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Clinical Immunology

Letter to the Editor

Targeting T-cell senescence and cytokine storm with rapamycin to prevent severe progression in COVID-19

Dear Editor-in-Chief,

Recently, *Zhang* et al. published "The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The perspectives of clinical immunologists from China" [1]. We have read with great interest this review. As mentioned, current knowledge of anti-inflammation treatment in COVID-19 patients supports the use of glucocorticoids, tocilizumab, JAK inhibitors, chloroquine and hydroxychloroquine [1].

The emergence of a new SARS-CoV-2 betacoronavirus has led to a major health-related crisis resulting in significant mortality in intensive care units (ICU), due to pulmonary complications of COVID-19 [1]. Mortality is also associated with advanced chronological age, diabetes, or cardiovascular disease [1].

Reduced counts and functional exhaustion of T lymphocytes, and cytokine release syndrome have been identified as adverse factors in patients affected by severe SARS-CoV-2 infection [2,3]. Severe COVID-19 can therefore mimic a state of immune senescence [4].

In COVID-19, the serine/threonine kinase mTOR (mechanistic Target Of Rapamycin) pathways may offer valuable targets to control cell injury, oxidative stress, and the onset of hyperinflammation [5]. mTOR is a central regulator of inflammation within the immune system [6,7] and a sensor of oxidative stress [8]. mTOR forms two complexes: mTORC1 mediates TH1 and TH17 differentiation at the time of viral antigenic presentation by dendritic cells (DC) [9]; mTORC2 mediates TH2 differentiation; while both complexes restrict regulatory T-cell (Treg) differentiation [7]. With regards to T cells, mTORC1 activation is consequence of oxidative stress, which can be blocked by *N*-acetylcysteine in Systemic Lupus Erythematosus (SLE) patients [10]. Consistent with its role in pro-inflammatory T-cell differentiation, mTORC1 activation is involved in SLE patients which can be blocked by rapamycin [7]. In addition, mTORC1 is thus known as the rapamycin-sensitive complex [9].

The aim of this letter is to discuss the potentiality for rapamycin (sirolimus), an mTOR inhibitor, to restore T-cell functionality and decrease cytokine storm.

Cytokine storm, a hyper-inflammatory reaction in which cytokines are produced rapidly and extensively by immune cells in reaction to endogenous or exogenous stress [11], is a major contributor to acute respiratory distress syndrome and multiple organ dysfunction syndrome [11].

In severe COVID-19 patients, IL-2, IL-6, IL-7, IL-10, TNF-α, G-CSF, IP-10, MCP-1, and MIP-1α levels increase significantly [1,2]. Among these, several cytokines are involved in TH17 type responses. IL-1β and TNF-α (TH17 and TH1 cells highly express TNF-α), both promote TH17 responses and vascular permeability and leakage [12]. COVID-19 expands TH17 cells further supporting a TH17 type cytokine storm in this disease [12]. Cytokine storm may promote T-cell apoptosis, necrosis or pyroptosis, causing reduced T-cell counts [2].

T-cell senescence is a state of T-cell dysfunction that occurs in chronic infections and cancer [2,4]. In COVID-19, patients over 60 years, and patients in ICU care have a decrease in CD4+, CD8+, and total T-cell numbers [2], and this is inversely correlated with patients' survival [2].

T cells play a vital role in viral clearance, particularly through secretion of cytotoxicity molecules such as perforin, granzyme and IFN-γ [3]. However, patients with severe form of COVID-19 have less multifunctional and more non-functional CD4+ T cells, as well as higher senescent CD8+ T cells, that are unable to secrete the cytotoxicity molecules, than patients with mild COVID-19 [3]. Also, reduced T-cell numbers are negatively correlated with serum IL-6, IL-10 and TNF-α [2] with higher levels of senescence markers PD-1 and Tim-3 [2], CTLA-4 and TIGIT [3]. These constitute a hallmark of severe forms of COVID-19 [2,3]. In addition, senescent cells are known to secrete a broad spectrum of molecules such as cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17, TNF-α...), chemokines, proteases and growth factors, which distinguish them from healthy cells present within tissues [13]. These factors represent a typical hallmark of senescence and therefore they have been defined as senescence-associated secretory phenotype (SASP) [13]. Taken together, T-cell senescence might be the *primum movens* of cytokine storm in severe COVID-19 [14].

