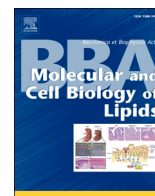




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Review

Lipid rafts as viral entry routes and immune platforms: A double-edged sword in SARS-CoV-2 infection?

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ABSTRACT

Lipid rafts are nanoscopic compartments of cell membranes that serve a variety of biological functions. They play a crucial role in viral infections, as enveloped viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can exploit rafts to enter or quit target cells. On the other hand, lipid rafts contribute to the formation of immune synapses and their proper functioning is a prerequisite for adequate immune response and viral clearance. In this narrative review we dissect the panorama focusing on this singular aspect of cell biology in the context of SARS-CoV-2 infection and therapy. A lipid raft-mediated mechanism can be hypothesized for many drugs recommended or considered for the treatment of SARS-CoV-2 infection, such as glucocorticoids, antimalarials, immunosuppressants and antiviral agents. Furthermore, the additional use of lipid-lowering agents, like statins, may affect the lipid composition of membrane rafts and thus influence the processes occurring in these compartments. The combination of drugs acting on lipid rafts may be successful in the treatment of more severe forms of the disease and should be reserved for further investigation.

1. Introduction

Lipid rafts represent a fascinating chapter in cell biology. These nanoscopic structures reside within cell membranes and are involved in a variety of physiological processes, including endocytosis and signal transduction [1]. Rafts are highly dynamic and their proteolipid composition is influenced by the external concentration of lipids, especially cholesterol. Lipid rafts appear to be critically involved in several steps of viral infections. Enveloped viruses, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can exploit rafts to enter target cells or to reassemble virion components before budding [2,3]. On the other hand, lipid rafts contribute to the formation of immune synapses and their proper function is a prerequisite for adequate immune response and viral clearance. Rafts harbor important molecules involved in the immune response, including Toll-like receptors (TLR), T- and B-cell receptors (TCR and BCR) and fragment crystallizable receptors (FcR) [4]. Additionally, lipid rafts play an important role in the control of coagulation, which is a crucial aspect of SARS-CoV-2 infection [5]. Lipid rafts can indeed promote platelet activation through the release of

extracellular vesicles that contain phosphatidylserine [6,7]. These vesicles are prothrombotic and form binding sites for coagulation complexes. Moreover, circulating microvesicles containing the tissue factor (TF) have been shown to originate from cholesterol-rich lipid rafts derived from the monocyte/macrophage lineage [8,9].

Given their dual role in viral infections, it is thought that the integrity and the proper functioning of lipid rafts may be associated with a more favorable course of CoronaVirus Disease 19 (COVID-19). COVID-19 pandemic has raised many questions regarding its high morbidity and mortality rates, but also because of its unpredictable clinical manifestations, ranging from asymptomatic cases to acute respiratory distress syndrome (ARDS) and death [10]. SARS-CoV-2 is transmitted via the air and, to a lesser extent, the gastrointestinal route [11]. Viral spike proteins recognize the molecule angiotensin converting enzyme 2 (ACE2), which is expressed in raft domains of the plasma membrane of target cells [12–14], thus enabling envelope fusion or endocytosis of the virus [15].

Recently, a so-called "lipid raft therapy" [13] showed promising results in the treatment of acquired immunodeficiency syndrome (AIDS)

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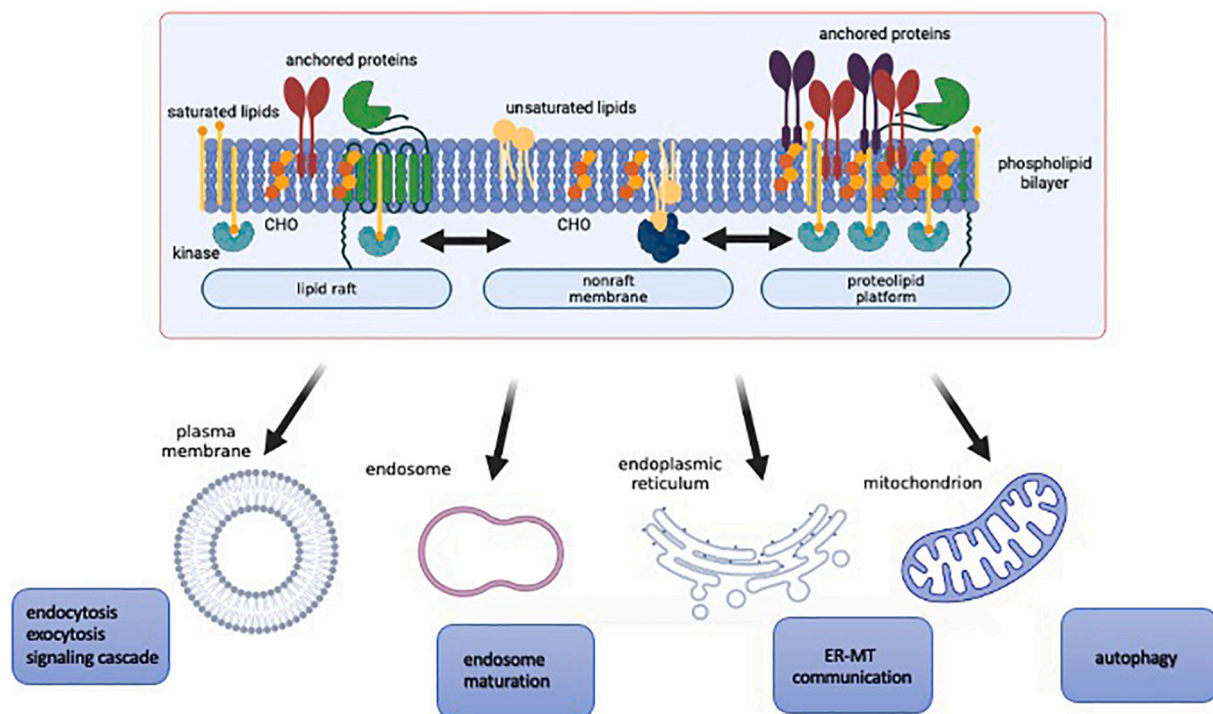


Fig. 1. Lipid rafts composition and biological function.

Lipid rafts are small compartments of cell membranes rich in sterols and other saturated lipids. These microdomains are highly dynamic and can exchange lipid content with adjacent nonraft membrane domains (double-headed arrow). Lipid rafts include multiple globular proteins, such as kinases, and can assemble into larger platforms that are used for specific functions in the plasma membrane or in organelles. Abbreviations: CHO: cholesterol.

and has also been proposed for COVID-19. Such therapy is based on the use of apoA-I binding protein (AIBP), a modulator of lipid rafts, which is able to stimulate cholesterol efflux and reduce its abundance in lipid rafts of cells of different tissues, which has modulatory effect on inflammation [13]. In addition, currently recommended treatments for COVID-19 may also act or be influenced by lipid raft perturbations. Drugs such as glucocorticoids and heparin, the most employed therapeutic options for COVID-19 since the beginning of pandemic [16–18], as well as antiviral agents, immunomodulatory drugs or monoclonal antibodies reserved for specific clinical conditions, may act through lipid rafts disruption, ultimately leading to prevention of crucial steps in the viral life-cycle or potentiation of the immune response. In this narrative review, we dissect the panorama focusing on this unique aspect of cell biology in the context of SARS-CoV-2 infection and therapy.

2. The role of lipid rafts in SARS-CoV-2 infection

2.1. Definition and biologic function of lipid rafts

Cell membranes are the primary interface between external and internal environments and between cytosol and organelles. Therefore, they play a crucial role in the regulation of many biological processes, such as endocytosis and exocytosis, and in the initiation of signaling pathways. According to the classical fluid mosaic model, cell membranes consist of an amphipathic lipid bilayer in which globular proteins float [19]. However, this traditional view is too simplistic to understand the complex composition and function of cell membranes. Indeed, lipid phases and lipid-protein ratios are extremely variable when considering plasma and organelle membranes, the top and the bottom of the plasma membrane of polarized cells, and the outer and inner layers [20]. Cell membranes contain several types of lipids, including cholesterol, glycerophospholipids, and sphingolipids. Lipids can organize differently to

form fluid-ordered or disordered phases and microdomains, which in turn are responsible for the heterogeneity of plasma membrane [21,22]. Cholesterol intercalating between acyl tails increases membrane fluidity and facilitates the lateral mobilization and clustering of globular proteins and microdomains. The process of compartmentalization, involving both lipid and protein components, likely occurs in all eukaryotic cells and is typically transient as it evolves according to the requirements of individual cells.

