Chapter

Cholesterol Recognition Motifs (CRAC) in the S Protein of Coronavirus: A Possible Target for Antiviral Therapy?

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Abstract

Some interactions of enveloped viruses with the host cell membrane have a cholesterol-dependent component, which may account for clinical manifestations of the infectious disease and can be used for the development of antiviral drugs. These cholesterol-dependent interactions can be mediated by cholesterol-recognition amino-acid consensus (CRAC) motifs present in viral proteins. The S protein of the SARS-CoV and SARS-CoV2 coronaviruses contains CRAC motifs that can be involved in the process of virus entry into the cell. Besides, during viral envelope formation, CRAC motifs can be responsible for binding of cell membrane cholesterol, leading to depletion of cell membrane cholesterol and subsequent malfunctioning of cellular cholesterol-dependent proteins, destabilization and permeabilization of cell membranes and, ultimately, to the death of infected cells. Understanding the mechanisms of cholesterol-dependent virus–cell interactions and the role of CRAC-containing viral proteins in the pathogenesis of the disease can serve as the basis for the development of new drugs that prevent both coronavirus entry into the cell and the damage of the infected cell during the viral morphogenesis. The target for such drugs can be the S-protein/cholesterol interface. CRAC-containing peptides derived from viral proteins may be among these agents. These peptides can also be used as experimental tools to study cholesterol-dependent virus–cell interactions.

Keywords: peptides, cholesterol-recognition motif (CRAC), CRAC-containing peptides, coronavirus, S-protein, SARS-CoV2, COVID-19

1. Introduction

The COVID-19 pandemic caused by the SARS-CoV2 coronavirus has resulted in almost hundred of millions of infections and about two millions of deaths in 2020 [1, 2]. According to the WHO data as of January 4, 2021, there have been 85 929 428 confirmed cases of COVID-19 reported to WHO, including 1 876 100 deaths [2]. It has been shown that in patients with laboratory-confirmed COVID-19, clinical results correlate with the presence of concomitant diseases, among which hypertension and diabetes mellitus, as well as old age, atherosclerosis, cardiovascular and cerebrovascular diseases worsening prognosis [3–9]. Notably, these factors are commonly associated with the impairments in the lipid/cholesterol metabolism and transport or are their direct consequence [3–5].

At present, a lot is known about the coronavirus SARS-CoV2. It is an enveloped single-stranded RNA virus belonging to the Betacoronavirus genus of the Coronaviridae family. The virus contains a 30 kb genome encoding four major viral structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins [9, 10]. According to modern concepts, the cellular receptor for S protein is angiotensin converting enzyme 2 (ACE2); binding of the S protein to this receptor allows the internalization of the virus and triggers the disease. Significant progress has been made in the development of vaccines against SARS-CoV2 and mass vaccination of people in different countries begins [11, 12]; this gives hope that the pandemic will stop. However, the issues of treating patients with various forms of COVID-19 and the development of drugs based on knowledge of the mechanisms underlying this pathology still need to be addressed. This chapter is mainly focused on the lipid aspects of the interactions of coronaviruses with host cells and, in particular, draws attention to the fact that interactions with host cells of many enveloped viruses, including coronaviruses SARS-CoV and SARS-CoV2, are cholesterol dependent. Moreover, these interactions lead to significant and potentially deleterious alterations in the cholesterol status of the infected cells. These cholesterol-dependent processes play a significant role both at the stage of the virus entry and during the development of severe respiratory syndrome (SARS) and other health problems caused by coronaviruses SARS-CoV and SARS-CoV2. Therefore, understanding this component is necessary for the development of additional approaches both to prevention and treatment of these diseases, and the attempts in this direction are being made (e.g., [13–15]). This review focuses on the fact that the coronavirus S protein, which is involved in cholesterol-dependent virus–cell interactions during entry and replication stages, contains the so-called cholesterol recognition amino-acid consensus (CRAC) motifs [16, 17] that can actually mediate these interactions. A hypothesis is put forward suggesting that binding of cell membrane cholesterol by CRAC-containing S protein (and possibly by other viral proteins) and subsequent removal of cholesterol from intracellular membranes by newly formed viral particles can affect normal functioning of cellular cholesterol-dependent proteins (receptors, ion channels, enzymes, etc.) and can eventually cause cell death due to destabilization and permeabilization of cell membranes. This deteriorating effect of CRAC-containing viral proteins can be counteracted by agents that prevent binding of membrane cholesterol to viral proteins and/or compensate for the membrane cholesterol depletion produced by the forming viral particles. It is possible that specially designed CRAC-containing peptides that specifically block interactions of S protein with cholesterol can expand the range of antiviral agents.

