure and function of ACE2 (*Prabakaran et al., 2004*).

For the positively charged receptor-binding domain (RBD) of the S-glycoprotein,which we recently identified.Several distinct patches of hydrophobic residues at the ACE2 surface

were noted at close proximity to the charged ridges that could contribute to binding.These results suggest a possible binding region for the SARS-CoV S-glycoprotein on ACE2 and could help in the design of experiments to further elucidate the structure and function of ACE2 (*Prabakaran et al*., 2004).

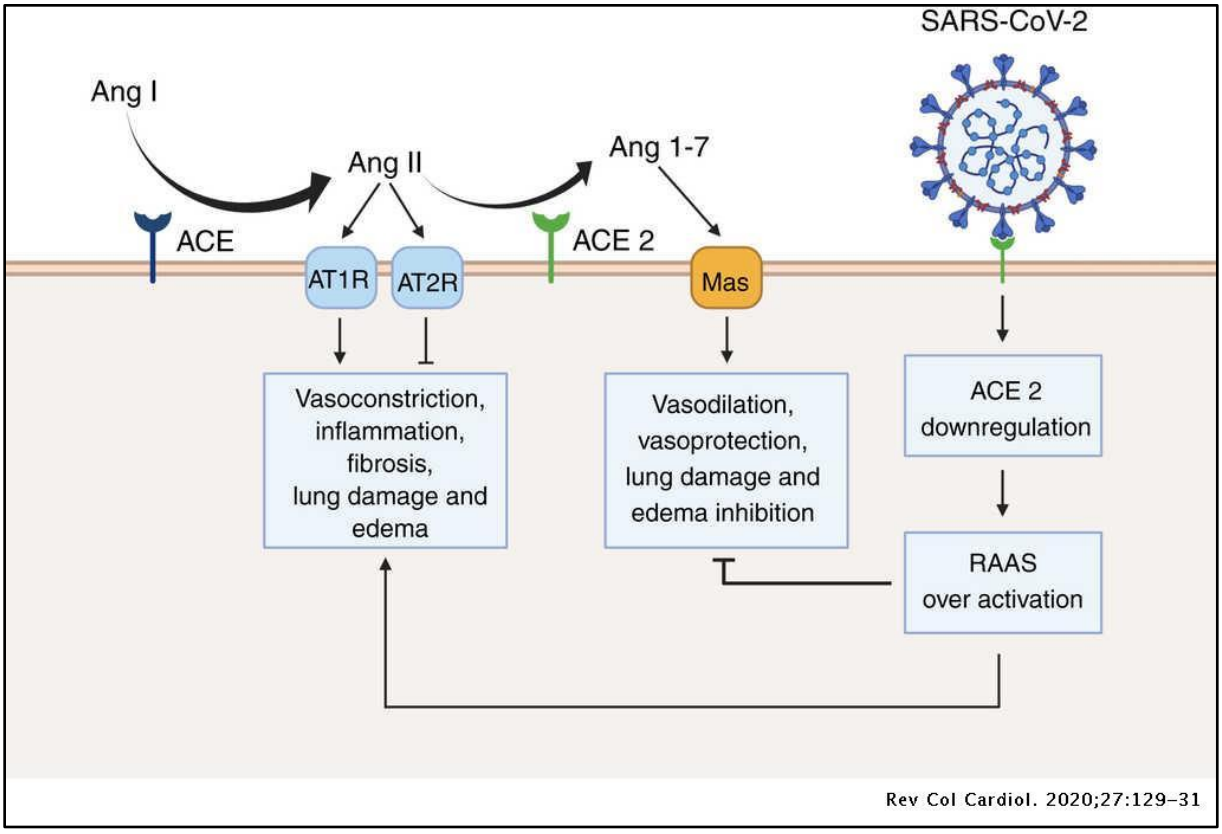

Figure 2: Renin angiotensin system (RAAS) overactivation as a result of SARS-CoV-2 infection.

In physiological conditions, the angiotensin converting enzyme (ACE) metabolizes angiotensin I (Ang I) to angiotensin II (Ang II), thus leading to increased vasoconstriction, inflammation, fibrosis, lung damage and edema. Conversely, angiotensin converting enzyme 2 (ACE 2) inactivates Ang I by generating 1-7 (angiotensin Ang 1-7), which then interacts with the G-protein-coupled receptor Mas.

1.2.4 Interaction between SARS-CoV-2 and ACE

Mechanisms involved in SARS-CoV-2/host cell contact are important for the infection and replication of the cell and lead to disease and associated destruction.In this case,the angiotensin converting enzyme 2 (ACE2),a central enzyme in the renin-angiotensin-aldosterone system (RAAS),hardly present in the circulation,nevertheless extensively expressed in organs can control blood pressure and fluid balance. ACE2 functions as an

ACE complement: it acts like a carboxypeptidase,eliminating single amino acids, transforming Ang II to its metabolite angiotensin-(1–7) (Ang1–7),stabilizing the consequences of Ang II. ACE2 is present in the apical surface of epithelial cells, differently from ACE,which is situated among the apical and basolateral membranes in polarized cells.ACE2 acts its essential role in controlling blood pressure and thus hypertension.This action is facilitated by the ACE2/Ang-(1–7)/Mas receptor axis,by the regulation of angiotensin and Ang-(1–7) and nitric oxide (NO) accessibility regulate blood pressure modifications,which damages the vascular tissue as atherosclerosis, hypertrophy and additionall,endothelial alterations.Indicatively,there are two forms of ACE2: (1) the full-length ACE2,that have a structural transmembrane domain that can anchor its extracellular domain to the plasma membrane,and (2) the soluble form of ACE2, that deficiencies the membrane anchor and circulates in the blood.SARS-CoV-2 pass in the cell by the fixation of spike (S) viral protein,a protein that compounds the viral envelope and with a “corona” like shape,to the ACE2 receptor.Firstly the viral admission is characterised by the binding of the N-terminal domain of the viral protein unit S1 to a pocket of the ACE2 receptor.Than, the receptor transmembrane protease serine 2 (TMPRSS2),that is stechiometrically attached to ACE2 receptor,makes the cleavage of the protein among the S1 and S2 units, with the aid of Furin which facilitates the entry of the virus into the cell after binding.After that, furin-mediated proteolytic cut of the S protein is essential for viral infection. Therefore,TMPRSS2 and Furin are fundamental for S activation.The importance of these two proteases was moreover established by a new study that presented that multicycle replication of SARS-CoV-2 in Calu-3 human airway cells was intensely repressed by stopping TMPRSS2 and Furin activit. But, almost, other human proteases, e.g., cathepsin L and B, elastase, trypsin and factor X, can be involved in the admission of SARS-CoV-2 into the human cell and in the loss of ACE2.A complex cell membrane protease associated with the endogenous shedding of ACE2 from membranes is the disintegrin metalloproteinase 17 (ADAM17.While TMPRSS2 cuts ACE2 and the S protein of SARS-CoV-2,due to membrane combination and the penetration of the virus, ADAM17 acts only on ACE2 and leads to ACE2 released into the extracellular cellular space.Therefore, ADAM17 and TMPRSS2 could have contrasting effects on ACE2 loss.Indications have exposed that the expression of TMPRSS2 blocks ADAM17-shedding of ACE2. Yet,it is uncertain exactly how TMPRSS2 excels ADAM17 to slice ACE2 through SARS-CoV-2 infection (*Salamanna et al*., *2020*).