This highly dynamic view of cell membranes led to the coining of the term *lipid* or *membrane rafts*. In 2006, membrane rafts were defined as small (10–200 nm) sterol- and sphingolipid-enriched membrane domains, characterized by heterogeneity and high dynamism, and whose role is to compartmentalize cellular processes [23]. Due to their nanoscopic dimension and the difficulties in isolating them from other cellular components, the composition and structure of lipid raft have been poorly characterized in detail. Cholesterol, sphingolipids and glycerophosphatidylinositol (GPI)-anchored proteins present in lipid rafts confer detergent resistance, but lipid rafts cannot be easily identified with detergent-resistant membranes [24,25].

Lipid rafts have a highly dynamic composition and are likely to exchange lipids and proteins with the surrounding nonraft membrane. Cholesterol is a lipid component of both raft and nonraft compartments, and rafts may occasionally harbor atypical lipids such as gangliosides [26]. As mentioned earlier, cholesterol plays a critical role in increasing membrane fluidity, inducing coalescence of small rafts and increasing the size of raft domains.

In addition, lipid rafts contain globular proteins that have various biological functions and are able to connect raft domains into larger platforms (>300 nm) [24].

Proteins can be bound to the lipid bilayer by lipid anchors: saturated lipids, generally found in rafts, anchor kinases and various transmembrane proteins [25,27], while unsaturated lipids or prenylated groups link other proteins to nonraft domains. Coalescing raft domains

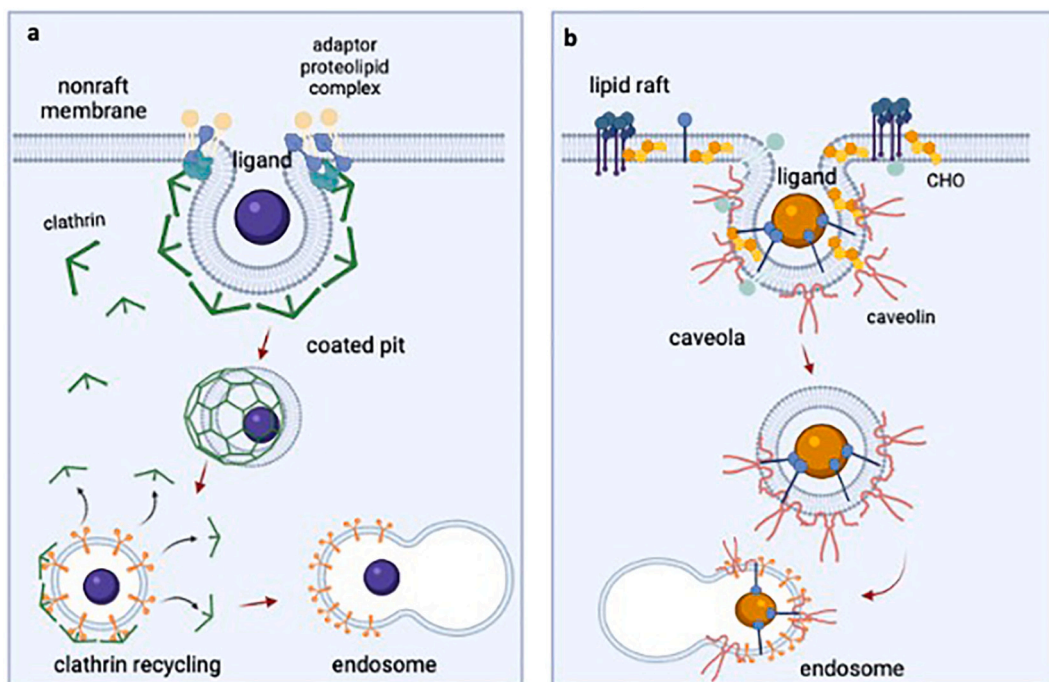


Fig. 2. Schematic representation of endocytosis.

a. Clathrin-mediated endocytosis. This type of endocytosis usually occurs in nonraft domains of the plasma membrane. After binding of a ligand to its receptor, clathrin molecules and other proteolipid components are recruited to the plasma membrane. This process results in invagination of the plasma membrane and formation of a coated pit that is transported to the endosome. Prior to fusion with the endosome, the clathrin molecules dissociate and are recycled to the cytosol.

b. Caveolae-mediated endocytosis. This type of endocytosis is thought to occur in lipid rafts. Once the receptors bind their ligand, the plasma membrane is invaginated thanks to the recruitment of caveolin molecules. The resulting vesicle, a caveola, can be transported to the endosome, but unlike clathrin-mediated endocytosis, caveolin molecules do not dissociate from the caveola membrane. The fusion of a caveola with an early endosome creates a caveosome in which the persistence of caveolin molecules appears to be critical for ligand sorting and ultimate fate.

Abbreviations: CHO: cholesterol.

and anchored proteins can form giant proteolipidic platforms that perform specific tasks: two nice examples are the brush border membrane and the immune synapse.

Lipid rafts can also be detected in the membrane of cellular organelles, such as the endoplasmic reticulum (ER), endosomes and mitochondria. An interesting experiment performed in human fibroblasts showed that mitochondrial lipid rafts are involved in the early steps of autophagy [28]. In particular, mitochondrial ganglioside GD3 can interact with proteins of autophagy and its depletion can affect this process by altering ER mitochondria communication. Lipid rafts composition may also determine endosome maturation: the reduction of sterols in the endosomal membrane and the increase of bis(monacylglycerol)phosphate are associated with the transition from early to late endosomal stages [20]. However, the exact biochemical composition of lipid rafts in organelles is unclear and it seems that cholesterol would play a lesser role than in the plasma membrane.

The biological characteristics of lipid rafts in plasma membrane and organelles are overall depicted in Fig. 1.

2.2. Lipid rafts in the mechanism of endocytosis

2.2.1. Clathrin-mediated endocytosis

The classical mechanism of endocytosis, or clathrin-mediated endocytosis, is exploited by the majority of antigens, pathogens, nutrients, and growth factors and occurs in nonraft regions of the plasma membrane. Therefore, classical endocytosis may be a potential target of drugs aimed at preventing viral infections.

Briefly, once the ligand (e.g., a virus) interacts with the receptor, soluble clathrin molecules in the cytosol are recruited to the inner surface of the plasma membrane and assembled, with the subsequent

formation of coated pits. These vesicles bud internally from the plasma membrane through a complex cross-talk involving phospholipids, amphiphysin, dynamin and other adaptor proteins [29], which stabilize the complex, bend the membrane, make the structure tubular and finally truncate the vesicle [30]. After endocytosis, clathrin is recycled into the cytosol, while uncoated vesicles fuse with early endosomes, which play a crucial role in directing cargo back to the plasma membrane or into late endosomes and lysosomes for further processing, Fig. 2. Early endosomes can also accommodate cargoes derived from non-classical endocytosis [31,32].

2.2.2. Caveolae-mediated endocytosis

Caveolae-mediated endocytosis is one of the non-classical pathways of endocytosis along with other non-clathrin/non-caveolin dependent mechanisms.

Caveolae consist of 60–80 nm plasma membrane invaginations [33] located within lipid rafts, rich in cholesterol and caveolin, and involved in lipid transport and signal transduction in addition to the process of endocytosis, Fig. 2b [25]. Once formed, caveolar vesicles can move to the Golgi complex, ER or early endosomes. Caveolae are of paramount importance in immune synapses and have been identified in macrophages, neutrophils, endothelial cells and fibroblasts.

Caveolin is a transmembrane protein that has a hairpin-loop conformation, exposing the N- and C-termini to the cytosol [34]. Three mammalian genes encoding caveolin (*cav-1*, *cav-2*, *cav-3*) have been identified and characterized in different tissues [35]. *Cav-1* and *cav-2* are diffuse in the majority of mammalian cells, while *cav-3* expression is restricted to neuroglial cells and myocytes. *Cav-1* exists in two distinct isoforms: *cav-1 α* and *cav-1 β* , the former being more capable of forming caveolae than the latter [34]. The two isoforms share

