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Statins: Could an old friend help the fight against COVID-19?

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Abstract

COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has overwhelmed Healthcare Systems requiring the rapid development of treatments, at least, to reduce COVID-19 severity. Drug repurposing offers a fast track. Here, we discuss the potential beneficial effects of statins in COVID-19 patients based on evidence that they may target virus receptors, replication, degradation and downstream responses in infected cells, addressing both basic research and epidemiological information. Briefly, statins could act modulating virus entry, acting on the SARS-CoV-2 receptors, ACE2 and CD147, and/or lipid rafts engagement. Statins, by inducing autophagy activation, could regulate virus replication or degradation, exerting protective effects. The well-known anti-inflammatory properties of statins, by blocking several molecular mechanisms, including NF-κB and NLRP3 inflammasome, could limit the "cytokine storm" in severe COVID-19 patients which is linked to fatal outcome. Finally, statin moderation of coagulation response activation may also contribute to improve COVID-19 outcomes.

Abbreviations

ACE2 -Angiotensin converting enzyme 2
ACEi -Angiotensin converting enzyme inhibitor
AMPK - AMP-activated protein kinase
Ang II -Angiotensin II
AP1 -Activator protein 1
ARB -Angiotensin II receptor blocker
ATG -Autophagy-related gene
CEACAM5 - Carcinoembryonic antigen-related cell adhesion molecule
cGAS -Cyclic GMP-AMP synthase
CI -Confidence interval
COPD -Chronic obstructive pulmonary disease
CoV - Coronavirus
CRP -C reactive protein
CYP3A4 -Cytochrome P450 3A4
DAMPs -Damaged-associated molecular patterns
DIC -Disseminated intravascular coagulation
DPP4 -Dipeptidyl peptidase-4
EP -Envelope protein
HBV -Hepatitis B virus
HCV -Hepatitis C virus
HIV -Human immunodeficiency virys
HMG-CoA - 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HR - Hazard ratio
ICU Intensive care unit
IL -Interleukin
INF -Interferon
IRFs -Interferon regulatory factors

ISG -Interferon stimulated genes
KLF2 -Kurppel-like factor 2
KSHV -Kaposi's sarcoma-associated herpesvirus
LC3 -Light chain 3
LDL -Low density lipoprotein
MERS -Middle-East respiratory syndrome
MHV -Murine γ-herpesvirus
MP -Membrane protein
mTOR -Mammalian target of rapamycin
MβCD -Methyl-β-cyclodextrin
NF-kB - Nuclear factor kappa-light-chain-enhancer of activated B cells
NLRP-3 - NACHT, LRR and PYD domains-containing protein 3
OR -Odds ratio
PAMPs -Pathogen-associated molecular patterns
PfRh5 -Plasmodium Falciparum reticulocyte binding-like homologue 5
PI3K - Phosphatidylinositol 3-kinase
PMA -phorbol-12-myristate-13 acetate
P-pg -P-glycoprotein transport system
PRISM - Platelet receptor inhibition in ischemic syndrome management
PRR -Pattern recognition receptor
PRRS -Porcine reproductive and respiratory syndrome
RAS -Renin angiotensin system
RBD -Receptor binding domain
RIG-I -Retinoic acid-inducible gene I
RLR -(RIG-I)-like receptor
ROCK -RhoA/Rho-kinase
SARS -Severe acute coronary respiratory syndrome
SKP-2 -S-phase kinase associated protein 2
SP -Spike protein
SPARCL - Stroke prevention by aggressive reduction in cholesterol levels
ssRNA -Single-stranded RNA
STAT - Signal transducer and activator of transcription
TF -Tissue factor
TLR -Toll-like receptors
TNF-α - Tumor necrosis factor α
TRAF-3 - TNF receptor-associated factor
ULK -Unc-51 like autophagy activating kinase
VSMCs -Vascular smooth muscle cells

Running title: Statins and COVID-19

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Introduction

Coronavirus (CoVs) are enveloped viruses from the order of Nidovirales that have a positive sense, single-stranded RNA genome (+ssRNA)(Schoeman and Fielding, 2019). Although primarily these viruses affect mammals and birds, the last decades have witnessed outbreaks of human infection, a process known as zoonosis(Schoeman and Fielding, 2019). In 2003, a zoonotic infection causing a severe acute respiratory syndrome (SARS) was reported in Guangdong province (China). Its cause was a novel virus, SARS-CoV-1(Singhal, 2020). Nine years later, another zoonosis called Middle-East respiratory syndrome (MERS) induced by the MERS-CoV was first identified in Saudi Arabia(Singhal, 2020), and more recently, by the end of 2019, again a coronavirus zoonosis inducing SARS was described for the first time in the city of Wuhan (China)(Singhal, 2020). This new SARS-CoV-2 induces a new disease called COVID-19. The recent events related to COVID-19 enhance the need of knowing what the virus is, where it comes from and how it can be defeated. Its high mortality rate and ease of transmission make SARS-CoV-2 one of the most important targets of research in recent years, forcing the scientific and medical community to undertake fast measures to understand the virus behavior. Currently, there is no effective, approved therapy for CoV infections, only palliative treatment of the symptoms and supportive care. In this point, background from SARS-CoV-1, MERS-CoV or other coronaviruses becomes important. Since the first outbreak of SARS, many studies have addressed the virus structure and the molecular basis of its interaction with the host. Developing a vaccine or an effective new antiviral treatment against an unknown virus needs many experimental studies, clinical trials and, what is more important in a critical situation, enough time to develop them. For this reason, in recent decades, several authors have pointed out the importance of drug repurposing, identifying already available drugs that may be used to treat future viral infections in order to be prepared for the next worldwide plague(Fedson, 2006; Phadke and Saunik, 2020). Unfortunately, now is "the day" and it is necessary to identify existing drugs that can be repurposed to help COVID-19 patients until an effective vaccine can be developed. Currently, there are more than 850 clinical trials using pharmacological interventions to treat COVID-19 (U.S. National Library of Medicine., 2020).