2. Some interactions of enveloped viruses with host cells are cholesterol-dependent

The viral life cycle includes four steps: entry, replication, assembly, and egress [18–21] (**Figure 1**). At the entry step, an enveloped virus binds to a target receptor, the viral envelope fuses with the host cell membrane, and the viral nucleic acids are released into the cytoplasm. At the replication step, the nucleic acid is replicated in cytoplasmic replication organelles and viral proteins are synthesized. At the assembly step, viral proteins and nucleic acids are packed into a viral particle and the viral envelope is formed. At the egress step, mature viral particles leave the cell through the cellular membrane [18–21].

Some interactions of enveloped viruses with the cell in the course of penetration and during assembly, budding, and exit of the virus from the cell are known to

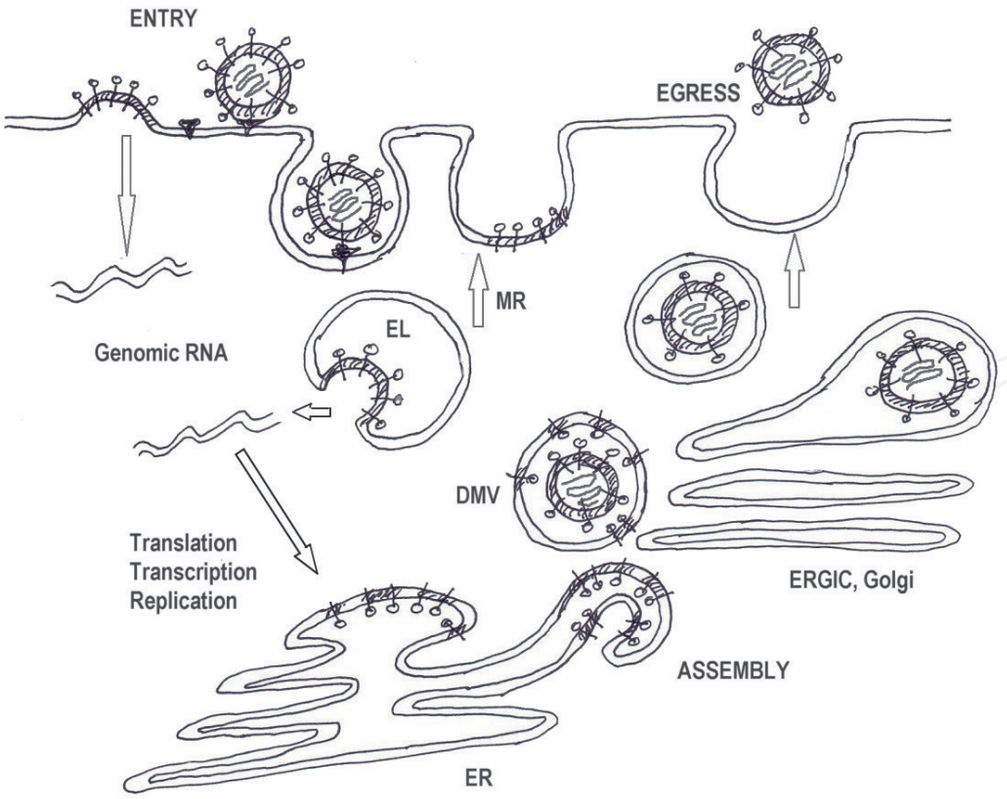

Figure 1.
Life cycle of an enveloped virus as exemplified by coronavirus (based on [18–21]). To enter a cell, virus binds via S protein to the receptors on the host cell and the viral envelope fuses with plasma membrane or membrane of endolysosome (EL) and releases the genomic nucleic acid into the cell cytoplasm. Viral membrane is shaded. Note that viral envelope remains in cell plasma membrane both after direct fusion and after endosomal membrane recycling (MR). At the replication stage, replication–transcription complex is formed and viral proteins and copies of RNA are produced. At the assembly stage, viral particles are formed from nucleocapsid (genomic RNA and N protein) and intracellular cell membranes (endopasmic reticulum (ER) and/or ER-to-Golgi intermediate compartment (ERGIC)), containing viral membrane-associated proteins (S, M, and E). During the assembly process, double-membrane vesicles (DMV) are formed. At the exit stage, viral particles are released by secretory pathway.

depend on the presence of cholesterol and lipid rafts in the membranes of the host cells [21–26]. This has been shown for immunodeficiency viruses (HIV) [27–31], influenza [32–37], herpes [38], Newcastle disease virus [39], and rotavirus [40], as well as hepatitis C virus (HCV) [41–43] and some other viruses of the Flaviviridae family (Yellow fever virus, Zika virus, Dengue virus, West Nile virus [44, 45]). For example, the vital need of cholesterol for replication of hepatitis C virus (HCV) was shown by different methods in [41, 43]. Lipid withdrawal from the medium considerably suppressed the virus replication, which was restored to normal levels upon addition of exogenous LDL. Moreover, virus replication was suppressed by knockdown or pharmacological inhibition of Niemann–Pick type C1 protein (NPC1) – cell protein mediating the endosomal cholesterol transport [41, 43].