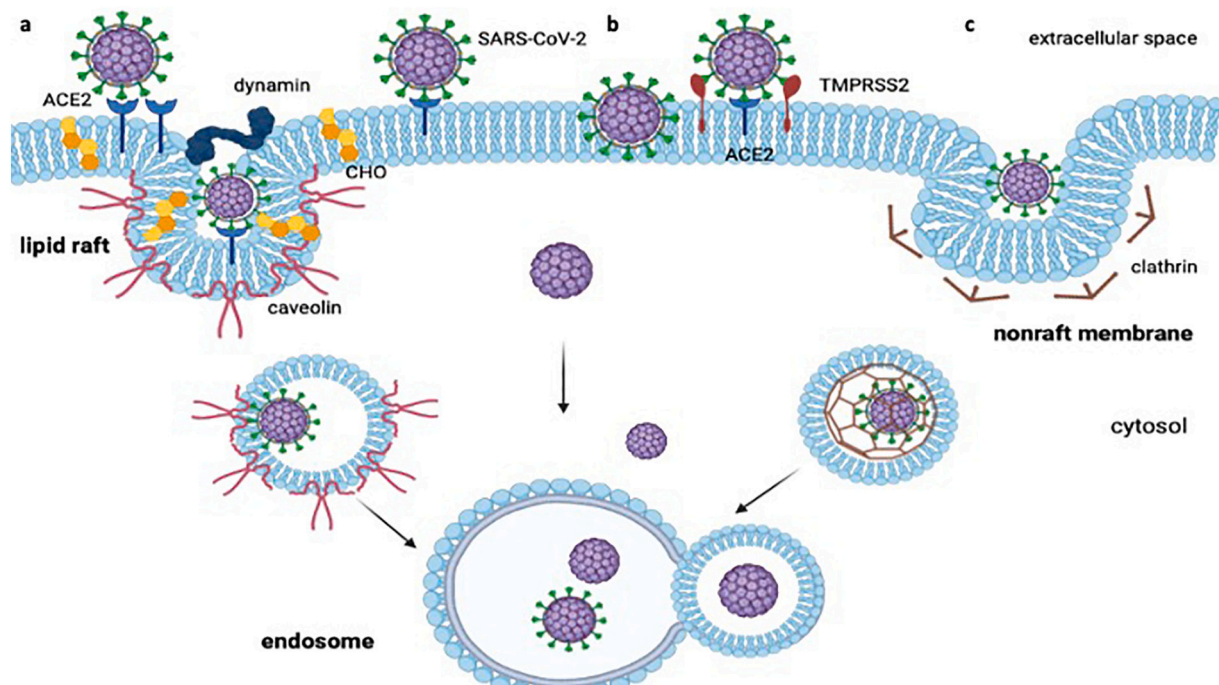


Fig. 3. The entry of SARS-CoV-2 into target cells.

The figure summarizes the plausible mechanisms of virus entry exploiting caveolae-mediated endocytosis (a), envelope fusion (b) and clathrin-mediated endocytosis (c). In the first scenario (a), SARS-CoV-2 binds to ACE2 located in lipid rafts. This may induce the subsequent formation of a caveola. Dynamin plays a central role in cutting the vesicle and enables its internalization. In the second scenario (b), the virus binds to ACE2 and TMPRSS2 on the plasma membrane of target cells, resulting in proteolytic priming and fusion of the envelope lipid bilayer with the cell membrane. In the third scenario (c), SARS-CoV-2 instead uses clathrin-mediated endocytosis to enter target cells. Following each of these entry mechanisms, SARS-CoV-2 can be transported to endosomes. The type of entry route, as well as the proteolipid composition of vesicles, may dictate a different processing pathway and is critical for viral clearance.

Abbreviations: ACE2: angiotensin-converting enzyme 2; CHO: cholesterol; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TMPRSS2: transmembrane protease, serine 2.

a hydrophobic sequence of amino acids, called the scaffolding domain (SD), which makes many connections with transmembrane receptors and other molecules that regulate their function.

Cav-1 appears to be critically involved in the intracellular-extracellular efflux of cholesterol [36] and may therefore contribute to membrane fluidity. Furthermore, cav-1 expression is a prerequisite for an adequate immune response and can be influenced epigenetically [4].

2.3. Lipid rafts as entry routes for viruses

Lipid rafts are of critical importance in the viral life cycle as they may be involved in envelope fusion, endocytosis, budding and release of viruses [1]. Enveloped viruses, such as SARS-CoV-2, can in fact use two main mechanisms of internalization: fusion and endocytosis, Fig. 3 [37]. In the first case, the viral glycoprotein spikes interact with surface cell receptors, allowing the connection between viruses and cell membranes [38]. Following this step, viruses may directly gain access to host cell albeit successful infection would additionally depend on extracellular environment pH, membrane rigidity and density of receptors, coreceptors and viral spike proteins.

In the second case, viruses are internalized into plasma membrane vesicles that fuse with endosomes. Lipid rafts would be necessary for either compartmentalization of plasma membrane receptors or invagination of vesicles containing viruses [13].

Concerning SARS-CoV-2, the spike protein was shown to dock the viral particle to ACE2 receptors for entry and to utilize the transmembrane protease, serine 2 (TMPRSS2) for protein priming [39]. The spike protein could also undergo enzymatic activation by furin, an enzyme belonging to the subtilisin-like proprotein convertase family. TMPRSS2-mediated cleavage of the spike protein has been shown to be an important requirement for endocytic entry of SARS-CoV-2 into the

host cell [40]. SARS-CoV-2 is thus internalized and undergoes intracellular transport within endosomes, which eventually fuse with mature lysosomes according to a strongly pH-dependent mechanism that is critical for viral membrane fusion and subsequent release of the viral RNA genome into the host cytoplasm. Although an in vitro study showed that ACE2 colocalizes with clathrin heavy chain in nonraft domains [41] and clathrin-mediated endocytosis is the best characterized route of entry for coronaviruses [30], the bulk of evidence suggests that ACE2 and TMPRSS2 are located in lipid rafts [13,14] and this may account for a preferentially caveolae-mediated transport of SARS-CoV-2 into target cells. A caveolae-mediated transport has been described for many viruses, such as Simian virus 40 (SV40), which can directly bind glycosphingolipids [42,43]. Several studies in vitro have also shown that coronaviruses can infect cells through caveolae-mediated endocytosis [14,30,44,45]. In this case, lipid rafts would play a central role especially in the early phase of viral infection. Interestingly, bioinformatic prediction models showed that SARS-CoV proteins, including the spike protein, contain at least 8 putative motifs that may interact with cav-1 [46]. It was also shown that replacement of ACE2 in a nonraft environment, after cholesterol depletion in Vero E6 cells, reduced pseudotyped SARS-CoV infectivity by 90% [14].

Unlike clathrin vesicles, which lose the clathrin envelope, caveosomes retain their caveolin content (and thus identity) when they fuse with endosomes, and this property appears to be critical in virus sorting [47]. Additionally, the small GTPase Rab5, which is associated with the plasma membrane and early endosomes, may determine the fate of the ligand in endosomes during both clathrin- and caveolae-mediated transport. Interestingly, clathrin- and caveolae-mediated endocytosis are two actively overlapping mechanisms and may inhibit each other [48].

Furthermore, preclinical evidence supports a close correlation

between membrane cholesterol content, ATP-binding cassette transporter A1 (ABCA1) expression and predisposition to viral infections. For instance, up-regulation of ABCA1, which in turn depends on loss of cholesterol and disorganization of lipid rafts, has been associated with reduced human immunodeficiency virus (HIV) infectivity [3]. Specifically, the overexpression of ABCA1 seems to interfere with the recognition and presentation of HIV by dendritic cells to susceptible T cells and to reduce in this way trans-infection [49]. Other experiments on HIV have shown that rafts could also be important for the formation of virological synapses, virion reconstitution and assembly of env and gag proteins [50,51]. However, the role of ABCA1 in SARS-CoV-2 infection is unknown. Using Vero E6 cells, Wang et al. showed that disruption of raft and nonraft cholesterol by methyl- β -cyclodextrin (M β CD) inhibited SARS-CoV pseudovirus infectivity in a dose-dependent manner, whereas the use of filipin or nystatin, which interferes more specifically with caveolae formation, had no effect [2]. The authors also observed the absence of colocalization between the virus and cav-1 in the dual immunostaining assay, leading to the hypothesis that entry of the SARS-CoV pseudovirus may occur via a cholesterol-related pathway, independent of caveolae formation.

Beside cholesterol, dynamin may also play a central role in classical endocytosis, caveolae-mediated endocytosis or other non-canonical pathways of endocytosis that have been proposed as alternative mechanisms of viral entry [30,52]. Drugs targeting dynamin may therefore disrupt the mechanism of virus internalization through either classical or non-classical pathways.

To conclude, the scenario underneath SARS-CoV-2 entry into target cells is characterized by the concomitant exploitation of different pathways, including caveolae- and clathrin-mediated routes or other non-canonical transports. Lipid composition, particularly plasma membrane cholesterol content, and key-proteins, like dynamin, could be of paramount importance in regulating these mechanisms, therefore representing potential pharmacological targets.

2.4. Lipid rafts as immune synapses

The plasma membrane is responsible for both the interface with the external environment and the mechanism of direct (cell to cell) or indirect (humoral) cell communication. Both the plasma membrane and organelle membranes are critically involved in many steps of the immune response, including pathogen defense, antigen processing and presentation, effector cell priming and activation, cytokine- and chemokine-mediated pathways, and initiation of intracytosolic cascades leading to apoptosis, autophagy and autoinflammation. Membrane lipid composition may influence the immune response, by relocating and accumulating receptors in specific plasma membrane regions of immune cells [25].