The HMG-CoA reductase inhibitors, usually known as statins, are a group of drugs commonly used to lower serum cholesterol by reducing its liver synthesis (Liao and Laufs, 2005; Rodrigues Diez et al., 2010). Besides its well-known lipid-lowering effects, statins have been postulated to possess pleiotropic beneficial actions by regulating numerous biological

pathways implicated in antioxidant, anti-inflammatory or anti-tumor cellular responses(Liao and Laufs, 2005). The pleiotropic effects of statins have been mainly demonstrated in cell cultures and experimental models but in humans is sometimes difficult to dissociate them from the statins hypolipemic effects(Oesterle et al., 2017). However, the anti-inflammatory non-lipid effects of statins have been confirmed in a wide range of clinical trials including the AFCAPS/TexCAPS or the JUPITER trials where statins lowered the acute inflammatory marker C reactive protein (CRP) independently of low-density lipoprotein (LDL) reduction(Ridker et al., 2001, 2008; Hansson, 2005). Additionally, the JUPITER clinical trial showed that rosuvastatin treatment might modestly reduce the incidence of pneumonia in healthy adults with low LDL cholesterol (below 130 mg/dL) and a high-sensitivity CRP level ≥2.0 mg/dL(Novack et al., 2012). These results support the hypothesis that statins can modulate other cellular responses independent of their main lipid lowering action. Since their discovery, statins have been proposed as therapeutic agents in different diseases including infections such as influenza virus or MERS-CoV(Fedson, 2006; Phadke and Saunik, 2020). Here, we describe different mechanisms through which statins could be potentially helpful in the fight against COVID-19.

SARS-CoV-2 infection entry pathways: the importance of ACE2 and CD147 receptors

CoVs genome encodes four major structural proteins: the spike protein, the nucleocapsid protein, the membrane protein and the envelope protein(Schoeman and Fielding, 2019). Recent studies have suggested that some CoVs do not require the full ensemble of the four proteins to form a complete infectious virion(Schoeman and Fielding, 2019) (**Figure 1**). Among them, the spike glycoprotein (SP) is known to be essential for virus binding to the host cells during the infection process(Shen et al., 2007). SP is a transmembrane protein that contains protrusions which confers their specificity for some host cell receptors. It is composed by two subunits: 1) S1, which contains the receptor binding domain (RBD) responsible for recognizing the cell surface receptors, and 2) S2, which is necessary for membrane fusion(Du et al., 2009). Several host cell receptors bind to S1 and help some coronaviruses to invade cells, such us dipeptidyl peptidase-4 (DPP4)(Raj et al., 2013), aminopeptidase N (APN)(Reguera et al., 2012), carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5)(Chan et al., 2016), or the angiotensin-converting enzyme 2 (ACE2)(Lan et al., 2020).

ACE2 is one of the best characterized receptors. It binds to the S1 domain and its relevant role in SARS-CoV-1-induced lung injury has been well established(Kuba et al., 2005). ACE2 is a component of the renin angiotensin system (RAS). This enzyme degrades Angiotensin II (Ang II), the effector RAS peptide, to Ang (1-7). Ang II regulates blood pressure and contributes to the pathogenesis of many cardiovascular diseases(Ruiz-Ortega et al., 2001). Drugs that block Ang II, including angiotensin converting enzyme inhibitors (ACEi) and/or angiotensin II receptor blockers (ARB), are currently used to treat many cardiovascular diseases, including hypertension and diabetes, two of the most prevalent COVID-19 comorbidities and clearly associated to the risk of admission to Intensive Care Unit, invasive ventilation, or death(Guan et al., 2020). RAS blockers may induce tissue ACE2 overexpression(Ferrario et al., 2005), and therefore their vascular beneficial effects, besides targeting Ang II actions, could be due to ACE2/Ang(1-7) vasoprotective effects. Importantly, experimental studies have demonstrated that ACE2 overexpression allowed SARS-CoV-2 infection(Zhang et al., 2020a). Based on this potential ACE2 activation, an early hypothesis stated that RAS blockers could be detrimental in COVID-19 and therefore, treatments with ACEi or ARB should be stopped (Fang et al., 2020). However, other studies and key scientific societies have argued that there is no empirical basis for this hypothesis and that stopping RAS blockade could be unfavorable(AlGhatrif et al., 2020; European Society of Hypertension, 2020; Zhang et al., 2020b). Nevertheless, the discussion on RAS blockers and COVID-19 is beyond the scope of the present review and future research is warranted to clarify this topic.

Although ACE2 is especially abundant in the heart and kidneys(Kuba et al., 2005) where it plays a major role in blood pressure control(Crackower et al., 2002), it is also present in other tissues, including lungs(Kuba et al., 2005). For this reason, modulating tissue ACE2 levels could lead to unwanted and fatal results. For instance, in lung diseases, an impaired ACE2 expression increased vascular permeability and lung edema, and did activate the RAS contributing to further lung injury progression(Imai et al., 2005). All these possible negative effects suggest that ACE2 suppression in COVID-19 should be carefully evaluated. In this sense, alternative solutions based on targeting ACE2 receptor has been proposed(Zhang et al., 2020a). The most promising approach consists of treatment with a soluble recombinant form of the human ACE2 (APN01), which potentially can bind SARS-CoV-2, block host cell infection and protect the lungs from injury (NCT04287686; NCT04324996).