1.3 Epidemiology of the coronavirus (2019-ncov)

1.3.1 Pandemic SARS-CoV-2/COVID-19

After only a month of the eruption at Wuhan, the SARS-CoV-2 virus prolonged speedily everywhere China at the period of the Chinese New Year.The virus was not restricted to a country.It was extremely infectious and proliferated to other 100 countries in the previous 2–3 months and affected 300,000 persons world wild.Such as on March 24, 2020,the affected population is as follows: the Western Pacific Region under which China,Republic of Korea, Australia,Malaysia, Japan,Singapore,New Zealand,etc,come informed a total of 96,580 established cases and 3502 deaths.On March 24,2020,943 new infected people and 29 deaths were showed in only one day.The European Region (Italy, Spain, Germany, the United Kingdom, Norway, etc.) accounted for more than 195,511 cases,among which 24,087 were reported in just 1 day.The number of confirmed cases has reached up to 10,189 and 1447 deaths in 1 day. In the Southeast Asia Region,1990 cases were reported with 65 deaths.In the Eastern Mediterranean Region,more than 27,215 people were contaminated and 1877 died due to this pandemic.In the Americas, 49,444 cases and 565 deaths were testified,with 12,428 novel cases and 100 deaths reported in a day.Lastly,in the African Region, 1305 cases and 26 deaths were confirmed *(Srivastava et al., 2020).*

Such as other regions and counties, Africa has not get away this disease.The first testified case was reported on 14 February 2020 in Egypt.then,more than 1.2 million cases and more than 28 000 deaths have been established.This characterizes about 5% and 3.4% of the whole cases and deaths world wild, correspondingly *(Lounis, 2020).*

Algeria,located in North Africa,is the biggest country in Africa with a population of 43 949 908 inhabitants.Ever since the first case,confirmed on 25 February 2020,it has presently reported 42 619 cases,making it one of the most contaminated countries in Africa beside South Africa,Egypt, Nigeria,Morocco and Ghana.It is too the third country in terms of deaths,with 1465 deaths, next South Africa (13 308) and Egypt (5298) (*Lounis*, *2020*).

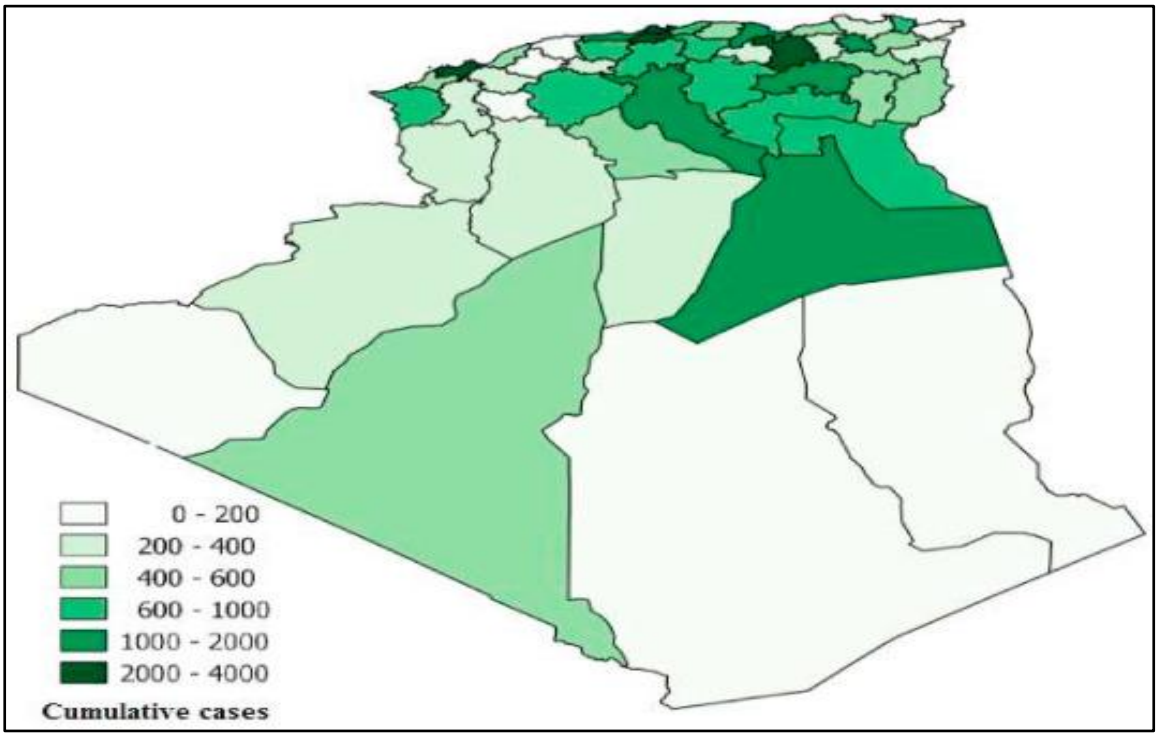

Figure 3 : Geographic repartition of COVID-19 cases in Algeria (On August 13th 2020). *(Lounis, 2020).*

1.4 Corona virus (2019-ncov) and vaccination

Vaccination could be used to avoid or to decrease the infection,viral excretion and thus spreading,therefore helping to regulate SARS-COV-2 epidemic.Several approaches have been elaborated to produce SARS-COV-2 vaccines,as well as DNA- and RNA-based vaccines,viral vector vaccines, deactivated virus vaccines,live-attenuated virus vaccines and recombinant protein vaccines inactivated virus vaccines,live-attenuated virus vaccines and recombinant protein vaccines.Allowing to reports from the WHO,there are 100 vaccines against SARS-CoV-2 at many periods of growth.Precisely,ten candidate vaccines are previously in experimental evaluation (Liu et *al.,* 202*0)*.