Polyunsaturated fatty acids (PUFA) and cholesterol have been shown to contribute to the recruitment of key-proteins in partitioned functional membrane domains. Acute cholesterol depletion, although not highly specific for lipid rafts, can activate some signaling pathways in human neutrophils, macrophages, and RBL-2H3 histamine-releasing cells [25]. Cholesterol present in lipid rafts may however regulate the shedding of lipid raft-associated proteins, including those responsible for cell adhesion and recruitment, cytokine signaling and apoptosis [53]. Cytokine receptor shedding can reduce cytokine signaling in target cells, but may also generate soluble receptors with agonistic or antagonistic effects [54]. For instance, cholesterol content in lipid rafts has been shown to be critical for shedding the receptors of interleukin-6 (IL-6) and tumor necrosis factor (TNF)- α and for activating of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) cascade in macrophages, although it had no effects on cell adhesion and migration [55,56]. It is known that IL-6 can bind the IL-6 soluble receptor and that this complex, circulating in the bloodstream, can eventually interact with a wide range of cells expressing the transmembrane receptor gp130 [57]. Furthermore, experiments on macrophages derived from mouse

models have shown that the increase in serum and plasma membrane cholesterol may enhance the responsiveness of TLR2, TLR4, TLR7 and TLR9 to ligands [58]. Since TLR3, TLR7 and TLR9 are responsible for the recognition of viral nucleic acids, it is likely that an increase in free and membrane cholesterol could potentiate the antiviral response by mobilizing these receptors into lipid raft domains.

Cholesterol, synthesized in ER or recycled in ER/Golgi compartments, is physiologically transported to membranes and exchanged with external lipids. Rafts can be enriched in cholesterol via a caveolae-dependent and a Niemann-Pick C1 (NPC1) protein-dependent pathway [25]. A deficit in the latter pathway would enrich late endosomes with cholesterol and promote aggregation of TLR3, lowering the activation threshold towards pathogens in macrophages, as demonstrated in preclinical studies on mouse models [59]. Extracellular acceptors of cellular cholesterol, such as lipid-free apoA-I and high-density lipoprotein (HDL), may further compromise raft domain integrity of immune cells and disrupt pro-inflammatory pathways depending on lipid rafts [60]. The level of membrane cholesterol seems also to regulate the expression of ABCA1, which in turn is negatively associated with pro-inflammatory responses in macrophages following stimulation of TLR, including TLR7 and TLR9 [4].

The increase in cholesterol and raft formation has been associated with B and T lymphocyte activation [4]. Increased cholesterol content has been reported in T cells from patients with systemic lupus erythematosus (SLE), where it may promote hyperactivation of pro-inflammatory signaling pathways and the imbalance between Th17/Treg cell subsets [61]. Specifically, the exchange of BCR, TCR, TNF receptor-1 (TNFR1) and CD40 from nonraft to raft domains and changes in lipid rafts after the binding of costimulatory molecules, such as CD28 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), would play a crucial role in the adaptive immune response [4,62]. In addition, immunological and proliferative signaling pathways that develop through the interferon (IFN) γ -Janus kinase (JAK)-signal transducer and activator of transcription (STAT), transforming growth factor (TGF) β -Smad and mitogen-activated protein (MAP) kinase signaling cascades would take place in lipid rafts and thus depend on adequate levels of membrane cholesterol [4]. However, other studies, based on different protocols and methodologies, have showed that moderate cholesterol depletion may induce the coalescence of lipid rafts in T cells and hence activate Ras-extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase signaling pathway [63,64].

In addition to cholesterol, other lipids, such as ceramide and phospholipids, have been shown to regulate the activity of TLR, major histocompatibility complex (MHC), and TNFR [25]. Preclinical studies have shown that eicosapentaenoic acid and docosahexaenoic acid can prevent the secretion of IL-1 β and IL-6 in mononuclear cells and the activity of natural killer (NK) cells [65,66], but the effects of supplementation in humans seem uncertain [67].

Caveolae are of major importance in immune synapses and have been identified in macrophages, neutrophils, endothelial cells, and fibroblasts but not in lymphocytes [25]. Cav-1, associated with lipid rafts, can interact with and affect the function of several proteins, promoting or downregulating the immune response. An example is the inhibition of the endothelial enzyme nitric oxide synthase (eNOS) by cav-1 SD [68], thereby reducing the production of nitric oxide (NO), which in turn prevents the activation of neutrophils, mast cells and platelets, decreases TLR4 expression in the lungs and regulates the expression of CD14, CD36 and myeloid differentiation factor 88 (MyD88) in macrophages [4]. Notably, preclinical data have shown that the exposure of bovine aortic endothelial cells to high cholesterol concentration may reinforce the inhibitory activity played by cav-1 on eNOS, whereas statins would have the opposite role [69]. The overproduction of NO in cav-1 deficient endothelial cells could however determine the tyrosine nitration of mitochondrial respiratory chain components [70], leading to reduced mitochondrial reserve capacity, oxidative stress, aerobic glycolysis, and inflammation through the activation of NF- κ B and activator protein-1

(AP-1) by reactive oxygen species (ROS). DNA damage provoked by ROS may activate poly-ADP-ribose polymerase (PARP) proteins and induce the expression of genes involved in cellular proliferation, angiogenesis, inflammation, and fibroblast activation [70].

Deficiency in cav-1 has been associated with fibroblast recruitment and pulmonary fibrosis in mouse models and patients affected by systemic sclerosis (SSc) [71], a scenario potentially mirroring the end-stage of COVID-19 ARDS. ARDS may also follow a surfactant deficit and thus be the result of altered metabolism of phospholipids, which are the major components of surfactant [72].

Finally, recent studies have shown that cav-1 may bind several microRNAs (miRNAs) capable of regulating its activity in lipid rafts [4]. Interestingly, some of them, such as miR-138 and miR-199-5p, have been associated with the NF- κ B pathway and lung fibrosis.

2.5. Lipid rafts and SARS-CoV-2 infection

A number of studies have examined the role of lipid rafts in the pathogenic steps of COVID-19. Although experimental results are conflicting, the majority of studies agree that ACE2 is a lipid raft protein, supporting the view that virus entry would preferably occur in these membrane microdomains [14,73,74]. Additionally, the SARS-CoV-2 spike protein contains a lateral N-terminal domain (NTD), capable of binding gangliosides within lipid rafts, which may subsequently allow the receptor binding domain (RBD) to interact with ACE2 [75]. Interestingly, an infection assay with a SARS-CoV-2 spike-bearing pseudovirus revealed that virus entry into HEK293T cells was not dependent on dynamin, clathrin, or caveolin, whereas it was enhanced by cholesterol-rich lipid rafts [76]. The authors also observed that the depletion of cholesterol content in rafts could reduce the infectivity of SARS-CoV-2, similar to what observed in other experiments with coronaviruses [3,14]. Other researchers have reported that disruption of lipid raft-associated sphingolipids by a spray containing α -cyclodextrin could counteract the binding of the virus to ACE2 [77].

While the role of lipid rafts in mediating SARS-CoV-2 endocytosis is gradually becoming more clearly defined, the link between lipid rafts disruption and antiviral response towards SARS-CoV-2 is largely unexplored. The integrity of lipid rafts appears to be a prerequisite for an adequate immunosurveillance against pathogens. As discussed before, lipid composition and relocation of receptors or coreceptors from non-raft to raft membrane may modulate either the innate or acquired immune response as well as immunotolerance mechanisms, whose balance is crucially important in dictating the outcomes of COVID-19 [78]. Patients with severe COVID-19 were shown to have a decrease in the levels of HDL cholesterol, CD8⁺ T lymphopenia or exhaustion and the hyperactivation of the innate immune response, occasionally culminating in a cytokine storm [79]. Therefore, targeting the immune response from a lipid raft perspective would be extremely difficult given the dual effect that these domains have in modulating innate and acquired immune cells as well as effector and regulatory elements of the immune response.

3. Lipid raft perturbation by pharmacological treatments

3.1. Anti-COVID-19 treatments used in clinical practice

3.1.1. Immunomodulators and immunosuppressants

Current guidelines recommend the use of systemic corticosteroids in the treatment of patients with severe COVID-19, while the use of other selective immunomodulatory or immunosuppressive drugs is suggested in certain clinical conditions [17,18]. Although the benefit of immunomodulatory drugs in COVID-19 is still being investigated in clinical trials and their combination is not sufficiently recommended in clinical practice, the World Health Organization (WHO) and the Food and Drug Administration (FDA) recently granted emergency clearance for baricitinib or tocilizumab in hospitalized patients on high-flow oxygen or

non-invasive ventilation in case of clinical deterioration and in most patients with severe or critical COVID-19 [18,80]. Interestingly, part of the mechanism of action of such agents may be explained on the basis of remodulation of target molecules in lipid rafts. Evidence in support of this will be discussed in the next sections.