CD147 is another cell surface protein that can act as a coronavirus receptor. The CD147, also known as basigin, EMMPRIN or leukocyte activation antigen M6, is a member of the immunoglobulin superfamily expressed in many epithelial, neuronal, lymphoid and myeloid

cell types in its different glycoforms(Grass and Toole, 2015). CD147 is a type I integral membrane receptor, that binds to many different ligands including cyclophilin proteins, integrins or the Plasmodium Falciparum reticulocyte binding-like homologue 5 (PfRh5)(Xiong et al., 2014). CD147 is overexpressed in several cancers, atherosclerosis, inflammation or microbial diseases(Grass and Toole, 2015). Accordingly, the role of CD147 in infections by pathogens such as human immunodeficiency virus (HIV), hepatitis B (HBV) and C viruses (HCV) or Kaposi's sarcoma-associated herpesvirus (KSHV) has been reported(Xiong et al., 2014). As described above, CD147 is an essential receptor in erythrocytes for plasmodium falciparum infection in malaria(Crosnier et al., 2011) and a clinical trial using an anti-CD147 antibody (Meplazumab) in malaria patients will start this year (NCT04327310). CD147 also facilitates human cytomegalovirus (HCMV) entry to epithelial and endothelial cells(Vanarsdall et al., 2018). More linked to SARS-CoV2-induced pulmonary damage, CD147 levels were found upregulated in chronic obstructive pulmonary disease (COPD) patients(Jouneau et al., 2011). Additionally, cultured primary bronchial epithelial cells from asthmatic patients showed higher CD147 levels after influenza A virus infection than cells from non-asthmatic patients(Moheimani et al., 2018). Regarding coronaviruses, CD147 is a receptor for the S protein in the SARS-CoV-1(Chen et al., 2005) and also for SARS-CoV-2(Ke Wang, Wei Chen, Yu-Sen Zhou, Jian-Qi Lian, Zheng Zhang, Peng Du, Li Gong, Yang Zhang, Hong-Yong Cui, Jie-Jie Geng, Bin Wang, Xiu-Xuan Sun, Chun-Fu Wang, Xu Yang, Peng Lin, Yong-Qiang Deng, Ding Wei, Xiang-Min Yang, Yu-Meng Zhu, 2020). In this new study, surface plasmon resonance and co-immunoprecipitation assays demonstrated a direct interaction between CD147 and the RBD region of the S1. Furthermore, CD147 blockade with Meplazumab inhibited SARS-CoV-2 replication in Vero E6 cells. All these data, including an open label clinical trial using the humanized CD147 antibody Meplazumab to treat COVID-19 pneumonia (NCT04275245), support the concept that CD147 is a potential therapeutic target to fight COVID-19.

Statin effects in the key SARS-CoV-2 entry pathways: ACE2 and CD147

Statins have been postulated to possess pleiotropic beneficial effects including the inhibition of the untoward effects due to an overactivated RAS such as inflammation and fibrosis(Ruperez et al., 2007; Rodrigues Diez et al., 2010). In this sense, both hypercholesterolemia and arterial hypertension are often observed in several clinical conditions such as obesity, type 2 diabetes, atherosclerosis and other cardiovascular diseases. For these

reasons, patients are frequently prescribed with statins and RAS blockers. Since ACE2 is a receptor for SARS-CoV-2 entry into host cells, an intense debate about the use of RAS blockers in COVID-19 patients has recently been generated, based on the fact that both ACEi and ARB are shown to modulate ACE2 tissue levels (South et al., 2020; Vaduganathan et al., 2020). Statins have also been included among the drugs that increase ACE2 levels(South et al., 2020). In a model of experimental atherosclerosis in rabbits, atorvastatin increased ACE2 protein levels in hearts and kidneys compared to untreated atherosclerotic animals(Dong et al., 2008; South et al., 2020). Similar results were observed using rosuvastatin or pravastatin in rat vascular balloon injury or diabetes(Li et al., 2013; Min et al., 2018). However, in those studies ACE2 levels were decreased in injured tissues compared to healthy groups, and therefore, ACE2 upregulation induced by statins is only described under disease situations. Thus, the reported upregulation of ACE2 by statins in preclinical studies could represent a normalization of ACE2 levels. Therefore, the clinical relevance of these findings is uncertain and probably negligible.

Another pleiotropic effect of statins is the modulation of the CD147 at different levels. Mechanistically, statins alter CD147 expression, structure and function by inhibiting protein isoprenylation and N-glycosylation(Sasidhar et al., 2017). In cultured THP-1 monocytes, pretreatment with atorvastatin, pravastatin or fluvastatin, impaired CD147 translocation to the cell surface, downregulating matrix metalloproteinase activity, and inhibiting THP-1 differentiation to macrophages after phorbol-12-myristate-13 acetate (PMA) administration(Sasidhar et al., 2017). Atorvastatin also downregulated CD147 levels and attenuated plaque vulnerability in experimental atherosclerosis in mice(Liang et al., 2017). Therefore, all these studies suggest that statins, by downregulating CD147 in human cells, including pulmonary cells, could impair the virus ability to infect cells and could be used as an add-on or coadjuvant therapy against COVID-19.

COVID-19 and lipid rafts

Lipid rafts, defined as small heterogeneous membrane domains enriched in cholesterol and sphingolipids, participate in the compartmentalization of several cellular processes(Lajoie and Nabi, 2007). A relevant role of membrane lipids in the attachment of viruses, including some coronaviruses, to host cells has been previously reported(Choi et al., 2005; Heaton and Randall, 2011). In this sense, in Vero E6 cells lipid rafts play an important role in the coronavirus life cycle during the early stage of SARS(Li et al., 2007). Closer to COVID-19,

one *in vitro* study addressed the role of cholesterol-rich membrane microdomains in the interaction of the S protein of SARS-CoV-1 with ACE2(Glende et al., 2008). ACE2 was present in detergent-resistant membranes, therefore cholesterol was required for efficient S-mediated binding to ACE2-containing cells. These data suggest that lipid raft modulation could be an option to reduce ACE2-mediated virus infection.