2 Monocyte

2.1 Monocyte heterogeneity and function

Monocytes derive from bone marrow hematopoietic stem cells and circulate in the blood. Monocyte extravasation and differentiation helps in many immune roles.The differentiation of monocytes into tissue macrophages at stable state may attend homeostatic roles.Monocytes are able to fuel acute inflammatory responses and anti-microbial immunity

through differentiating into inflammatory macrophages.Lastly,monocytes moreover actively help in the resolution of inflammation and tissue restoration *(Guermonprez et al., 2021).*

The division of monocytes is an emerging field.Current evolution in single-cell genomics and high dimensional approaches in phenotyping have highlighted extra subsets of monocytes.Monocytes can as well adopt novel dynamic transcriptional states related with inflammation and reflecting their subset heterogeneity.This carries the researchers to face a significant challenge of attributing monocyte heterogeneity to precise roles *(Guermonprez et al., 2021).*

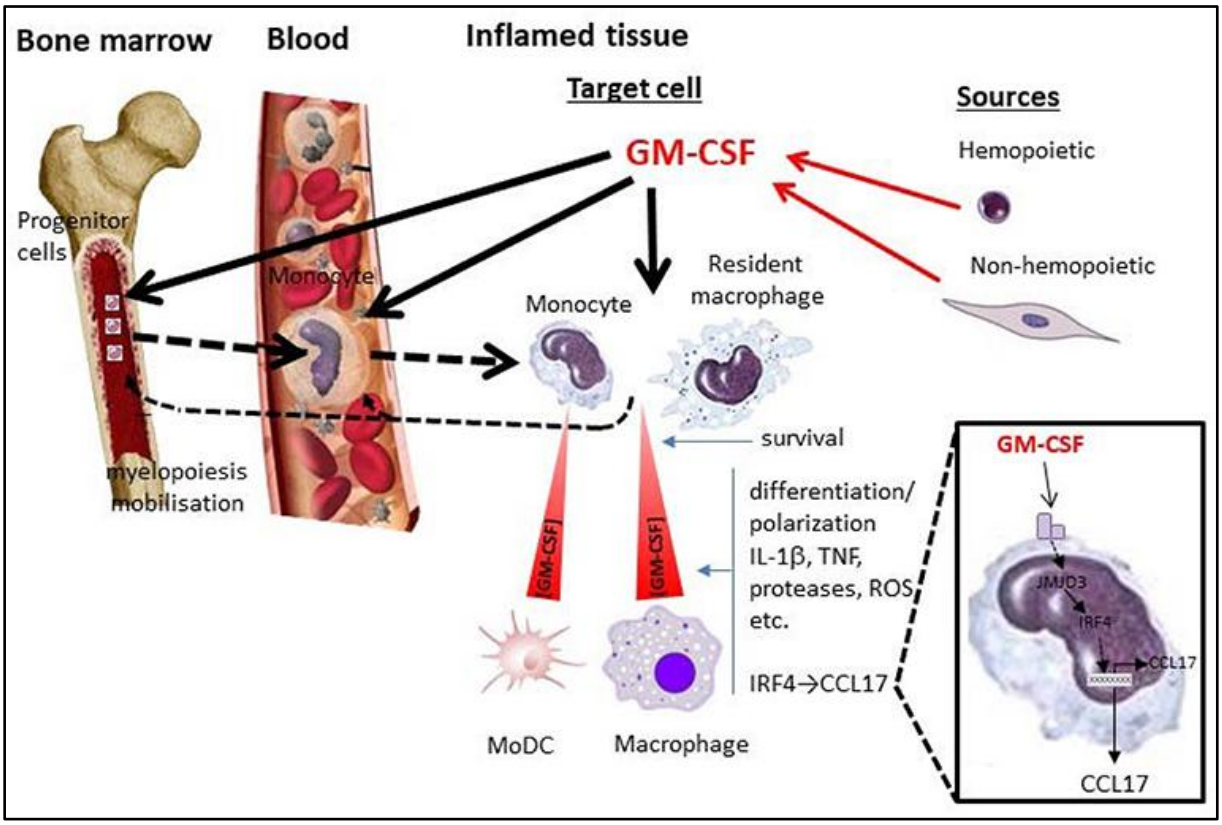

Figure 4 : GM-CSF and monocytes/macrophages in inflammation *(Hamilton, 2019)*

2.2 Control of Monocyte Subset Production and Functional Programming

Signals from the microenvironment is able to stimulus monocyte gene expression and role in a tissue-specific way,however the being there of several subsets of monocytes or monocyte-derived cells in the same tissue shows that they could have independent roots.We will study how signals identified by HSPCs,like cytokines and microbial constituents,shape the repertoire of monocytes producedboth in the steady-state and under emergency

conditions. This may occur via epigenetic and metabolic programming of the differentiating cells,which is argued in this section,and/or via selective elevation of particular differentiation pathways that yield different monocyte subsets,which is considered in the next section *(Wolf et al., 2019).*

Cytokines released by hematopoietic or non-hematopoietic cells in the bone marrow niche,or originating from outside the bone marrow via the circulation,can influence monocyte production and functional programming upon exposure by progenitors *(Wolf et al., 2019)*.

2.3 Monocyte Subsets and Phenotypes

The development of flow cytometry in the 1970s allowed the plan of a monocyte-specific antibody panel founded on the surface protein levels of the pattern recognition receptor CD14 and the Fc gamma III receptor CD16*(Kapellos et al., 2019)*.