3.1.1.1. Glucocorticoids. Glucocorticoids represent a reference class of drugs in the treatment of severe respiratory distress syndrome. Data from the RECOVERY [81] and SOLIDARITY [82] studies showed that dexamethasone was the only drug that reduced mortality in people with COVID-19, making it the first drug approved for the treatment of this disease. Although the results of more recent studies do not support the use of these drugs in the treatment of early and uncomplicated COVID-19 [18,83], the use of dexamethasone, along with other glucocorticoid molecules, can still be recommended in hospitalized patients with severe COVID-19 who require oxygen therapy (ranging from supplemental oxygen to mechanical ventilation) [80]. The early use of corticosteroids in SARS-CoV-2-positive patients is questionable due to their deleterious effect on immune response and viral clearance [84]. Similarly, there is no strong evidence to recommend corticosteroids in not severe COVID-19, diabetic or immunocompromised patients [16]. All evidence from clinical trials refers to oral or intravenous administration for up to ten days, while there are no recommendations for the use of inhaled or transdermal corticosteroids or with different regimens in terms of dose, treatment initiation and/or duration [16]. Of note, the anti-inflammatory function of these drugs may depend in part on altering the lipid composition of immune cell membranes. An experiment performed on murine T cell hybridomas showed that steroids can increase membrane fluidity, reduce palmitic acid content, and repress TCR by interfering with signaling proteins localized in lipid rafts [85]. These effects occurred early and appeared to be mediated by non-genomic mechanisms, including binding of glucocorticoid receptors placed in the lipid raft domains of the membrane and associated with cav-1 [86].

3.1.1.2. IL-6 inhibitors. COVID-19 is characterized by increased activation of monocyte-macrophage cells and consequent hyperproduction of systemic cytokines [87]. Among these, IL-6 is a pro-inflammatory cytokine with a broad spectrum of activity. IL-6 promotes the development of innate and acquired immune responses and can also induce endothelial dysfunction, fatigue, anemia, thrombocytosis and hepatic release of acute phase proteins [88]. IL-6 inhibitors (e.g., sarilumab, tocilizumab, siltuximab) approved for the treatment of rheumatoid arthritis (RA) and other rheumatic diseases showed efficacy in case series of patients with severe COVID-19 and may be recommended in combination with dexamethasone in selected hospitalized patients experiencing rapid respiratory decompensation due to COVID-19 [80]. Indeed, the results of several studies suggest that critical hospitalized patients and overall most patients with severe or critical COVID-19 benefit in terms of mortality from the addition of tocilizumab or sarilumab to corticosteroids [81,89], with no differences in terms of efficacy or safety between the two IL-6R inhibitors [89,90].

The effect of IL-6 inhibitors on lipid raft integrity and composition is not clear. In studies conducted in RA cohorts of patients, treatment with IL-6 inhibitors has been associated with increased blood HDL/low density lipoprotein (LDL) ratios and reduced levels of pro-inflammatory markers, such as C-reactive protein (CRP) [91,92]. It may be postulated that the increased availability of circulating cholesterol would influence lipid rafts composition and membrane fluidity, which ultimately would have an anti-inflammatory effect.

3.1.1.3. Janus Kinase-inhibitors. The JAK-STAT pathway consists of a signaling cascade that mediates the action of various cytokines and growth factors [93,94]. JAK-inhibitors (JAKi), such as baricitinib, tofacitinib and ruxolitinib, are recommended in COVID-19 management as these drugs can counteract the development of cytokine storm, while

preventing the angiotensin II-angiotensin receptor-1 (AT-1) signaling pathway, which has been associated with ARDS and worse disease outcomes [95]. In addition, JAKi interfere with viral endocytosis by controlling the AP2-associated protein kinase 1/cyclin-G-associated kinase-pathway [83]. Notably, it has been shown that the assembly of lipid rafts in response to the binding of cytokines to their receptors precedes the activation of the JAK/STAT signaling cascade in human lymphocytes [96]. Treatment with JAKi in patients affected by RA has been associated with increases in blood LDL and HDL [97], and experiments *in vitro* have shown that tofacitinib, a pan-JAKi, can inhibit intracellular lipid accumulation in macrophages by upregulating the expression of ABCA1 [98]. It is plausible that JAKi may affect the raft composition of organelles and the plasma membrane, which in turn is critical for their biological effects, but whether this event could affect the viral lifecycle or clearance is unknown. Consistently, WHO guidelines strongly recommend baricitinib in case of severe or critical COVID-19, as an alternative to IL-6 inhibitors, while they make a conditional recommendation against other JAKi, due to limited data from clinical trials [18].

3.1.1.4. IL-1 β inhibitors. Immunotherapies that neutralize IL-1, such as anakinra, have been used with success in cases of COVID-19 complicated by systemic inflammation [99]. These agents are approved for RA and autoimmune inflammatory syndromes. To our knowledge, there is no evidence linking IL-1 antagonists to lipid rafts, although IL-1 β signaling requires a lipid raft-mediated mechanism to generate oxidative stress and systemic inflammation [100]. Prenylation of RhoA kinase is an important step in preventing the activation of inflammasome platforms that precede the proteolytic activation of IL-1 β . Deficient prenylation of RhoA kinase may be a risk factor for autoinflammation [101]. However, prenylation would occur in domains other than lipid rafts [102].

3.1.1.5. Antimalarials. Antimalarials are lipophilic and weak basic compounds used in the treatment of rheumatic diseases [103]. The use of antimalarials such as hydroxychloroquine or chloroquine has been considered for prevention and treatment of COVID-19 [104], but eventually not recommended by most recent evidence [18]. However, a number of interesting observations indicate that these medications may prevent some steps of viral infection via a lipid raft-mediated pathway. These mechanisms may include: modification of lysosomal pH and interference with intracellular TLR function, the improvement in lipid profile [105–107], and the competition for the binding of sialic acid and gangliosides placed in lipid rafts with the NTD domain of the spike protein [108].

3.1.2. Antiviral drugs

The European Medicines Agency (EMA) and the FDA granted conditional approval for the antiviral treatment remdesivir for the treatment of patients with severe COVID-19 who require oxygen therapy, excluding patients on high-flow oxygen, noninvasive ventilation, mechanical ventilation, or extracorporeal membrane oxygenation [18,109]. However, remdesivir cannot be considered an established standard of care because currently available data are inconsistent and overall show no clear clinical benefit in terms of mortality or use of mechanical ventilation [17,18]. In addition to its main mechanism of action, which is the inhibition of the viral RNA-dependent RNA polymerase, remdesivir may also synergize with NPC1-inhibitors in regulating the lysosomal trafficking of cholesterol and sphingolipids [40].

Conversely, there is a strong recommendation against the use of the anti-retroviral protease inhibitor lopinavir, alone or in combination with ritonavir, for SARS-CoV-2 infection, due to its unfavorable efficacy and safety profile [110,111]. Similarly, evidence indicates controversial efficacy of oseltamivir, a sialidase/neuraminidase inhibitor approved for influenza [112,113]. *In vitro* studies have however shown that oseltamivir modulates the sialoglycosphingolipid GM1 and endogenous

sialidases [114,115]. Sialoglycosphingolipid GM1 is a marker of lipid rafts and accumulates in the immunological synapses [116]. By inhibiting endogenous sialidases and GM1 synthesis, oseltamivir may have an anti-inflammatory effect on T cells involved in antiviral immunity. Indeed, oseltamivir has been shown to reduce the number of lipid-raft-associated GM1 on the surface of T-cells [116] and may thus prevent the expansion of CD4⁺ Th1 cells during COVID-19-related hyper-inflammation [117].

While the use of intravenous immunoglobulins (IVIG) or convalescent plasma led to conflicting results in COVID-19 [118–120] being therefore not supported by most recent evidence, monoclonal antibodies targeting the SARS-CoV-2 spike protein (bamlanivimab, bamlanivimab-etesevimab, casirivimab-imdevimab, sotrovimab) have been approved as monotherapy or combination therapy for early-use in patients with mild to moderate COVID-19 who do not require supplemental oxygen but are at higher risk for severe disease [17,18,109]. Although studies are lacking, it has been reported that the interaction between antibodies and FcR would occur in rafts and that raft disruption with M β CD may inhibit the binding and phagocytosis of IgG immune complexes [121,122]. Thus, the integrity of lipid rafts appears to be an essential prerequisite for the success of antibody-based antiviral treatment.

3.1.3. Heparin

Heparin can be used both prophylactically and therapeutically. Prophylactic use of heparin is recommended by current guidelines for patients with acute respiratory infections and limited mobility, who are therefore at higher risk for thromboembolic events. Preliminary evidence suggests the use of medium-high doses of heparin in patients with severe COVID-19 and with D-dimer levels 4 to 6 times above average and/or sepsis-induced coagulopathy (SIC) ≥ 4 , elevated ferritin (>1000 $\mu\text{g/L}$), or body mass index >30 kg/m^2 [123]. It is unclear whether the effects of heparin might be mediated in part by lipid rafts. However, an isolated report showed that heparin may suppress the MAP kinase Erk signaling in vascular smooth muscle cells and prevent phosphorylation of endothelial growth factor receptor (EGFR), which is localized in caveolin-enriched lipid rafts [124].