Statins modulate lipid rafts: potential role in SARS-CoV-2 infections

Statins inhibit the cholesterol biosynthesis pathway by inhibiting HMG-CoA reductase and modulate cell membrane lipid raft composition. Statins have been proposed to treat disorders associated with lipid rafts changes. Thus, atorvastatin reversed many of the lipid raft-associated signaling defects characteristic of autoreactive T cells in systemic lupus erythematosus(Jury et al., 2006). In the viral context, viruses could subvert cholesterol homeostasis generating a protective membrane environment that facilitates virus assembly and proliferation(Deng et al., 2010). Therefore, some authors propose targeting host cell lipid flow as a potential new antibacterial and antiviral strategy(Fernandez-Oliva et al., 2019). Accordingly, the use of methyl-β-cyclodextrin (MβCD) for cholesterol depletion and lipid raft disruption decreased the infectivity of several viruses, such as HCV or bovine parainfluenza virus, mainly through blocking virus entry into host cells(Fernandez-Oliva et al., 2019). Similar results were observed using gemfibrozil as a lipid-lowering drug(Bajimaya et al., 2017). Studies performed in cells infected by several +ssRNA viruses, including from the Coronaviridae family, suggested that viruses induce changes in cell cholesterol metabolism through activation of cellular HMG-CoA reductase. In 2005, transmission electron microscopy evidenced that SARS viral infection can result in alterations to the host subcellular membrane inducing a gyroid cubic structure that could modulate viral severity, persistence and free radical production(Almsherqi et al., 2005). Thus, plasma membrane structural changes in the host cells seems to be playing a key role in SARS-CoV infection(Almsherqi et al., 2005). All these data support the potential use of statins to prevent or reverse host cell lipid raft alterations induced by COVID-19 infection, which could reduce both cell infection and viral replication.

SARS-CoV-2 and autophagy

Macroautophagy, thereafter referred to as autophagy, is a very conserved process in which damaged cellular material is enclosed into a double-membrane structure called

autophagosome, which finally fuses with lysosomes and forms the autolysosomes for degradation. The main aim of this process is to recycle cellular material, maintain energy levels and promote cell survival(Yang and Shen, 2020). Canonical autophagy can be divided into three different steps. The first one is the initiation step, where an isolation membrane, also called phagophore, is formed. The second step is the elongation, in which this isolated membrane enlarges and forms the autophagosome. During the third step, maturation, autophagosomes merge with lysosomes forming the autophagolysosomes(Cong et al., 2017). Several proteins closely regulate these processes, being the mammalian target of rapamycin (mTOR) signaling network a central hub in autophagy(Munson and Ganley, 2015). Autophagosome formation is mainly controlled by a cluster of genes called autophagy-related genes (ATG). Furthermore, the unc-51 like autophagy activating kinase (ULK) complex, as well as the class III hVPS34 phosphatidylinositol 3-kinase complex, which includes BECN1 (Beclin 1), are essential in the initiation of the autophagy and autophagosome formation(Wirth et al., 2013). Microtubule-associated protein light chain 3 (LC3) is involved in elongating and enclosing the phagophore(Yang and Klionsky, 2010). LC3 forms a complex with Atg8, and is cleaved by Atg4, generating LC3-I which has a glycine residue in the C-terminal side. Then Atg7 conjugates LC3-I with a phosphatidylethanolamine resulting in LC3-II, which is initially attached to both faces of the phagophore membrane, although later on it will be only present in the inner face, enabling autophagy to continue(Yang and Klionsky, 2010; Deretic, 2016). Apart from its role in cellular homeostasis, autophagy also participates in the innate immunity response by degrading intracellular pathogens(Maier and Britton, 2012). Regarding viruses, autophagy could act as pro-viral or anti-viral process, depending on the virus(Jackson, 2015). Autophagy inhibition increased virulence and replication of some viruses, such as herpes simplex virus 1 (HSV1)(Orvedahl et al., 2007) and the Sindbis virus(Orvedahl et al., 2010). Moreover, some viruses can modulate the autophagy pathway as a mechanism to increase their own replication(Maier and Britton, 2012). This is the case of HSV1, Kaposi's sarcoma associated herpesvirus (KSHV) and murine γ-herpesvirus (MHV) which inhibit autophagosome formation by inhibiting Beclin1(Maier and Britton, 2012). The interaction between the order of Nidovirales and autophagy has been mostly investigated in the coronavirus and arterovirus families(Cong et al., 2017). One of the most studied arteroviruses causes the porcine reproductive and respiratory syndrome (PRRS). In PRRSV, autophagosome and lysosome fusion are decreased, suggesting that viruses can promote an incomplete autophagy, an abnormal process that may benefit viral replication(Sun et al., 2012). Coronaviruses and other RNA viruses exploit the autophagy for their own replication using the

double-membrane compartments formed during autophagy as a platform for their viral replication machinery which protects viral RNA from the innate immune system of the host cell(Choi et al., 2018). A recent study demonstrated that autophagy inhibition favored MERS-CoV viral replication. Thus, S-phase kinase-associated protein 2 (SKP2) promoted ubiquitination and degradation of Beclin1, while SKP2 inhibition enhanced autophagy and reduced MERS-CoV replication up to 28,000-fold(Gassen et al., 2019).

Interestingly, CD147 is also related to autophagy. The small-molecule compound AC-73, which targets CD147, elicits autophagy and reduces cell proliferation by inhibiting the ERK/STAT3 pathway(Spinello et al., 2019). Moreover, in human prostate cancer PC-3 cells CD147 inhibited autophagy via the PI3K/Akt/mTOR signaling pathway, preventing cell death from unrestrained autophagy(Fang et al., 2015). Taking into account all the available information suggesting a relevant role of autophagy in coronavirus infection and, potentially, in SARS-CoV-2 infection, autophagy should be considered as a potential target to treat COVID-19.