The cholesterol dependence of virus–cell interactions has also been demonstrated for various coronaviruses [14, 15, 46–52], including SARS-CoV and SARS-CoV2 [50–52]. Li et al. 2007 [50] reported that the production of SARS-CoV particles released from the infected Vero E6 is notably suppressed following cholesterol depletion by cell pretreatment with methyl-β-cyclodextrin (mβCD), and the addition of cholesterol to the culture medium reversed this effect. Later, Glende et al. 2008 [51] showed that the removal of cholesterol from cell membranes using mβCD reduces the efficiency of infection of cells not only with the SARS-CoV but also with a non-replicating pseudotype of vesicular stomatitis virus containing the surface glycoprotein S of the SARS-CoV virus (VSV-ΔG-S), which confirms the key

role of the S protein in the virus entry. The authors also reported that the cellular receptor of the SARS-CoV virus, angiotensin-converting enzyme (ACE2), is co-localized with Flotilin2 and LAMP2, the protein markers of the detergent-resistant membrane domains (rafts) [51].

The issues concerning the importance of the host cell membrane lipids, rafts, and cholesterol at different stages of the virus life cycle have been addressed in numerous comprehensive reviews, and the dependence of the viral life cycle on cellular cholesterol, as well as the impact produced by viruses on cellular lipids and cholesterol in particular is regarded as a basis for antiviral therapy [15, 30, 31, 45, 53]. For example, cholesterol-lowering treatments are considered as a possible prophylactic or preventive measures [45, 54]. However, alterations in the cell lipid status produced by viruses that have entered a cell impose more complex requirements on potential medicines.

3. Virus-induced modulations of the lipid composition of cell membranes. Formation of viral envelope can result in a hazardous decrease in the cholesterol content in cell membranes

Viruses not only depend on cholesterol but also significantly modulate the lipid composition of cell membranes [53, 55–63]. This occurs both at the stage of virus internalization and during the synthesis of viral proteins and intracellular assembly of new viral particles. The consequences of these changes can determine the clinical course and severity of the disease. The release of the gene material of many enveloped viruses into the cytoplasm of the cell occurs by fusion of the viral envelope with plasma membrane or with membranes of late endosomes (endolysosomes) formed during receptor-mediated endocytosis [18–21, 64–70]. The inclusion of viral envelopes into the host cell membrane (either after direct fusion of after endosomal membrane recycling) should change both the lipid and protein composition of the cell membrane and cause rearrangements in the lipidic milieu and antigenic profile of the host cell membrane (**Figure 1**). Further, at the stage of the assembly of new viral particles, their envelopes are formed from cellular membranes [14, 15, 45, 55, 57–65, 67, 68]. Some viruses bud from the plasma membrane (e.g., togaviruses, rhabdoviruses, paramyxoviruses, orthmyxoviruses, and retroviruses, including HIV), others use the endoplasmic reticulum (ER) (coronaviruses and flaviviruses) or/and a Golgi complex (bunyaviruses), some (e.g., herpes virus) have more complicated budding scenario [59–62]. The formation of the viral envelopes can involve lipid sorting and, in particular, accumulation in the viral envelope of cholesterol and sphingolipids that are acquired from the host cell membranes [33, 61–65, 69, 70]. For example, HIV-1 selectively buds from membrane domains enriched in cholesterol and sphingolipids (rafts); as a result, host cell rafts become a viral coat and the level of cholesterol and sphingolipids and the cholesterol/phospholipids ratio in the viral envelope is higher than in the plasma membrane where they originate, and also notably higher than in the intracellular membranes [53, 61, 62, 65]. Another example – bovine viral diarrhea virus (BVDV) of the Flaviviridae family budding from the endoplasmic reticulum (ER): the content of cholesterol, sphingomyelin, and hexosyl-ceramide in the BVDV particles was shown to be more than twofold higher than in the infected cells [69]. As the cholesterol concentration in the ER is significantly (several times) lower than in the plasma membrane [71–74], the loss of cholesterol due to the formation of viral envelopes can be destructive for the ER membranes.