3.1.4. Azithromycin

The use of azithromycin was initially supposed to improve COVID-19 outcomes when added to usual care [125]. Evidence from preclinical studies showed that azithromycin could prevent the interaction between SARS-CoV-2 NTD and gangliosides found in lipid rafts in a manner similar to antimalarials [75]. However, due to potential arrhythmogenic side effects, the use of this antibiotic is solely recommended in COVID-19 cases complicated by bacterial superinfection [126].

3.2. Agents with only preclinical evidence for the treatment of COVID-19

3.2.1. Modulators of ACE2 or TMPRSS2 expression

Another goal of therapeutic interventions against COVID-19 could be to reduce the availability at the plasma membrane of ACE2 or other molecules that allow internalization of the virus. Several molecules have been hypothesized to downregulate ACE2 receptors, such as estradiol, spironolactone, isotretinoin, and retinoic acid, as well as the TMPRSS2 inhibitors bicalutamide, bromhexine, camostat mesilate, and nafamostat. Camostat mesilate has been shown to significantly reduce viral entry into cultured lung cells [39] and to specifically block the spread of SARS-CoV-2 in human lung tissue in *ex vivo* experiments [127].

It has also been reported that the expression of TMPRSS2 may be strongly upregulated by androgens in the prostate [128] and by both androgens and glucocorticoids in human lung cells [129]. According to some authors, the strong dependence of TMPRSS2 on androgens may contribute to the increased risk of highly symptomatic forms of COVID-19 observed in men [130]. On the other hand, estrogens are known to modulate the expression of ACE2 [131], possibly preventing the release of cytokines and the subsequent activation of the innate or acquired

immune response, thrombosis and fibrosis, which overall condition a worse COVID-19 outcome [132]. Based on this consideration, several clinical trials are currently underway to investigate the potential benefits of estrogen treatment on COVID-19 (ClinicalTrials.gov ID NCT04359329, NCT04539626, NCT04365127). Raloxifene, a selective estrogen receptor modulator, has also recently been proposed to be repurposed for COVID-19 treatment [133].

3.2.2. Dipeptidyl peptidase-4 inhibitors

Dipeptidyl peptidase-4 (DPP-4), originally known as lymphocyte cell surface protein CD26, has been proposed as another interesting pharmacological target for COVID-19. DPP-4 is expressed in various tissues such as lung, intestine, liver, and kidney. Due to its localization in the plasma membrane and its chemical properties that allow it to bind the S1 domain of SARS-CoV-2 spike proteins, DPP-4 has been proposed as an alternative pathway to mediate SARS-CoV-2 cell adhesion [134]. Furthermore, DPP4 is involved in T-cell signaling and functionality [135]. CD26-mediated signaling leading to T-cell activation occurs in lipid rafts through its association with CD45RO [136]. In clinical practice, DPP-4 inhibitors (DPP-4i) are commonly used in patients with type 2 diabetes mellitus (T2DM), including the frail population, due to their efficacy and safety profile [137]. Therefore, patients treated with DPP-4i may represent an interesting study population to evaluate whether molecular inhibition of DPP-4 could represent an effective pharmacological target for COVID-19. Although current data from observational studies are inconclusive [135], a cohort study from Northern Italy found a lower risk of death in patients receiving sitagliptin than in those on standard of care [138], while a subsequent study from England failed to replicate this result and found a potentially, although small, association between DPP-4i and the risk of death from COVID-19 [139].

3.2.3. Opioids

Opioid use has been associated with increased susceptibility to COVID-19 as well as morbidity, mortality, and utilization of healthcare resources [140,141]. Patients receiving long-term opioid therapy may have a weaker immune response and impaired respiratory function [142–144], both of which predispose to SARS-CoV-2 infection and severe outcomes. Additionally, large use of opioids in the context of COVID-19 pandemic, as analgesics during orotracheal intubation or for pain management, could expose patients to dangerous drug-drug interactions and side effects [145].

Surprisingly, an unexpectedly low incidence of COVID-19 in patients receiving opioid substitution-treated patients was reported elsewhere [146,147]. In this regard, it was hypothesized that the interplay among long-acting opioids or opioid antagonists, ACE-2 signaling, TLR4, and SARS-CoV-2 would play a protective role against the development of COVID-19 [146]. This evidence led to the design of clinical trials on the efficacy of some opioids for the treatment of COVID-19 which are currently ongoing (ClinicalTrials.gov ID NCT04604704; NCT04604678; NCT04365985; NCT04454307).

Cholesterol is an important determinant of the lateral organization of κ - and μ -opioid receptor within lipid raft domains [148] and may also influence μ -opioid receptor signaling in cell models and analgesia in mice and humans by supporting lipid raft integrity and facilitating G-protein coupling [149]. It was shown that lowering brain cholesterol levels by simvastatin impaired the analgesic effect of opioids in mice, whereas the analgesic effect of opioids was enhanced in mice fed with a high-cholesterol diet. Furthermore, an inverse correlation between cholesterol levels and the dose of fentanyl used for anesthesia was found, suggesting that these mechanisms may also be applicable to humans [149].

3.2.4. Antidepressants and antipsychotics

In late 2021, some evidence of the efficacy of selective serotonin reuptake inhibitor (SSRI) antidepressants against COVID-19 emerged. In the TOGETHER trial, investigators assessed the efficacy of fluvoxamine

compared with placebo in preventing hospitalization in symptomatic high-risk Brazilian adults who were confirmed positive for SARS-CoV-2 infection [150]. The study included 1497 subjects and showed a lower hospitalization rate in patients taking 100 mg of fluvoxamine twice daily for 10 days. In another multicentre cohort study of 83,584 patients diagnosed with COVID-19, a reduced relative risk of mortality was associated with fluoxetine use [151].

Previous studies have shown that chronic treatment with antidepressants may enhance adenylyl cyclase activity by partially translocating G α s (G α s) from rafts to nonraft domains of the plasma membrane, where they may more easily stimulate adenylyl cyclase [152,153]. Sustained treatment of cells with various antidepressants, including SSRIs, may lead to an accumulation of these compounds in lipid rafts and correlates with a redistribution of G α s in the membrane, resulting in lipid raft disruption [154]. This event may ultimately affect SARS-CoV-2 infection: indeed, some preclinical studies have shown that antidepressants are able to prevent infection of Vero E6 cells with SARS-CoV-2 [155].

Moreover, SSRIs belong to the class of functional inhibitors of acid sphingomyelinase (FIASMA) [156]. Acid sphingomyelinase (ASM) is a lysosomal glycoprotein located in the inner lysosomal membrane. In response to stimulation, ASM is able to migrate to the outer leaflet of the cell membrane and catalyze the hydrolysis of sphingomyelin into ceramide and phosphorylcholine. Sphingomyelin is associated with cholesterol in lipid rafts and is abundant in these structures. ASM inhibition induced by SSRIs may alter the formation of lipid rafts, resulting in a modification of the cell membrane structure. This modification could lead to impairment of lipid raft-mediated endocytosis of SARS-CoV-2, explaining the beneficial role of these molecules on COVID-19 [157].

Chlorpromazine, an antipsychotic, was used to prevent clathrin-mediated endocytosis in vitro [158]. Together with chloroquine, loperamide, and lopinavir, chlorpromazine was shown to inhibit replication of Middle-Eastern respiratory syndrome coronavirus (MERS-CoV) and human coronavirus 229E at a 50% effective concentration (EC₅₀) of 3–8 μ M [159]. The beneficial effect of chlorpromazine in COVID-19 patients is currently being investigated in a phase III randomized controlled trial [160]. An in vitro experiment has shown that chlorpromazine prevents SARS-CoV infection of HepG2 cells by arresting clathrin-mediated endocytosis [41], but the effects on lipid raft-dependent endocytosis are unknown.

3.2.5. Glycyrrhizin

Glycyrrhizin, a natural product with a triterpenoid saponin structure derived from the root of *Glycyrrhiza glabra*, has been extensively studied for its pharmacological effects, which include anti-inflammatory and antiviral properties. Part of these effects could be explained on the basis of a lipid raft perturbation. Specifically, thanks to its saponin structure, glycyrrhizin can interact with membrane cholesterol and reduce membrane fluidity, preventing the movement of viral receptors within the membrane and the formation of fusion pores [161].

3.3. Agents targeting lipid components

Lipid rafts can be targeted by drugs that act on their lipid components, with important repercussion on viral entry and immune response.