Role of statins in the autophagy response

Some of the pleiotropic effects attributed to statins may related to their potential role regulating essential proteins involved in autophagy(Ashrafizadeh et al., 2020). Atorvastatin induced autophagy by enhancing Beclin1 and LC3-II(Gao et al., 2016) gene and protein expression or via AMPK/mTOR pathway(Zhang et al., 2013). In cancer cells, lovastatin induced autophagy by up-regulating LC3-II(Shi et al., 2012), and atorvastatin through LC3-I to LC3-II conversion(Hu et al., 2018). In the same way, pitavastatin stimulated autophagy in melanoma after also increasing LC3-II levels(Al-Qatati and Aliwaini, 2017). Interestingly, not only in tumoral tissues statins trigger autophagy. In coronary arterial myocytes simvastatin increased autophagy by mTOR pathway inhibition(Wei et al., 2013). In relation to lung, the most affected tissue in SARS-CoV-2 infection, fluvastatin induced autophagy in two lung adenocarcinoma cell lines (A549 and SPC-A-1), by increasing LC3-II levels(Yang et al., 2017). Additionally, statins could increase autophagy by indirect mechanisms. Thus, *in vitro*

studies showed that lovastatin and simvastatin elicited SKP2 degradation and, therefore, an increase in Beclin1 levels and autophagy(Vosper et al., 2015; Wang et al., 2017). Altogether, these studies demonstrate that statins modulate autophagy, and therefore add another target supporting their potential beneficial effects in SARS-CoV2 infection.

SARS-CoV-2 and NLRP3 inflammasome activation.

Viruses infecting host cells need to survive and to replicate. Following infection, host cells activate the innate immune response trying to eliminate the viruses and prevent virus replication(Thompson et al., 2011). To this purpose, host cells have developed highly conserved sensors to recognize viral infection and trigger antiviral immune responses. These sensors, known as pattern recognition receptors (PRRs), include Toll-like receptors (TLRs), several DNA sensors such as cyclic GMP-AMP synthase (cGAS) and retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs). The aim of the PRRs is to identify different pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) from invading viruses. PRRs binding to their ligands recruit different pathways through activation of the transcription factors NF-κB, activator protein 1 (AP1), and interferon regulatory factors (IRFs)(Lamkanfi and Dixit, 2014). While IRFs lead to secretion of type I interferons (INFs), which exert their function by signaling through the JAK-STAT pathway and the subsequent interferon stimulated genes (ISG) synthesis, NF-κB activates the production of proinflammatory factors, including interleukin 6 (IL-6), and also initiates the first stage of inflammasome activation(Yang et al., 2019; Zhao and Zhao, 2020). Among the PPRs, the host cell response to an RNA viral infection, usually involves the activation of the NACHT, LRR and PYD domains-containing protein 3 (NLRP3), and the NLRP3 inflammasome(Kelley et al., 2019). NLRP3 inflammasome activation is a complex process initiated by caspase-1 activation, followed by the maturation of interleukin 1 beta (IL-1β) and IL-18, leading to inflammation and some mechanisms of cell death, such as pyroptosis(Man and Kanneganti, 2016). In this sense, the open reading frame 3a from the SARS-CoV-1 protein activates the canonical NF-κB pathway and the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of the p105/NF-κB subunit(Chen et al., 2019; Siu et al., 2019). Accordingly, an increased expression of several pro-inflammatory cytokines, including IL-1β and IL-6, has been observed and related to the pathogenesis of acute lung injury in SARS-CoV-1 patients(He et al., 2006). Similar results have been observed in SARS-CoV infection in mice, in which NF-κB inhibition increased survival(DeDiego et al., 2014). Analogous mechanisms are proposed for the new

SARS-CoV-2, in which an exacerbated inflammatory response leading to a cytokine storm syndrome is responsible for COVID-19 severity and mortality(Mehta et al., 2020). In agreement with this hypothesis, clinical trials intended to modulate the inflammatory response have been proposed. Some examples are the use of the anti-interleukin drugs anakinra (IL-1 receptor antagonist), tocilizumab and sarilumab (blocking antibodies against the IL-6 receptor) and colchicine, which disrupts NLRP3 inflammasome activation and downregulates IL-1β, IL-18 and IL-6 levels(Martinez et al., 2015) or even tranilast that targets the NACHT domain of NLRP3 blocking the NLRP3 complex formation(Huang et al., 2018). Additionally, following pilot clinical trials, the antimalaria drugs chloroquine, hydroxychloroquine or mefloquine are also being used to treat COVID-19(Chen et al., 2017; Tang et al., 2018)[,92]. Several potential mechanisms of action have been proposed for these drugs, including modulation of ACE2 expression and anti-inflammatory effects including decreased NLRP3 inflammasome activation.

Statins regulate NLRP3 inflammasome-mediated inflammation

Probably, one of the best-characterized pleiotropic actions of statins is their anti-inflammatory effects(Blanco-Colio et al., 2003; Liao and Laufs, 2005; Hothersall et al., 2006). Attenuation of vascular inflammation, on top of their lipid-lowering effect, is thought to contribute to the beneficial effect of statins on cardiovascular outcomes(Albert et al., 2001; Blanco-Colio et al., 2003). At the molecular level, atorvastatin inhibits NF-κB activation induced by Ang II or tumor necrosis factor-α (TNF-α) in cultured rat vascular smooth muscle cells (VSMCs) and mononuclear cells by a redox-mediated inhibition of IKK-1/-2(Ortego et al., 2005). Similar results were observed in cultured human endothelial cells, in which cerivastatin prevented TNFα-induced NF-κB pathway activation by inhibiting PI3K/Akt signaling(Holschermann et al., 2006). Several studies have shown a direct regulation of NLRP3 inflammasome by statins(Parsamanesh et al., 2019). In THP-1 monocytes, atorvastatin inhibited NLRP3 inflammasome by suppressing the TLR4/MyD88/NF-κB pathway(Kong et al., 2016). In patients with cardiovascular diseases, treatment with statins downregulated the expression of NLRP3 and the downstream cytokines, IL-18 and IL-1β(Satoh et al., 2014; Altaf et al., 2015). Accordingly, in human peripheral blood mononuclear cells obtained from patient

with hyperlipidemia or healthy controls, stimulation with cholesterol crystals caused NLRP3 inflammasome activation and release of IL-1β, that was abolished by simvastatin pretreatment(Boland et al., 2018). Regarding NLRP3 inflammasome, administration of atorvastatin during 8 months in patients with coronary artery disease resulted in a decrease in NLRP3 inflammasome levels(Satoh et al., 2014). There are also some studies suggesting the potential therapeutic role for statins in respiratory diseases such as COPD and asthma(Hothersall et al., 2006; So et al., 2018). A recent study revealed that statins markedly reduced the risk of subsequent hospitalized exacerbations in COPD frequent exacerbators(Lin et al., 2020). Therefore, taking into account the demonstrated anti-inflammatory actions of statins, both in NF-κB-mediated cytokine induction and NLRP3 inflammasome activation, these drugs could be considered as a potential way of impairing uncontrolled inflammation in the treatment of COVID-19 patients.