For lipid sorting necessary for the virus envelope formation, various mechanisms are used to manipulate synthesis, metabolism, and transport of host lipids,

cholesterol in particular, and lead to significant changes in the lipid status of the host cell. HIV-1 infection is known to induce various alteration of cellular lipids, including increased cholesterol synthesis and uptake [75], suppressed cholesterol efflux [76], as well as a shift in phospholipid synthesis to neutral lipids and peroxidation of polyunsaturated fatty acid [53, 65, 75, 76]. Hepatitis C virus (HCV) also causes massive rearrangements of intracellular membranes leading to the formation of double-membrane vesicles (DMVs) enriched with cholesterol. As was shown in [41], HCV 'usurps' cholesterol transporter proteins, such as NPC1, in order to deliver cholesterol to the viral replication organelle where cholesterol is needed, and blockage of this transporter suppresses the virus replication. Coronaviruses, like Flaviviruses, are assembled and bud from the membranes of Golgi complex and ER [41, 60] and also form double-membrane vesicles [77]. At the same number of newly formed viral particles, the consequences of removing cholesterol from ER membranes by double-membrane vesicles can be more severe than in the case of single membrane vesicles; a quantitative assessment of this process is necessary.

A possible mechanism stimulating the delivery of cholesterol to ER from plasma membrane during coronavirus replication was demonstrated by Wang et al. 2020 [78]. The authors reported that SARS-CoV2 activates the host cell gene encoding cholesterol 25-hydroxylase and induces the formation of 25-hydroxycholesterol, which increases cholesterol availability [79] and triggers its delivery from the plasma membrane to the endoplasmic reticulum, where cholesterol is required for the viral envelope formation. Although, as is known [14, 23–29, 32–41, 46–55], the depletion of cholesterol in the plasma membrane suppresses the virus entry into the cell, the observed trafficking of cholesterol into the ER (normally the flow goes in the opposite direction [71, 72]) can reflect an increased uptake of cholesterol for the formation of envelopes of new viruses. After the release of newly formed viruses, this depleted cell will not be susceptible to new infection.

Thus, the formation and release of viral particles from the cell cannot but affect the composition of the host cell membranes. It can be expected that after a full replication cycle of viruses with high envelope cholesterol, the level of cholesterol in the membranes of the host cell will be reduced, and this depletion of cholesterol can lead to a significant deregulation of cholesterol-dependent processes, including intracellular signaling and metabolic pathways. At a high rate of assembly of a large number of viruses, infected cells may not be able to compensate for the loss of cholesterol in their membranes, and this can lead to cell death due to destabilization and permeabilization of cell membranes. Indeed, ample evidence indicates that lowered membrane cholesterol is associated with altered mechanical properties and increased permeability of the membrane [80–83]. Some viral and bacterial proteins trigger apoptosis through lysosomal membrane permeabilization leading to release of cathepsins [81]. The human immunodeficiency virus type 1 (HIV-1) protein Nef is one of such proteins: when entering mammalian cells, it causes permeabilization of the lysosomal membrane [81]. It seems appropriate at this point to recall that permeabilization of intracellular membranes due to cholesterol depletion underlies the cytotoxic effect of some anticancer drugs [81, 84–87]. As was shown by Appelqvist et al. 2011 [84], the mechanism of action of cisplatin and some other lysosomotropic drugs at least partially is based on the permeabilization of lysosomal membranes leading to cell death; cholesterol accumulation in lysosomal membranes caused by inhibition of cholesterol transporting protein NPC1 prevented the lysosome-dependent cell death [84]. Note that in the case of virus infection, inhibition of the NPC1-dependent cholesterol transport suppressed the virus replication [41] and rescued the infected cells. When NPC1 functions normally and cholesterol is delivered from lysosomal compartment to ER for the formation of viral envelopes, lysosomal membranes lose cholesterol and become leaky; in a way,

virus acts like a lysosomotropic drug. Cholesterol supplementation was also shown to reverse a strong cytotoxic effect on colon cancer cells caused by a low molecular weight compound TASIN-1 producing cholesterol-dependent ER stress triggering oxidative stress and JNK-dependent apoptosis [85].