Two populations were recognised; the classical ($CD14^{++}CD16^{+}$) and the non-classical ($CD14^{dim}CD16^{+}$).Subsequently,an intermediate for CD14 and CD16 ($CD14^{+}CD16^{+}$HLA-$DR^{+}CD86^{+}CD11c^{+}$) monocyte population with a different transcriptomic profile (LYZ, S100A8, CD14, S100A10, HLA-DRA, CD74, IFI30, HLA-DPB1, CPV) was exposed.Now, it was too suggested that this population could be divided from non-classical monocytes by the expression of 6-sulfo LacNAc (SLAN).These “intermediate” monocytes showed comparable ROS production and phagocytosis potential,lesser adhesion to surfaces,however established higher class II molecule expression and IL-12 production than classical monocytes.In mice, two monocyte subsets were recognised in the bloodstream by flow cytometry and intravital microscopy; a short-lived $Gr\text{-}1^{+}CCR2^{+}CX_3CR1^{lo}$ which migrates to tissues during inflammation and a $Gr\text{-}1^{-}CCR2^{-}CX_3CR1^{hi}$ one,which carries out CX_3CR1-dependent patroling of the vasculature through homeostasis *(Kapellos et al., 2019)*.

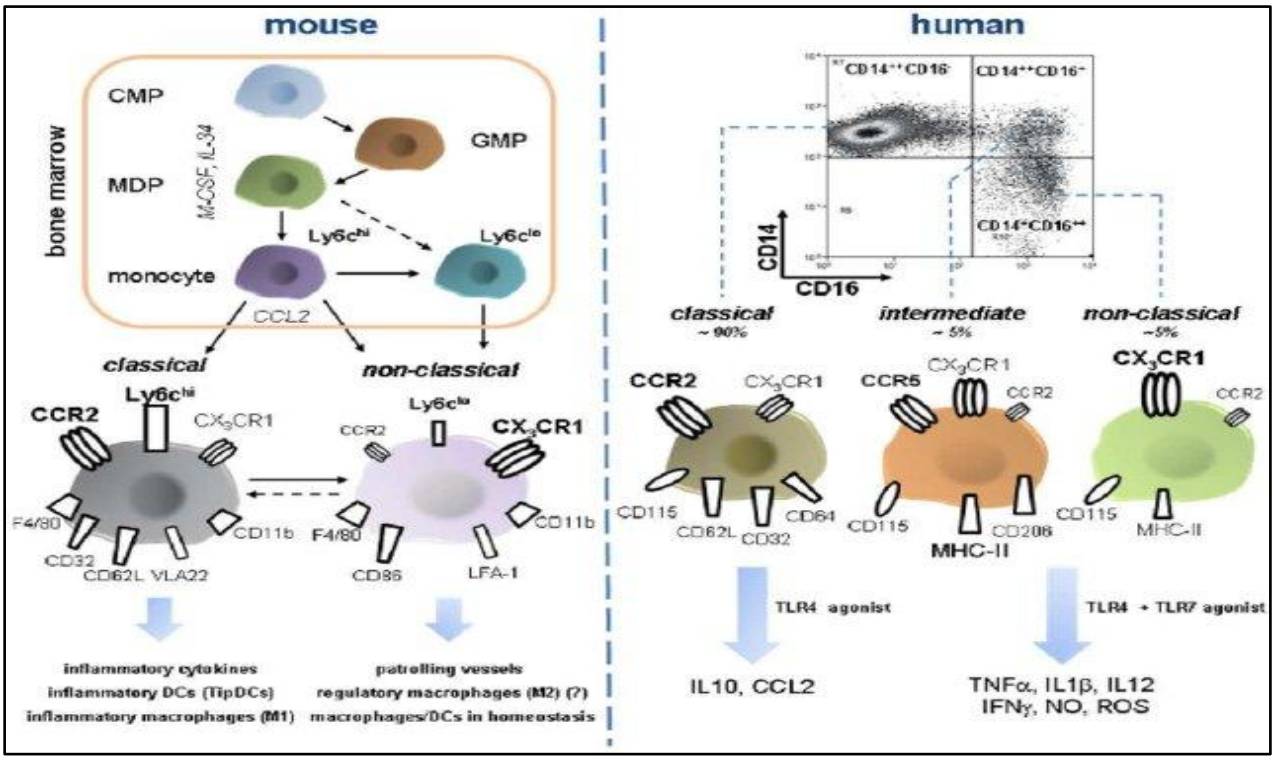

Figure 5 : Development and features of murine and human monocyte subsets *(Zimmermann et al., 2012).*

3 Monocyte and Covid 19

3.1 Hyperinflammation in severe COVID-19

Higher levels of inflammatory signs in blood (including C-r eactive protein, ferritin, and D-dimers), an amplified neutrophil- to- lymphocyte ratio1,12,13 and increased serum levels of different inflammatory cytokines and chemokines3,14–17 have been related with disease severity and death.The total cytokine profiles detected in patients with severe COVID-19 display resemblances to those detected in cytokine release disorders,as well as macrophage activation disease,with augmented production of cytokines like IL-6, IL-7 and tumour necrosis factor (TNF) and also of inflammatory chemokines including CC- chemokine ligand 2 (CCL2), CCL3 and CXC-c hemokine ligand 10 (CXCL10),such as the soluble form of the α-c hain of the IL-2 receptor.This has contributed to the theory that dysregulated activation of the mononuclear phagocyte (MNP) compartment led to COVID-19-related hyperinflammation8,18. Levels of IL-6 are frequently augmented in the sera of patients with severe infection,however almost studies have not seen augmented serum IL-1β levels in patients with COVID-19,as well as in patients with severe infection.Whether the difference

between IL-6 and IL-1β heights realised in patients with COVID-19 reveals practical restrictions or limited tissue production of cytokines or shows the lack of inflammasome activation involves additional explanation. Amount of systemic IL-18 such as local measurement of IL-1β and IL-18 must better assess the fonction of inflammasome activation in COVID-19 disease *(Merad)*.