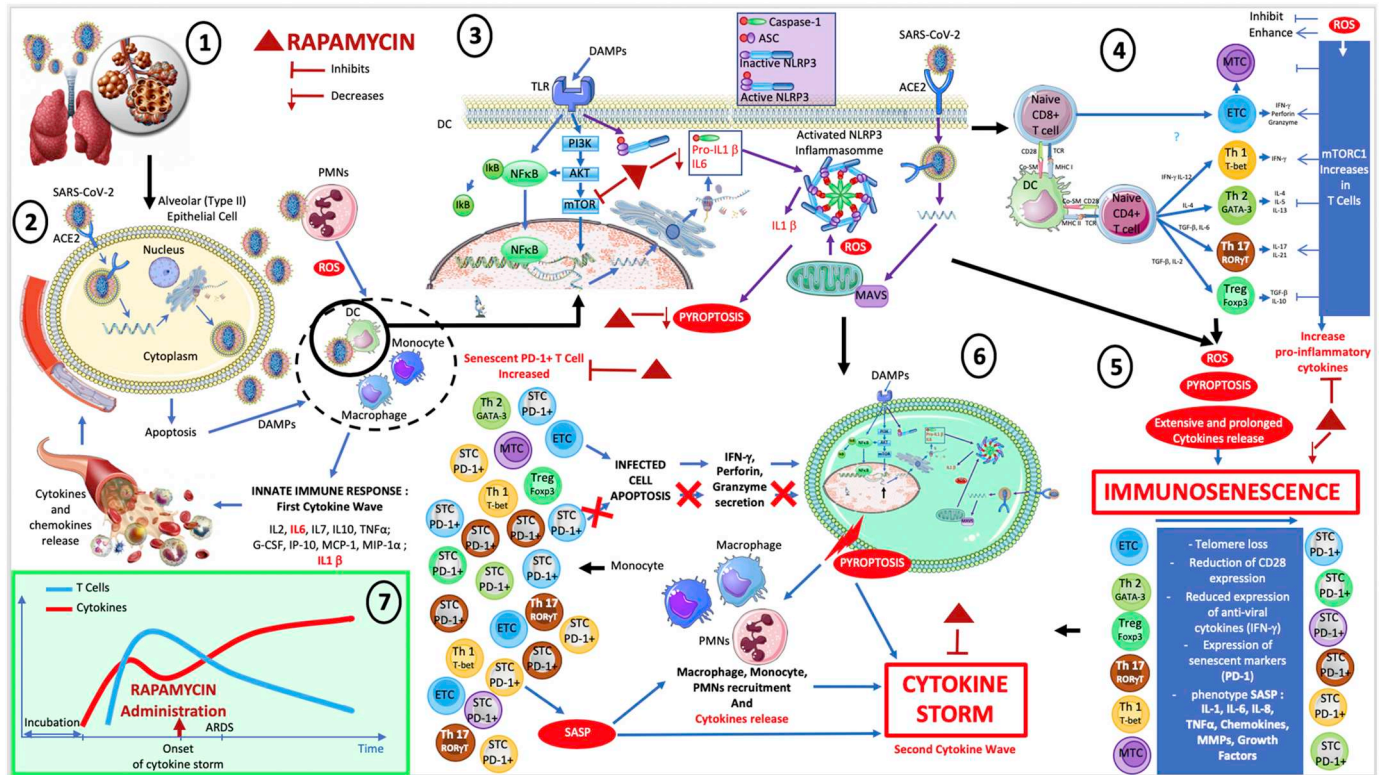In these patients, the potential of rapamycin, a specific mTOR

Fig. 1. Rapamycin use in COVID-19. ①SARS-CoV-2 entry into lungs through respiratory droplets ②Alveolar (Type II) Epithelial cell zoomSARS-CoV-2 bindingACE2 and entry into Alveolar (Type II) Epithelial cell SARS-CoV-2 replication into Alveolar (Type II) Epithelial cell Cell apoptosis releasing DAMPs Innate immune cells recruitment ROS releasing Innate immune response with chemokines and cytokines release ③ DC Zoom Binding SARS-CoV-2 to TLR Activation of NFkB signaling pathway Activation of PI3K/AKT/mTOR signaling pathway Activation NLRP3 inflammasome pathway by the ROS as result of SARS-CoV-2 binding ACE2 Production of IL-1β by Caspase-1 from pro- IL-1β Caspase-1 mediate cell pyroptosis Rapamycin blocks mTOR and finally limits IL-1β and IL-6 production as well as pyroptosis ④ Preferential differentiation of ETC, TH1 and TH17 by activation of mTORC1 pathway by ROS ⑤ ROS, Pyroptosis, extensive and prolonged cytokines release lead to immunosenescence Expression of senescent markers such as PD-1 Senescent Associated Secretory Phenotype (SASP) with IL-1, IL-6, IL-8, TNFα, Chemokines, MMPs, and Growth Factors ⑥ Critical phase of SARS-CoV-2 infection with Cytokine Storm and immunoscenescence SASP and Pyroptosis lead to Macrophages, Monocytes, PMNs recruitment and cytokines release. SASP, Pyroptosis, and cytokines released by Macrophages, Monocytes, and PMNs are the main components of CYTOKINE STORM CD8 + T lymphocytes senescence under cytokine storm and extensive SARS-CoV-2 replication : Cytokine storm increases the numbers of STC expressing the senescent marker PD-1 PD-1 + STC become unable to secrete IFN-γ, Perforin, and Granzyme, and eventually kill the infected cell ⑦ Kinetics of SARS-CoV-2 infection and window of opportunity for rapamycin treatment Abbreviations: DAMPs: Damage Associated Molecular Paterns; PMNs : Polymorphonuclear Leukocytes ; ROS: Reactive Oxygen Species; DC : Dendritic Cell ; TLR : Toll Like Receptor ; ACE2 : Angiotensin Converting Enzyme 2; MAVS : Mitochondrial Anti-Viral Signaling; STC : Senescent T Cell; ETC: Effector T Cell; MTC: Memory T Cell; ARDS : acute respiratory distress syndrome.