Coagulation complications in COVID-19 patients

The coagulation system could be regulated by host defenses to limit the spread of pathogens during severe infections, as exemplified by a large variety of viruses such as HIV, Dengue virus or Ebola(Antoniak and Mackman, 2014). Nevertheless, in acute viremia this situation could lead to disseminated coagulation contributing to multiorgan failure and mortality(Antoniak and Mackman, 2014). Tissue factor (TF) is an essential cofactor component of the TF-factor VIIa complex enzyme. TF is transmembrane protein mainly expressed in the vascular adventitia in normal conditions(Butenas et al., 2009). However, during viral infection, TF can be expressed by endothelial cells (and monocytes) and, when exposed to blood, it can activate the coagulation cascade. TF binds to plasma factor VIIa forming the TR-Factor VIIa complex enzyme which triggers blood coagulation by proteolysis activation of the zymogens factor IX and factor X to the serine proteases, factor IXa and factor Xa(Butenas et al., 2009). Coagulation disorders have been also reported in SARS-CoV-1 and MERS-CoV infections associated with thrombotic complications and hematologic manifestations. Different complications, such as vascular endothelial damage (in both small- and mid-sized pulmonary vessels), disseminated intravascular coagulation (DIC), deepyvenous thrombosis and pulmonary embolism, leading to pulmonary infarction, have been observed in SARS-Cov-1

infected patients(Wong et al., 2003; Chong et al., 2004; Hwang et al., 2005; Giannis et al., 2020). Similarly, DIC was one of the major complications reported in fatal MERS-CoV cases. Clinical reports include a stable MERS patient who developed MERS-induced DIC, intracerebral hemorrhage, and multiorgan failure(Giannis et al., 2020).

Accordingly, coagulopathy complications are one of the most recent discoveries in COVID-19 patients. Some authors have suggested that clot formation in COVID-19 patients could be related to the exacerbated inflammatory responses but, like for other viruses, the direct participation of the SARS-CoV-2 virus should not be discarded (Connors and Levy, 2020). First evidence of abnormal coagulation parameters in COVID-19 patients appeared in China where elevated partial thromboplastin time and prothrombin time (a parameter of how long it takes the blood to clot) were found. In addition, D-dimer (a fibrin degradation fragment produced after blood clot dissolving) levels and other inflammation biomarkers such as IL-6, erythrocyte sedimentation rate and CRP, were increased in COVID-19 patients.(Connors and Levy, 2020). More recent cohort studies from different countries evaluated clotting factors and/or coagulation function in COVID-19 patients with acute respiratory illness and found increased fibrinogen levels as well as prothrombin time prolongation(Di Micco et al., 2020). However, D-dimer levels elevation and mild thrombocytopenia are the most consistent hemostatic abnormalities in COVID-19 patients and are associated with a higher risk of requiring mechanical ventilation, ICU admission or death(Bikdeli et al., 2020; Klok et al., 2020). Based on these data it is recommended to consider the preventive and therapeutic use of antithrombotic agents in COVID-19 patients(Bikdeli et al., 2020). Indeed, autopsies from patients who died of COVID-19 showed a high incidence of deep venous thrombosis(Wichmann et al., 2020) and anticoagulation treatment was associated with survival in COVID-19 hospitalized patients(Paranjpe et al., 2020; Tang et al., 2020). At present, a wide range of clinical trials are evaluating the use of low-molecular-weight heparin to treat COVID-19 patients (e.g., NCT04372589; NCT04345848).

Role of statins in the thrombotic process

Among the wide range of proposed pleiotropic effects of statins, the interference with the activation of the clotting system and the coagulation cascade is one of the most studied. In 1997, *in vitro* studies showed that fluvastatin dose-dependently, impaired TF activity, and therefore the coagulation process(Colli et al., 1997). Preclinical studies have addressed the

potential mechanisms involved. *In vitro* data suggest that the inhibition of small G (Rho, Rac and Ras) protein isoprenylation by statins is key to inhibit the coagulation cascade(Eto et al., 2002; Holschermann et al., 2006). Importantly, the RhoA/Rho-kinase (ROCK) pathway is a key regulator of TF(Ding et al., 2017). Statins can also downregulate clot formation by other mechanisms, including the thrombomodulin augmentation, via the transcription factor Kruppel-like factor 2 (KLF2)(Sen-Banerjee et al., 2005; Lin et al., 2007). Thrombomodulin binds thrombin and promotes protein C activation, lowering factors Va and VIIIa plasma levels and thus having a potent anticoagulant effect(Maruyama, 1999). The anti-thrombotic effects of statins have also been confirmed in preclinical studies(Undas et al., 2002). Interestingly CD147 inhibition, an effect also attributed to statins as we described in other section, diminished acute ischemic stroke in mice by reducing thrombo-inflammation(Jin et al., 2017).