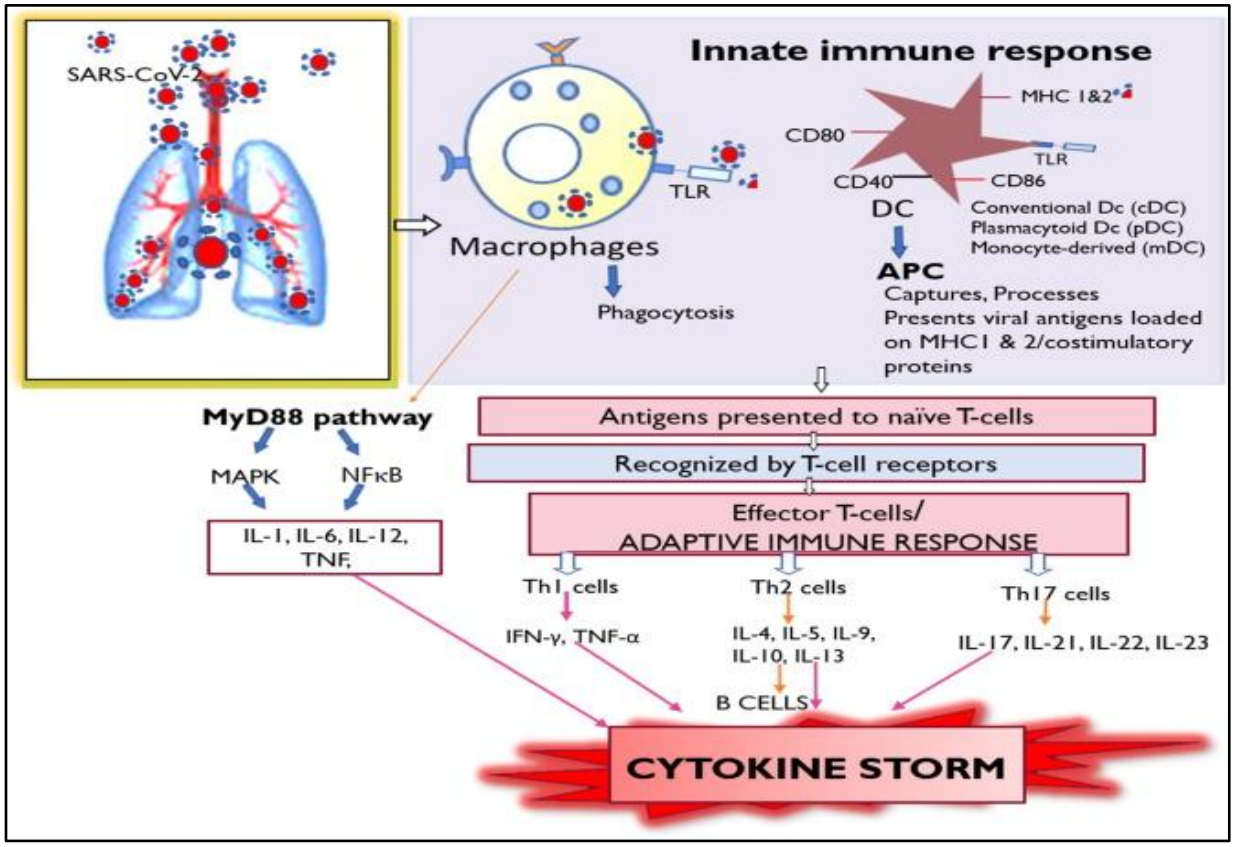

Figure 6 : The entry of the virus leads to activation of the innate immune system which comprises of macrophages and dendritic cells *(Banji et al., 2021).*

4 Trained immunity/ monocytes

4.1 WHEN TRAINED CELLS ENCOUNTER SARS-COV-2

Innate immune memory is recognised in circulating,local or tissue-resident cells such as in progenitor stem cells through epigenetic reprogramming.We may hypothesise that when trained airway epithelial cells would be exposed to SARS-CoV2,they would produce IL-8 and recruit neutrophils to the infected epithelia,which would then induces the eradication of infected cells.Circulating monocytes would be recruited to the lung in reaction to MCP1 and GM-CSF. Meanwhile innate immune memory moreover occurs at the progenitor stem cell level, GM-CSF and GCSF induce the differentiation of innate immune cells and prolong

myelopoiesis, which in turn rises the number of cells migrating to the contamination location, thus improving immune response and exciting operational resident activation of the innate immune system.[3,15] TLR3 activation is encouraged by SARS-CoV. The BCG-trained monocytes may as well yield IL10 via TLR3 activation.[62] Reactivation of this pathway by the virus may effect in inflection of resident inflammation *(Sohrabi et al., 2020)*.

4.2 Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity

4.2.1 Epigenetic Regulation

Epigenetic regulation is the mechanism in which diverse cells and tissues in an organism execute cell-type-specific programs even though holding nearly similar DNA in each cell.This is how each organ in the body creates different phenotypic characters and is how trained immunity is controlled as well.Trained immunity is mediated by modifications in chromatin accessibility to differentially methylated DNA and alterations in histone tail modifications,mutually result in chromatin remodeling.Chromatin remodeling through these changes modulates the augmentation or decrease of immune cytokine release.Moreover, TF fixation is a important feature of epigenetic modifications,lineage-specific activity,and cellular memory reacting to environmental stimulation.These three mechanisms work in concert to induce modifications in cell destiny and identity,such as cells do not approve new phenotypes or terminal differentiation without dynamic chromatin modifications *(Adams et al., 2020)*.

4.3 Innate Immune Training Is Dependent on Histone Modification

Numerous supressed enhancers are exposed to stimulation-specific transcription factors that may stay in place for a long period of time.These dormant enhancers become poised to respond quickly to a secondary contact.Genes related to H3K4me1-bound enhancer sites trend to low expression,whereas enhancer sites also bound with H3K27ac are upregulated.H3K27ac(−) enhancers are considered poised,while H3K27ac(+) enhancers are active.This is significant in trained immunity,as prolonged exposure to training stimuli is not important for long-term memory.Furthermore, the dose and nature of the training provocation may yield opposing histone profiles *(Adams et al., 2020).*