Lipid-lowering agents can act on the composition of lipid rafts by i) depriving them of cholesterol or sphingomyelin, lipids responsible for their stability, ii) stimulating physiological pathways responsible for the efflux of cholesterol, iii) reducing the supply of cholesterol and sphingolipid by inhibiting their biosynthesis [9].

The first approach could be pursued by using the food additive M β CD, which has already been shown to inhibit the entry of HIV into host cells and to have anti-inflammatory properties. Disruption of raft cholesterol could prevent translocation of ACE2 to the apical pole of epithelial cells, thereby dramatically reducing its availability for

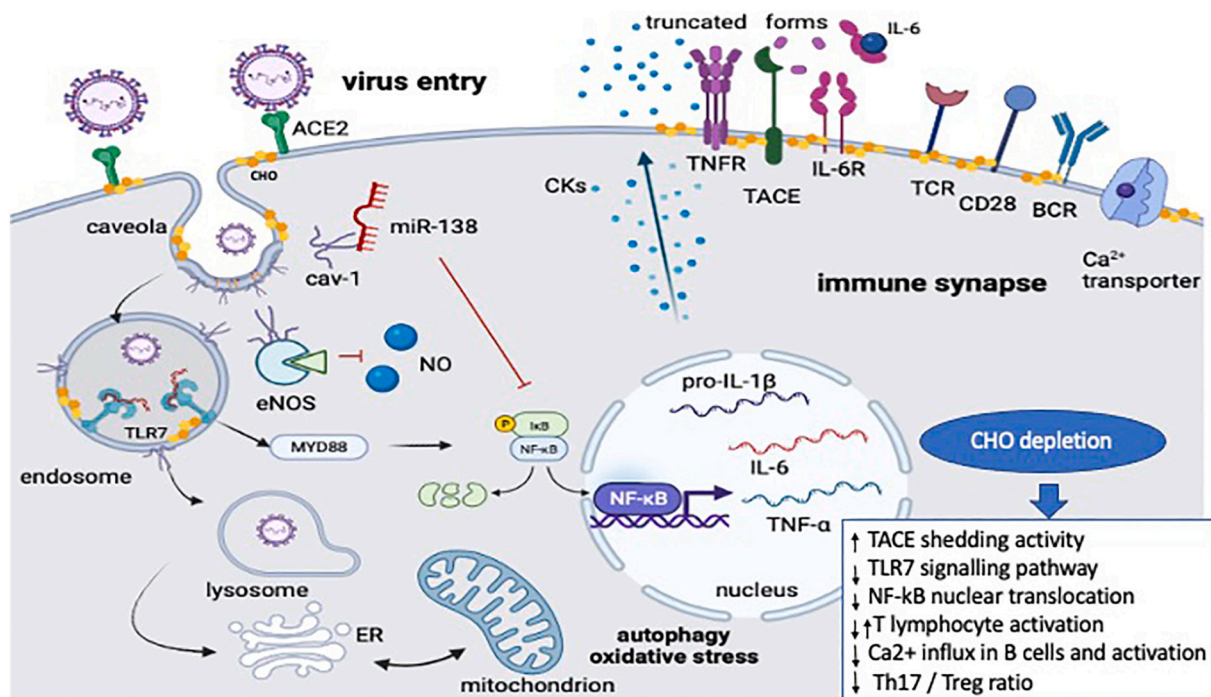


Fig. 4. Potential mechanisms linking lipid rafts to the pathogenesis of COVID-19.

In this scenario, SARS-CoV-2 may enter target cells via binding of ACE2, which is located in lipid rafts, or via caveolae-mediated endocytosis, which also occurs in raft compartments of the plasma membrane. The lipid and protein composition of rafts is critical for the subsequent transport of viruses to endosomes and lysosomes. The raft-associated protein cav-1 can inhibit eNOS in endothelial cells and the subsequent production of NO, which plays an important role in inflammation. Lipid rafts and cholesterol in the endosomal membrane may enhance TLR7 activity and promote nuclear translocation of NF- κ B via the MYD88 pathway. This event leads to transcription of genes encoding the pro-inflammatory cytokines pro-IL-1 β , IL-6, and TNF- α . Importantly, cav-1 may bind miR-138, which suppresses the NF- κ B-mediated pathway. Moreover, lipid rafts in the mitochondrial membrane may contribute to autophagy and oxidative stress, both of which occur in infected cells. Lipid rafts in the plasma membrane of immune cells also harbor important protein complexes involved in the immune response, such as receptors for pathogens, antigens or cytokines or costimulatory molecules. Rafts also regulate the activity of TACE, a transmembrane enzyme that generates truncated forms of cytokine receptors. Cholesterol depletion in the immune synapse may profoundly alter the ability of cells to counteract SARS-CoV-2 infection and promote the improper generation of a dysfunctional immune response, characteristics of the most severe outcomes of COVID-19.

Abbreviations: ACE2: angiotensin-converting enzyme 2; BCR: B cell receptor; cav-1: caveolin 1; CD28: Cluster of Differentiation 28; CHO: cholesterol; CKs: cytokines; eNOS: endothelial enzyme nitric oxide synthase; ER: endoplasmic reticulum; I κ B: inhibitors-of-kappaB; IL-6: interleukin 6; IL6R: interleukin-6 receptor; miR: microRNA; MYD88: myeloid differentiation primary response 88; NF κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; NO: nitric oxide; P: phosphorylation; pro-IL1 β : pro-interleukin 1 beta; T17: T helper 17 cell; TACE: tumor necrosis factor-alpha converting enzyme; TCR: T cell receptor; TLR7: Toll-like receptor; TNFR: tumor necrosis factor receptor; TNF- α : tumor necrosis factor alpha; Treg: Regulatory T cell.

activation of the SARS-CoV-2 receptor. Priming of furin is also dependent on cholesterol [162]. Preliminary evidence of a possible beneficial effect of lipid-lowering therapy based on M β CD or mevastatin against coronaviruses has been described for infectious bronchitis virus (IBV) [163] and for porcine deltacoronavirus (PDCoV) [164], suggesting a possible role also in the context of SARS-CoV-2. By using in vitro cell models expressing ACE2, researchers showed that depletion of cholesterol by M β CD was associated with a significant decrease in the number of bindings to the ACE2-spike protein, along with a dose-dependent reduction in ACE2 expression that resulted in reduced SARS-CoV replication [165]. In another study on coronavirus infectious bronchitis [45], it was reported that M β CD or mevastatin-induced disruption of lipid rafts reduced the expression of structural viral proteins associated with cav-1 in Vero cells, limiting viral infectivity in the early stage of infection. However, treatment with M β CD was ineffective in the late stage and did not prevent virion release. M β CD depletes membrane cholesterol in a nonspecific manner and act on both raft and nonraft domains; in addition, the compound is also capable of altering ion permeability and inducing cytotoxicity. Phytosterols, fumonisin, myriocin and sphingomyelinase could also affect viral infectivity by interacting with other lipid components of lipid rafts such as sphingolipids [166,167].

The second strategy could be achieved by stimulating of the expression of ABCA1 and G1 using liver X receptor (LXR) agonists or heterologous overexpression of these transporters [13,168].

The last option could be achieved by using inhibitors of cholesterol and sphingolipid biosynthesis, such as statins and miglustat, respectively [169]. In addition to inhibiting the cholesterol biosynthesis pathway and disrupting lipid raft composition, the use of statins may also lead to a reduction in CD147 translocation to the cell surface [170]. CD147 is a transmembrane glycoprotein associated with lipid rafts and plays a critical role in SARS-CoV-2 infection by promoting viral entry and modulating T cell activation. Additionally, it can increase the expression of GLUT1 transporters and shift cellular metabolism towards a glycolytic pathway [171]. As cellular cholesterol increases with age, inflammation, smoking, and metabolic disorders (e.g., obesity and diabetes), the plasma membrane may become a more favorable environment for SARS-CoV-2 engagement, contributing to the severity of COVID-19 [171,172]. Statins may have further benefits in COVID-19 treatment thanks to their anticoagulant and anti-inflammatory effects. The use of statins and/or anti-hypertensives was associated with a 32% lower mortality rate in patients hospitalized for COVID-19 and with a history of cardiovascular disease and/or hypertension [173]. However, a recent multicenter randomized clinical trial comparing the efficacy of atorvastatin vs placebo performed in patients with COVID-19 admitted to the intensive care unit (ICU) showed no effect of this statin on the composite outcome of venous or arterial thrombosis, treatment with extracorporeal membrane oxygenation, or all cause mortality within 30 days, compared with placebo [174].