Importantly, several human studies support the anti-thrombotic effects of statins, In the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) study, in patients with acute coronary syndrome statin administration reduced the risk of clinical outcomes and statin treatment discontinuation abrogated statin-related beneficial effects(Heeschen et al., 2002). In the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial, efficacious in preventing stroke was found in patients with acute cerebral ischemia treated with statins(Amarenco et al., 2009). In addition to these clinical trials, some other human studies have been performed in this regard. Patients with stable atherosclerotic plaque treated with high-dose (80 mg/d) atorvastatin, showed a reduction in the ROCK activity vs. placebo that could modulate the coagulation process(Nohria et al., 2009). In hypercholesterolemic patients rosuvastatin, but not atorvastatin, reduced TF(Panes et al., 2017). Moreover, atorvastatin as well as simvastatin prolonged prothrombin time in patients with hypercholesterolemia reducing the tendency to clot generation(Kadikoylu et al., 2003). Additionally, a meta-analysis study suggests that statins reduce plasma D-dimer levels after three months suggesting their potential use in some coagulation disorders(Sahebkar et al., 2015). Summarizing, all these proposed anti-thrombotic effects of statins can be another way of exerting beneficial effects in COVID-19 patients and their associated clinical complications.

Statins against COVID-19: a hypothesis worthy of consideration

Statins are used, or have been proposed to be used, either alone or as adjuvant drugs, in several diseases. These pathologies include hypercholesterolemia, diabetes, hypertension, cardiovascular diseases, chronic kidney diseases, different types of cancer, rheumatoid

arthritis, asthma or COPD(McCarey et al., 2004; Zhou and Liao, 2009; So et al., 2018; Fatehi Hassanabad, 2019), as well as other infective diseases induced by pathogenic microorganisms such as malaria, Ebola, influenza virus related diseases or MERS(Taoufiq et al., 2011; Mehrbod et al., 2014; Yuan, 2015; Shrivastava-Ranjan et al., 2018). Unfortunately, some of the potential protective effects have not yet been evaluated in some of these diseases or more thorough studies are needed. Supporting our hypothesis, while writing this review, other authors have also suggested the add-on therapy of statins in COVID-19 patients due to their anti-inflammatory and immuno-modulatory effects, their ability to curb down cholesterol in lipid rafts, and of course for their extended worldwide use(Bifulco and Gazzerro, 2020; Castiglione et al., 2020). In addition, several clinical trials in COVID-19 patients, using simvastatin combined with the JAK-1/2 inhibitor ruxolitinib to treat COVID-19 patients (NCT04348695), using atorvastatin alone (NCT04380402) or combined with other drugs (NCT04333407) are currently underway.

Here, we review some pleiotropic effects of statins such as the downregulation of CD147 expression and function, lipid raft disruption, autophagy activation, and attenuation of both the inflammatory response and the coagulation activation (**Figure 2**). All these processes are thought to be relevant in the infection and replication of SARS-CoV-2 in host cells. Although the use of statins would require to consider potential interactions with other experimental therapies for COVID-19 (The University of Liverpool, 2020), taking into account their effectiveness, safety, low cost and worldwide distribution, it is worth considering their potential to fight COVID-19. Additionally, *in-silico* studies to identify FDA approved drugs targeting SARS-CoV-2 positioned rosuvastatin as the sixth potentially usable drug that may have clinical utility in COVID-19(Farag, Ayman; Wang, Ping; Ahmed, Mahmoud; Sadek, 2020). As rosuvastatin does not use the cytochrome P4503A4 (CYP3A4) and the P-glycoprotein transport system (P-pg), its inferior interference with various drugs employed in these patients, such as remdesivir or chloroquine, would favor its choice as the appropriate statin. For this potential to be realized, first steps would be a) to analyze COVID-19 infection databases for potential differential severity or mortality, after adjusting for cofounders, of patients already on statins, and b) providing a biological plausibility basis by studying the impact of statins on viral replication and numbers in cultured cells. This may be followed by a pilot clinical trial that, given the known safety profile of the drugs, may be started without awaiting the results of basic science and epidemiological studies if these are delayed. So far, only observational evidence on the impact of statins in COVID-19 patients is available. Although the high mortality among elderly people, who are more likely to have cardiovascular

risk factors and be taking statins, may argue against any benefit of these drugs, it is also true that a high percentage of elderly people with COVID-19 survive or may even have asymptomatic infection(Spiegeleer et al., 2020). In this regard, a recent large multinational report of 96.032 hospitalized patients with COVID-19 supports the potential beneficial effects of statins. Thus, treatment with statins was more frequent among surviving patients than among those patients who died (10.0% vs 6.9%, p <0.0001). Indeed, statin use was an independent predictor of low in-hospital mortality (HR, 95% CI: 0.793, 0.736–0.855), although it was not associated with a differential risk of ventricular arrhythmias during hospitalization (Mehra et al., 2020) These results are in line with a smaller study in 154 nursing home residents in which there was a statistically significant association between statin intake and the absence of symptoms during SARS-CoV-2 infection (unadjusted OR 2.91; CI 1.27-6.71; p=0.011), which remained statistically significant after adjusting for age, sex, functional status, diabetes mellitus and hypertension (Spiegeleer et al., 2020).

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander *et al.*, 2019).

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Figure 1. <u>SARS-CoV-2 complete infectious virion</u>. RNA genome encodes a spike protein (SP), an envelope protein (EP), a membrane protein (MP), and a nucleoprotein, being the spike protein the most important surface membrane protein of the SARS-CoV-2

Figure 2. <u>Schematic summary of SARS-CoV-2 entry into host cells, replication, effect on host cells and postulated impact of statins</u>. ACE2 and CD147 are located in the plasma membrane, associated to lipid rafts and can act as SARS-CoV-2 receptors. Statins, by inhibiting cholesterol synthesis, modify lipid rafts composition. Statins can also downregulate CD147 expression and its translocation to the cell surface. Autophagy in host cells is altered during SARS-CoV-2 infection, by a mechanism that involves SKP2 upregulation and subsequent BECN1 degradation. Statins decrease SKP2 levels and induce BECN1 and LC3 II synthesis, which trigger autophagy activation. Another process modulated by SARSCoV-2 is the activation of the NF-κB pathway leading to proinflammatory cytokine synthesis, including IL-6, and NLRP3 inflammasome activation. Statins can downregulate NF-κB pathway activation, proinflammatory cytokine synthesis and NLRP3 inflammasome activation. Anti-thrombotic effects of statins by TF modulation could also be beneficial in COVID-19 patients. **<u>Purple, discontinuous lines</u>**: viral entry and release. **<u>Black lines</u>**: cell processes. **<u>Green lines</u>**: positive regulation of the process. **<u>Red lines</u>**: negative regulation of the process. **<u>Continuous green or red lines</u>**: process regulated by statins. **<u>Discontinuous green or red lines</u>**: process regulated by virus. **<u>Viral proteins</u>**: EP: Envelope protein, NP: Nucleocapsid protein, MP: Membrane protein, SP: Spike protein. **<u>*:</u>** Denotes targets of specific ongoing clinical trials against COVID-19.