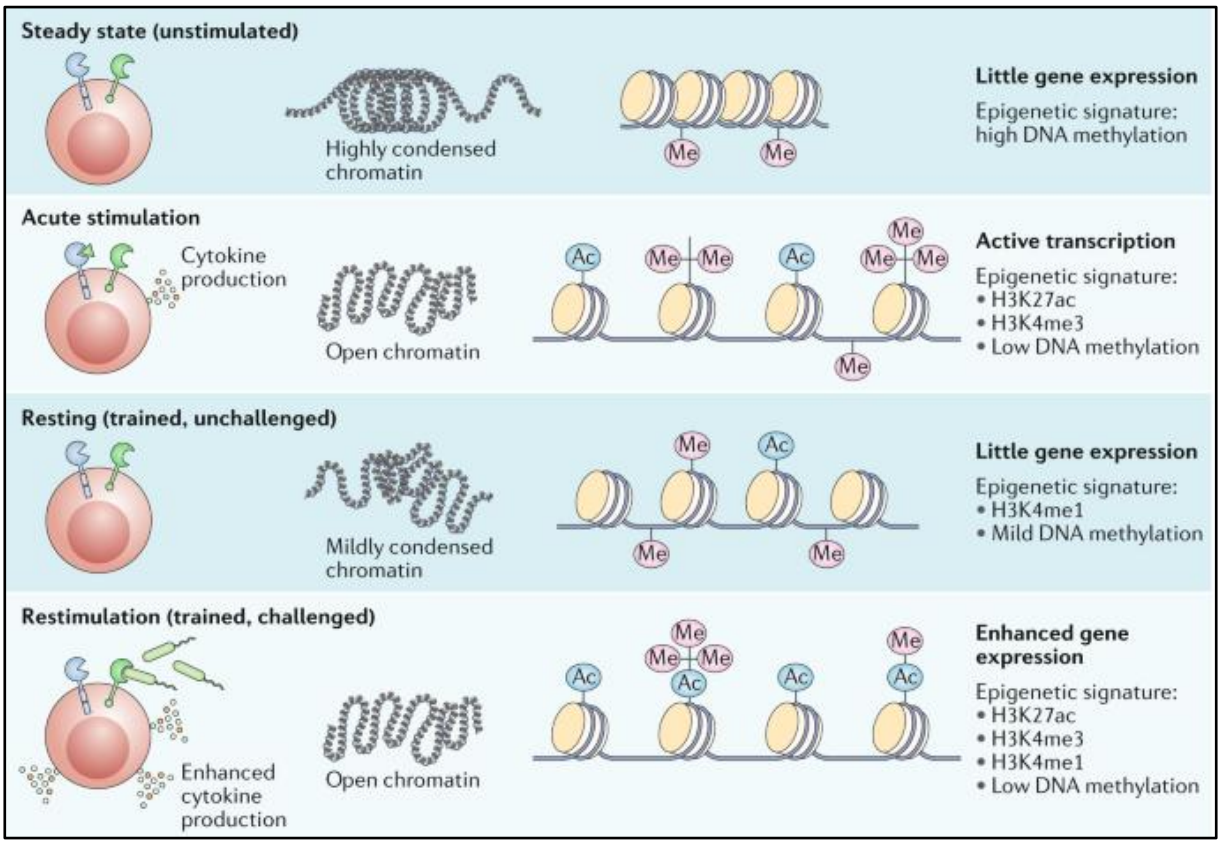

Figure 7: Epigenetic reprogramming underlies the induction of trained immunity *(Netea et al., 2020).*

Monocyte differentiation into macrophages represents a cornerstone procedure for host defense.Concurrently,immunological imprinting of both tolerance and trained immunity concludes the functional destiny of macrophages and vulnerability to secondary contagions. We characterized the transcriptomes and epigenomes in four primary cell types: monocytes and in vitro–differentiated naïve,tolerated,and trained macrophages.Inflammatory and metabolic pathways were modulated in macrophages,counting reduced inflammasome activation,and we recognized pathways functionally implicated in trained immunity.b-glucan training elicits an exclusive epigenetic signature,revealing a complex network of enhancers and promoters.Analysis of transcription factor motifs in deoxyribonuclease I hypersensitive sites at cell-type–specific epigenetic loci unveiled differentiation and treatment-specific repertoires.Altogether,we provide a reserve to realize the epigenetic changes that trigger innate immunity in humans *(Saeed et al., 2014)*.

5 Problematic and objective

5.1 Problematic

Trained immunity was hypothesized to be a tool for reducing susceptibility to SARS-CoV-2.It is now proposed that the boosting of the innate immune response by inducing trained immunity might protect against severe SARS-CoV-2 infection at least for a limited amount of time (*Bekkering et al.,* 2021).

5.2 Objective

we evaluated the level of expression of H3k4me3 by monocyte following its infection with SARS-CoV 2

5.3 Purpose

show that SARS-CoV-2 is able to induce trained immunity in monocytes.

2 Chapter 2. Materials and methods

2.1 Preparation of PBMC

PBMCs were isolated from fresh venous blood collected into ethylenediamineteraacetic acid (EDTA)-containing tubes (BD, Belliver Industrial Estate, United Kingdom). Primary, 3,5mL of Histopaque-1077 (Sigma Aldrich Co., St. Louis, MO) was introduced in conical centrifuge tube and brought back to room temperature. PBMCs were then isolated by using density gradient, after carefully layering whole blood onto an equal volume of Histopaque-1077, and centrifugation at 400 g during 30 min at room temperature *(Miliani et al., 2018)*.

The opaque interface containing mononuclear cells were recovered and washed 2 times with consecutive volume of isotonic phosphate-buffered saline (PBS) solution (10, 5, and 5mL) by centrifugation at 100 g for 10min. Cells were suspended in 1mL (0.5mL/each isolated cell pellet) of RPMI 1640 culture medium (Sigma Chemical Co., St. Louis, MO), supplemented with 2mM l-glutamine, 1mM of sodium pyruvate, 50mg/mL gentamicin, and 10% fetal calf serum (FCS). Cell viability was assessed by trypan blue exclusion test (TBET) *(Miliani et al., 2018*).

2.2 MOs isolation

MOs were isolated from PBMCs based on differential plastic adherence. For a short time, PBMCs were cultivated in RPMI-1640 complemented with 10% FCS and 50 µg/ mL gentamicin, and seeded at 2 x 106 cell/mL into 24-well plates. Cells were allowed to adhere for 2 h at 37˚C before removal of non-adherent cells were and treatment of adherent MOs with MET. Cells were counted microscopically using trypan blue staining*(Dahmani et al., 2020)*.