Table 1

Drugs which may prevent SARS-CoV-2 infection by exploiting a lipid raft-mediated mechanism. Abbreviations: ABCA1: ATP-binding cassette A1; ACE2: angiotensin-converting enzyme 2; ASM: acid sphingomyelinase; cav-1: caveolin 1; DPP-4: dipeptidyl peptidase-4; EGFR: endothelial growth factor receptor; ER: endoplasmic reticulum; Erk: Extracellular signal-regulated kinase; FcR: fragment crystallizable receptor; HDL: high density lipoprotein; IgG: Immunoglobulin G; IL-1 β : interleukin-1 beta; IL-6R: interleukin-6 receptor; IVIG: intravenous immunoglobulins; JAKi: Janus kinase-inhibitor; MAP: mitogen-activated protein; moAbs: monoclonal antibodies; M β CD: methyl- β -cyclodextrin; NPC1: Niemann–Pick C1; NTD: N-terminal domain; PCSK9: proprotein convertase subtilisin/kexin type 9; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; SSRIs: selective serotonin reuptake inhibitors; STAT: signal transducer and activator of transcription; TCR: T cell receptor; TLR: Toll-like receptors.

Type of treatment according to the level of evidence or recommendation	Drug	Category	Main findings on lipid rafts
Pharmacological treatments used for COVID-19 in clinical practice	Glucocorticoids	Immunosuppressants	- Increase in the fluidity of membranes and reduction in the content of palmitic acid leading to the displacement of TCR and other signaling proteins localized in lipid rafts in murine T cell hybridomas [85] - Rapid effects mediated by the binding of glucocorticoids to membrane receptors placed in lipid rafts and associated with cav-1 [86]
	IL-6R inhibitors	Biological immunosuppressants	- Increased availability in circulating cholesterol that may influence the composition of lipid rafts and membrane fluidity with final anti-inflammatory effects [92]
	JAKi	Immunosuppressants (small molecules)	- Assembly of lipid rafts in response to the binding of cytokines to their receptors preceding the activation of the JAK/STAT signaling cascade in human lymphocytes [96] - In vitro inhibition of intracellular lipid accumulation in macrophages, through the up-regulation of ABCA1 [98]
	IL-1 β inhibitors	Biological immunosuppressants	- Prevention of the IL-1 β signaling that may generate oxidative stress and systemic inflammation through a lipid raft-mediated mechanism [100]
	Antimalarials	Immunomodulators	- Improvement in lipid profile, with an increase in serum HDL and a reduction in atherogenic lipoproteins [105–107]. - Competition with SARS-CoV-2 spike protein for the binding of sialic acid and gangliosides placed in lipid rafts [108]
	Remdesivir	Antiviral agents	- Synergic action with NPC1-inhibitors in regulating the lysosomal trafficking of cholesterol and sphingolipids [40]
	Monoclonal antibodies targeting SARS-CoV-2 spike protein	Antiviral agents	- Integrity of lipid raft interface to guarantee the interaction between antibodies and FcR placed in rafts, binding and phagocytosis of IgG immune complexes [121,122]
	Heparin	Anticoagulants	- Suppression of MAP kinase/Erk signaling in vascular smooth muscle cells and prevention of the phosphorylation of EGFR localized in caveolin-enriched lipid rafts [124]
	Azithromycin	Antibiotics	- Prevention of the interaction between SARS-CoV-2 NTD and gangliosides found in lipid rafts [75]
	Drugs potentially affecting SARS-CoV-2 infection according to preclinical evidence but currently not recommended for treating COVID-19 in clinical practice	Camostat mesilate	Serine protease inhibitors
Estrogens		Hormones	- Modulation of the expression of ACE2 in lipid rafts [131]
DPP-4 inhibitors		Anti-diabetic agents	- Prevention of T cell activation by blocking DPP-4 association with CD45RO in lipid rafts [136]
Opioids		Analgesics	- Interplay among long-acting opioids or opioid antagonists, ACE-2 signaling, TLR4, and SARS-CoV-2 [146] - Immune modulation that may depend on cholesterol-related lateral organization of κ - and μ -opioid receptors within lipid raft domains [148]
SSRIs		Antidepressants	- ASM inhibition with following disruption of lipid rafts and impairment of the lipid raft-mediated SARS-CoV-2 endocytosis [157]
Glycyrrhizin		Anti-inflammatory natural compounds	- Interaction with membrane cholesterol and reduction of membrane fluidity that limits translocation of virus receptors within the membrane and the creation of fusion pores [161]
M β CD		Lipid-lowering agents	- Reduction of cholesterol in raft and non-raft domains, eventually decreasing SARS-CoV pseudovirus infectivity in Vero E6 cells in a dose-dependent manner [2] - Decrease in the number of bonds between ACE2 and SARS-CoV-2 spike protein along with a dose-dependent reduction in ACE2 expression leading to reduced SARS-CoV replication in in vitro cell models expressing ACE2 [165] - Reduced expression of structural viral proteins associated with cav-1 in Vero cells, leading to limited viral infectivity in the early but not late stage of infection [45]
Statins		Lipid-lowering agents	- Reduced expression of structural viral proteins associated with cav-1 in Vero cells, leading to limited viral infectivity in the early but not late stage of infection [45] - Disruption of lipid rafts through the inhibition of cholesterol biosynthesis leading to reduced CD147 translocation to the cell surface and T cell activation [170]
PCSK9 inhibitors		Lipid-lowering agents	- Cholesterol accumulation in macrophages and other immune cells potentially improving lipid raft composition and augmenting TLR function [175]

Recently, it was hypothesized that proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, another lipid-lowering therapy, could potentially suppress SARS-CoV-2 infection by either blocking virus entry into host cells or inhibiting replication [162]. PCSK9, which stimulates low-density lipoprotein receptor (LDLR) degradation and inhibits reverse cholesterol transport, is frequently upregulated in sepsis and is associated with worse outcome. PCSK9 may cause preferential cholesterol accumulation in macrophages and other immune cells, potentially affecting lipid raft composition and increasing the function of TLR and thus the inflammatory response [175].

4. Concluding discussion

This narrative review aims to highlight the contribution to lipid raft perturbation of already commercialized drugs, which could potentially be useful as a rationale for drug repurposing or explain different severities or prognoses of COVID-19 disease in specific patients' groups.

The crucial role of lipid rafts in viral infections as virus entry pathways and in immune response as immune synapses make these domains a double-edged sword in the pathogenesis of COVID-19, as they contribute to both viral spread and inflammation, Fig. 4. Lipid rafts are indeed required for pathogen endocytosis and endosomal processing, as well as for the recruitment of receptors and other transmembrane proteins involved in immune pathways. Human coronaviruses can enter host cells via a clathrin-mediated, caveolae-mediated, or non-canonical mechanism, all of which are directly or indirectly dependent on membrane cholesterol content and proper lipid rafts functioning [2,44]. Lipid rafts also harbor many receptors and molecules involved in the immune response, such as TLR, TCR, BCR, and costimulatory molecules that help regulate viral spread and inflammation in various pathological contexts. For this reason, a "lipid raft therapy" has been proposed with the aim of both modulating drug delivery to the rafts and allowing structural modifications of lipid rafts to mitigate the infection [13]. Although there is not yet clear evidence linking lipid raft disruption to the antiviral response associated with COVID-19, some evidence highlights that lipid rafts may represent the site of initial binding, activation, internalization, and cell-to-cell transmission of SARS-CoV-2 [13]. Moreover, dysregulation of the immune response leading to uncontrolled inflammation, which also appears to be mediated by lipid rafts, is a key-feature of COVID-19 [176,177]. It has been shown that disruption of raft lipid content with membrane cholesterol reduction prevents coronavirus entry into target cells [2,45]. Lipid modification may also affect the translocation and recruitment of proteins that play a central role in either SARS-CoV-2 entry, such as ACE2, or immune synapse formation [165,170]. Proteins located within lipid rafts may be targeted by anti-COVID-19 drugs including steroids, antimalarials, immunosuppressants, antiviral agents, or heparin [40,85,86,92,96,98,100,108,116,121,122,124], Table 1. Adjunctive therapies, such as estrogens, lipid-lowering or glucose-lowering drugs, may also lead to better COVID-19 outcomes, although it is unclear whether such effects may depend on the protective mechanisms exerted on the components of the lipid rafts [131,178].

This intricate scenario underlines the central role of lipid rafts in SARS-CoV-2 infection and suggests that combined strategies targeting these microdomains and aimed at simultaneously preventing viral entry and modulating the immune response may prove highly beneficial for the treatment of the disease. Indeed, further research is needed to better unravel the extent to which lipid rafts contribute to COVID-19 pathogenesis and may be selected as ideal candidates for therapy.

CRedit authorship contribution statement

RT conceived the idea of the manuscript, performed bibliographic research, wrote the first draft of the manuscript, drew the figures and the table. RR performed bibliographic research, wrote the first draft of the manuscript and edited its final version. JA and AP helped perform bibliographic research and write the manuscript. All the authors

critically revised the paper according to the comments of the Referee and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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