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SARS-CoV-2 Complete infectious virion

- ◎ Membrane protein (MP)
- ◯ Nucleocapsid protein (NP)
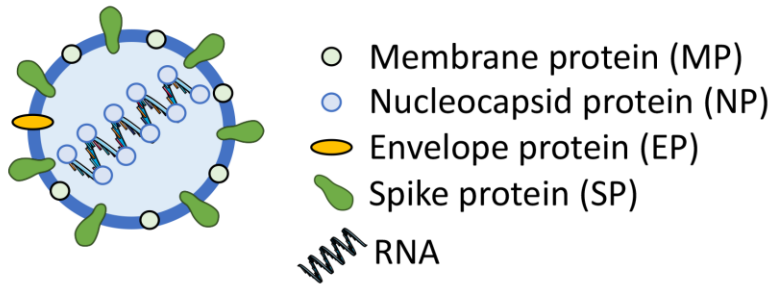- ⬭ Envelope protein (EP)
- 🌿 Spike protein (SP)
- 〰 RNA

Figure 1. SARS-CoV-2 complete infectious virion. RNA genome encodes a spike protein (SP), an envelope protein (EP), a membrane protein (MP), and a nucleoprotein, being the spike protein the most important surface membrane protein of the SARS-CoV-2
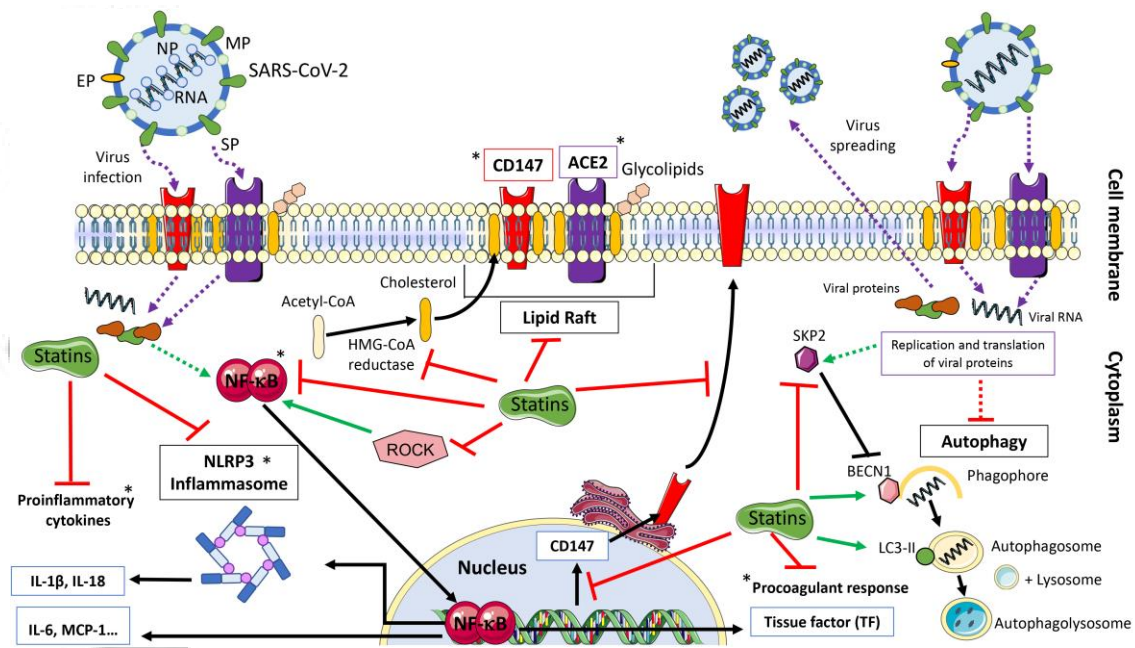
Figure 2. Schematic summary of SARS-CoV-2 entry into host cells, replication, effect on host cells and postulated impact of statins. ACE2 and CD147 are located in the plasma membrane, associated to lipid rafts and can act as SARS-CoV-2 receptors. Statins, by inhibiting cholesterol synthesis, modify lipid rafts composition. Statins can also downregulate CD147 expression and its translocation to the cell surface. Autophagy in host cells is altered during SARS-CoV-2 infection, by a mechanism that involves SKP2 upregulation and subsequent BECN1 degradation. Statins decrease SKP2 levels and induce BECN1 and LC3 II synthesis, which trigger autophagy activation. Another process modulated by SARSCoV-2 is the activation of the NF-κB pathway leading to proinflammatory cytokine synthesis, including IL-6, and NLRP3 inflammasome activation. Statins can downregulate NF-κB pathway activation, proinflammatory cytokine synthesis and NLRP3 inflammasome activation. Anti-thrombotic effects of statins by TF modulation could also be beneficial in COVID-19 patients. Purple, discontinuous lines: viral entry and release. Black lines: cell processes. Green lines: positive regulation of the process. Red lines: negative regulation of the process. Continuous green or red lines: process regulated by statins. Discontinuous green or red lines: process regulated by virus. Viral proteins: EP: Envelope protein, NP: Nucleocapsid protein, MP: Membrane protein, SP: Spike protein. *: Denotes targets of specific ongoing clinical trials against COVID-19.