2.3 Quantify the level of histone H3 lysine 4 methylation

2.3.1 Procedure

Cell Wash

Harvest 1 □ 10^7 cells and wash twice with ice cold Phosphate Buffered Saline (PBS).

Resuspend the pellet in 1 mL of PBS and transfer cells to a 1.5 mL tube. Spin cells at 600 □ *g* for 10 minutes in a microfuge and aspirate supernatant.

Tissue Wash

Cut 100 mg of tissue of interest into 2 mm^3 sections and wash twice in a 1.5 mL tube with 1 mL of ice-cold PBS. Centrifuge the cells at 600 □ g for 10 minutes for each wash step and discard the supernatant.

Cell Lysis

Resuspend washed cells in 1 mL of ice-cold Lysis Buffer (optional: containing 2 mM PMSF) and lyse for 10 minutes on ice with intermittent gentle mixing (7–10 tube inversions). Centrifuge the lysate at 600 □ *g* for 10 minutes at 4 □C. Remove the supernatant and wash the pellet with 0.5 mL of Lysis Buffer Stain 5 □L of cell lysate with Trypan Blue and view under a microscope at 20□ on a glass slide. At least 80–90% of the cells should be lysed. Centrifuge the lysate and discard the supernatant. Repeat the wash step with 0.5 mL of Lysis Buffer and remove supernatant.

Tissue Lysis

Resuspend the washed tissue in 1 mL of ice-cold Lysis Buffer (optional: containing 2 mM PMSF) and homogenize it with a Dounce homogenizer on ice to fully disperse the cells. To check for the homogenization efficiency in the tissue sample, view the homogenized sample under a microscope. A uniform suspension should be observed. Typically for soft tissues 10–15 strokes and for hard tissues 15–20 strokes are sufficient. Transfer the lysate into a 1.5 mL tube and incubate on ice for 10 minutes. Spin the minced tissue in a table top microfuge at 600 □ *g* for 10 minutes. Remove the supernatant and wash with 0.5 mL of Lysis Buffer. Centrifuge as before and discard the supernatant.

Extraction

Completely resuspend pellet in 0.25 mL of ice-cold Extraction Reagent and incubate on ice for 1 hour.

Centrifuge at 10,000 □ *g* for 10 minutes at 4 □C and collect the supernatant. Add 0.1 mL of ice-cold Neutralizing Buffer containing 1 mM DTT directly to the supernatant and mix well. This isolate contains the core histones. Quantify the histones isolated with any protein quantitation assay. BSA can be used as a standard.

3 Chapter 3. Results and discussion

Results are confidential until publication

4 Chapter 4.Conclusion

The first contact with SARS-COV-2 activates monocyte, that will lead to upregulation of target gene transcription related to a speedy acquisition of activating histone modification. Once the elimination of the stimulus,though some of these epigenetic characters persevere,which leads to earlier and stronger activation of gene transcription upon next exposition.Significant epigenetic marks involved in trained immunity are H3K4me3.

Epigenetics modifications of H3K4Me3 induces trained immunity in the monocyte.trained immunity is a very promising approach against COVID-19 and its Induction could possibly improve antiviral-host defence and better train COVID-19 patients to fight the infection it may be useful to reduce the propagation of the virus and the mortality rates.

Chapter 5. Bibliography

Adams, K., Weber, K.S., and Johnson, S.M. (2020). Exposome and Immunity Training: How Pathogen Exposure Order Influences Innate Immune Cell Lineage Commitment and Function. Int. J. Mol. Sci. *21*, 8462.

Bekkering, S., Domínguez-Andrés, J., Joosten, L.A.B., Riksen, N.P., and Netea, M.G. (2021). Trained Immunity: Reprogramming Innate Immunity in Health and Disease. Annu. Rev. Immunol. *39*, 667–693.

Dahmani, Z., Addou-Klouche, L., Gizard, F., Dahou, S., Messaoud, A., Chahinez Djebri, N., Benaissti, M.I., Mostefaoui, M., Terbeche, H., Nouari, W., et al. (2020). Metformin partially reverses the inhibitory effect of co-culture with ER-/PR-/HER2+ breast cancer cells on biomarkers of monocyte antitumor activity. PLOS ONE *15*, e0240982.

Esakandari, H. (2020). A comprehensive review of COVID-19 characteristics. 10.

Guermonprez, P., Ginhoux, F., Mildner, A., Gautier, E.L., Schlitzer, A., Jakubzick, C., Varol, C., and Bain, C.C. (2021). Monocyte Heterogeneity and Function. Front. Immunol. 205.

Kapellos, T.S., Bonaguro, L., Gemünd, I., Reusch, N., Saglam, A., Hinkley, E.R., and Schultze, J.L. (2019). Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front. Immunol. *10*, 2035.

Liu, X., Liu, C., Liu, G., Luo, W., and Xia, N. (2020). COVID-19: Progress in diagnostics, therapy and vaccination. *10*, 15.

Lounis, M. (2020). Epdemiology of coronavirus disease 2020 (COVID-19) in Algeria. 7.

Merad, M. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. 8.

Miliani, M., Nouar, M., Paris, O., Lefranc, G., Mennechet, F., and Aribi, M. (2018). Thymoquinone Potently Enhances the Activities of Classically Activated Macrophages Pulsed with Necrotic Jurkat Cell Lysates and the Production of Antitumor Th1-/M1-Related Cytokines. J. Interferon Cytokine Res. *38*, 539–551.

Netea, M.G., Domínguez-Andrés, J., Barreiro, L.B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L.A.B., van der Meer, J.W.M., Mhlanga, M.M., Mulder, W.J.M., et al. (2020). Defining trained immunity and its role in health and disease. Nat. Rev. Immunol. *20*, 375–388.

Platto, S., Wang, Y., Zhou, J., and Carafoli, E. (2021). History of the COVID-19 pandemic: Origin, explosion, worldwide spreading. Biochem. Biophys. Res. Commun. *538*, 14–23.

Prabakaran, P., Xiao, X., and Dimitrov, D.S. (2004). A model of the ACE2 structure and function as a SARS-CoV receptor. Biochem. Biophys. Res. Commun. *314*, 235–241.

Saeed, S., Quintin, J., Kerstens, H.H.D., Rao, N.A., Aghajanirefah, A., Matarese, F., Cheng, S.-C., Ratter, J., Berentsen, K., van der Ent, M.A., et al. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science *345*, 1251086–1251086.