inhibitor that can promote autophagy and suppress the SASP, to reverse T-cell senescence can be discussed [15].

In elderly with increased senescent PD-1+ T-cells, everolimus (an analog of rapamycin) enhanced immune function, and improved T-cell responses to antigenic stimulation with an acceptable risk/benefit balance [4]. In elderly with coronary artery disease, rapamycin reduced serum senescence markers through IL-6 suppression [16].

In patients infected with the H1N1 influenza virus, early adjuvant rapamycin therapy during a short period (2 mg/day for 14 days) was significantly associated with an increased viral clearance, a greater improvement in lung injury (i.e. less hypoxemia), and a decrease of multiple organ dysfunction. The duration of ventilation in survivors was also shortened [17].

In a mouse model, H1N1 causes acute lung injury in an IL-17-dependent manner [18]. mTOR blockade with rapamycin might inhibit the expansion of Th17 cells in COVID-19 patients such as in Systemic Lupus Erythematosus patients [19,20].

H1N1 and SARS-CoV-2 both activate mTOR, and NLRP3 inflammasome pathway [5,21] leading to the production IL-1β, the mediator of lung inflammation, fever and fibrosis [5,17] and induces pyroptosis, a hyperinflammatory form of cell death [22]. Rapamycin inhibits H1N1-induced mTOR pathway activation, and thus IL-1β secretion [21]. In COVID-19, the binding of SARS-CoV-2 to Toll Like Receptor (TLR), which leads to IL-1β production, could be reversed by rapamycin [23].

Furthermore, rapamycin promotes de novo expression of Foxp3 in naive T cells, leading to Treg proliferation and survival in vivo and in vitro [9]. As a result, rapamycin inhibits effector T-cell proliferation and promotes Treg accumulation [9].

In addition, rapamycin was recently identified in a network-based drug repurposing study as a candidate for potential use in COVID-19 [23].

When given at the early onset of the cytokine storm phase, rapamycin, through the down-regulation of the SASP, of the mTOR-NLRP3-IL-1β axis, of the IL-6 pathway, and of senescent T-cell number, might prevent progression to severe forms of COVID-19 (Fig. 1).

The adverse effects of rapamycin are well known and include leukopenia, thrombocytopenia, diarrhea, stomatitis, hypercholesterolemia, and rarely interstitial pneumonitis [24].

Associated to antiviral therapy, rapamycin could optimize the treatment of COVID-19 patients with advanced chronological age, and/or comorbidities, or those with reduced T-cell counts who are more likely to progress to severe disease.

To date, there is one registered clinical trial of rapamycin: "Sirolimus Treatment in Hospitalized Patients With COVID-19 Pneumonia (SCOPE)" (NCT04341675).

Author contribution statement

LO: Conception, design, literature analysis, and manuscript redaction.

AJ: Analysis, manuscript redaction, revising the manuscript for important intellectual content.

FP: Drafting the figure, and revising the manuscript for important intellectual content.

BL: Analysis, revising the manuscript for important intellectual content.

OM: Analysis, revising the manuscript for important intellectual content.

GM: Analysis, revising the manuscript for important intellectual content.

Each author revised the report and approved the submitted version of the manuscript.

Each author has agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Permission information

The authors do hereby declare that all illustrations and figures in the manuscript are entirely original and do not require reprint permission.

Ethics approval

Not applicable.

Declaration of Competing Interest

All authors declare they have nothing to disclose, and no competing interests.

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