Salamanna, F., Maglio, M., Landini, M.P., and Fini, M. (2020). Body Localization of ACE-2: On the Trail of the Keyhole of SARS-CoV-2. Front. Med. *7*, 594495.

dos Santos, W.G. (2020). Natural history of COVID-19 and current knowledge on treatment therapeutic options. Biomed. Pharmacother. *129*, 110493.

Shah, V.K., Firmal, P., Alam, A., Ganguly, D., and Chattopadhyay, S. (2020). Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. Front. Immunol. *11*, 1949.

Sohrabi, Y., Godfrey, R., Netea, M.G., and Joosten, L.A. (2020). Trained immunity as a novel approach against COVID-19 with a focus on Bacillus Calmette–Guérin vaccine: mechanisms, challenges and perspectives. *9*, 17.

Srivastava, N., Baxi, P., Ratho, R.K., and Saxena, S.K. (2020). Global Trends in Epidemiology of Coronavirus Disease 2019 (COVID-19). In Coronavirus Disease 2019 (COVID-19), S.K. Saxena, ed. (Singapore: Springer Singapore), pp. 9–21.

Wolf, A.A., Yáñez, A., Barman, P.K., and Goodridge, H.S. (2019). The Ontogeny of Monocyte Subsets. Front. Immunol. *10*, 1642.

Résumé

Introduction : Le coronavirus 2 du syndrome respiratoire aigu sévère (SARS-CoV-2) de la maladie COVID-19 qui fait des ravages dans le monde entier est causé par le virus SARS-CoV-2. De même que d'autres virus,La réaction immunitaire innée montre un rôle important dans la défense contre COVID-19. Les cellules du système immunitaire inné affichent la capacité d'acquérir des caractéristiques de mémoire après une incitation transitoire, provoquant une réaction améliorée lors d'une exposition secondaire. Ce phénomène a été nommé immunité entraînée. L'immunité entraînée est conditionnée par différents programmes épigénétiques. Marques épigénétiques importantes impliquées dans l'immunité entraînée sont H3K4me3.

Objectif : Tester l'activité et les modifications épigénétiques de H3K4Me3 sur le macrophage/monocytes dans le contexte de COVID 19.

Matériels et méthodes : Les PBMC ont été isolées à partir de sang veineux frais provenant de donneurs de volontaires sains. Les MO ont été isolés à partir de PBMC sur la base d'une adhérence plastique différentielle. Le niveau de méthylation de l'histone H3 lysine 4 a été quantifié.

Conclusions : Les modifications épigénétiques de H3K4Me3 induisent une immunité entraînée dans le monocyte. L'immunité entraînée est une approche très prometteuse contre COVID-19 et son induction pourrait éventuellement améliorer la défense antivirale de l'hôte et mieux former les patients COVID-19 à combattre l'infection, il peut être utile de réduire la propagation du virus et les taux de mortalité.

Mots clé: COVID-19, SARS-CoV-2, Trained immunity, epigenetic, H3K4Me3

Abstract

Introduction: The severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) of the COVID-19 disease that is damaging all over the world is caused by the SARS-CoV-2 virus. Likewise other viruses,The innate immune reaction shows an important role in defence against COVID-19. The Cells of the innate immune system display the ability to gain memory features after transitory inducement,causing an improved reaction upon secondary exposition.This phenomenon has been named trained immunity.Trained immunity is conditioned on different epigenetic programs.Significant epigenetic marks involved in trained immunity are H3K4me3.

Objective: To test the activity and epigenetics modifications of H3K4Me3 on the macrophage/monocyte in the context of COVID 19.

Material and methods: PBMCs were isolated from fresh venous blood from healthy volunteers' donors.MOs were isolated from PBMCs based on differential plastic adherence.the level of histone H3 lysine 4 methylation were quantified.

Conclusions : Epigenetics modifications of H3K4Me3 induces trained immunity in the monocyte.trained immunity is a very promising approach against COVID-19 and its Induction could possibly improve antiviral-host defence and better train COVID-19 patients to fight the infection it may be useful to reduce the propagation of the virus and the mortality rates.

Key words: COVID-19, SARS-CoV-2, Trained immunity, epigenetic, H3K4Me3

ملخص

مقدمة: إن فيروس كورونا المتلازمة التنفسية الحادة الوخيمة 2 (SARS-CoV-2) لمرض COVID-19 الذي يعيث فساداً في جميع أنحاء العالم ناجم عن فيروس .SARS-CoV-2 مثل الفيروسات الأخرى ، تُظهر الاستجابة المناعية الفطرية دورًا مهمًا في الدفاع ضد-COVID 19.تُظهر خلايا الجهاز المناعي الفطري القدرة على اكتساب خصائص الذاكرة بعد تحفيز عابر ، مما يتسبب في استجابة محسنة عند التعرض الثانوي. هذه الظاهرة تسمى المناعة المدربة. تكون المناعة الناتجة مشروطة ببرامج الوراثة اللاجينية المختلفة. العلامات التجارية اللاجينية الهامة المشاركة في المناعة المستحثة هي.H3K4me3

الهدف: اختبار النشاط والتعديلات اللاجينية لـ H3K4Me3 على البلاعم / وحيدات في سياق19 COVID.

المواد والطرق: تم عزل PBMCs من الدم الوريدي الجديد الذي تم الحصول عليه من متبرعين لمتطوعين أصحاء. تم عزل MOs من PBMCعلى أساس التصاق البلاستيك التفاضلي. تم قياس مستوى مثيلة هيستون H3 ليسين 4.

الاستنتاجات: التعديلات اللاجينية لـ H3K4Me3 تحفز المناعة في الخلية الوحيدة. تعد المناعة المدربة نهجًا واعدًا للغاية ضد COVID-19 ويمكن أن يؤدي تحريضها إلى تحسين دفاع المضيف المضاد للفيروسات وتدريب مرضى COVID-19 بشكل أفضل على مكافحة العدوى ، وقد يكون من المفيد تقليل انتشار الفيروس ومعدلات الوفاة.

الكلمات المفتاحية: COVID-19، SARS-CoV-2 ، مناعة مدربة ، التخلق المتوالي ، H3K4Me3