Thus, virus–cell interactions lead to significant modulations in the lipid composition of cell membranes. A decrease in cholesterol in cell membranes owing to the formation of viral envelopes can be one of the most dangerous consequences of the virus particle assembly, as the amount of cholesterol removed from the cell membranes by newly formed viruses can exceed the compensatory resources of the cell. If the delivery of cholesterol to the cells is insufficient, deregulation of cholesterol-dependent processes can lead to massive cell death, which manifests itself in the clinical course of the disease and a poor prognosis. In this connection, it should be noted that in patients infected with COVID-19, a significant decrease (several fold) in total cholesterol and low-density lipoprotein (LDL) cholesterol levels was recorded [13, 88], and cholesterol-lowering treatments (such as statins) may not be advisable for patients with life-threatening COVID-19 infection, at least until they recover from the infection [88]. Such a drop of the LDL cholesterol level in COVID-19 patients may reflect an enhanced recruitment of circulating cholesterol by the cells to compensate for its loss associated with virus reproduction. Perhaps the clinical prognosis depends on the timely and successful delivery of cholesterol required for cell membrane repair. Another direction in the development of drugs for the treatment of the disease is the search for agents that interfere with the interactions of viral proteins with cholesterol, and this search should be based on an understanding of the mechanisms of these interactions. So far, the only drugs for which clinically significant results have been demonstrated against COVID-19, are dexamethasone and some other corticosteroids [89–91]; secosteroids (vitamin D) are shown to help, too [92]. The use of dexamethasone led to a reduction in mortality to one third of hospitalized patients with severe respiratory complications from COVID-19. It seems possible that the action of steroids may be associated with the repair of a cell membrane damaged by virus-induced depletion of cholesterol.

How does the virus manage to bind and remove cholesterol from cell membranes?

4. Cholesterol recognition/interaction amino acid consensus (CRAC) motifs in viral proteins. Possible uses of CRAC-containing peptides

As was shown earlier [16, 17, 93], some proteins involved in cholesterol-dependent cell functions possess the so-called cholesterol recognition/interaction amino acid consensus (CRAC) motifs – small regions with a specific set of amino acid residues involving a branched apolar aminoacid residue (Val (V), Leu (L), or Ile (I)), aromatic residue (Tyr (Y)), and cationic aminoacid residue (Arg (R) or Lys (K)); these motif-forming amino acids are separated by short segments of any 1–5 amino acid residues. In subsequent discussions, more candidates of aromatic amino acid residues were proposed, and the general formula for the CRAC motifs presumably involved in the interaction of protein with cholesterol presently appears as follows: $V/L/I–X_{(1–5)}–W/Y/F–(X)_{(1–5)}–R/K$, where X stands for any amino acid residue [94–100]. Although the predictive value of this formula has been questioned [95–97], the presence of this motif in many proteins and its participation in the protein–cholesterol interactions has been confirmed by different methods [16, 17, 93–95]. The formula of the CRAC motif can be further developed [94]; important is the very concept of a motif mediating the interactions of cholesterol-dependent proteins with cholesterol.

CRAC motifs are found in many viral proteins, and their role in cholesterol-dependent virus–cell interactions have been demonstrated. For example, CRAC motifs are present in the HIV matrix protein p17, which was shown to participate in virus entry through the raft domains of the cell membranes [27, 101]. The α-helical domain of the hepatitis C virus nonstructural protein NS5A, which is anchored at the cytoplasmic leaflet of the endoplasmic reticulum and is involved in replication hepatitis C virus, also contains CRAC motif [102]. Importantly, peptides derived from this domain were shown to exhibit a broad-spectrum anti-viral activity. Cheng et al. 2008 [103] reported that peptide C5A containing amino acid residues 3–20 of the amphipathic α-helical N-terminal domain of hepatitis A virus protein NS5A suppressed the virus replication by more than 5 orders of magnitude. The authors did not mention the CRAC concept; however, the active peptide C5A (SWLRDIWDWICEVLSDFK) clearly contains the CRAC motif: RDIWDWICEV. Later, the antiviral activity of this peptide C5A against HIV was also demonstrated [104]. It is possible that peptide C5A, owing to the presence of the CRAC motif, binds cholesterol and competes for cholesterol binding with viral protein and thus inhibits the formation of the viral particle.

CRAC motifs are found in alpha-helices of matrix protein M1 of influenza A virus [105–107]. An important role of these CRAC motifs in the organization of the raft structure of the virion membrane was substantiated by using the method of directed point mutations in the CRAC-containing α-helices in the M1 protein [106, 107]. Further studies revealed that M1-derived peptides containing CRAC motifs LEVLMEWLKTR, NNMDKAVKLYRKLK, GLKNDLLENLQAYQKR, corresponding to α-helixes 3 (aa 39–49), 6 (aa 91–105) and 13 (aa 228–243), respectively, to a different extent modulate cholesterol-dependent interactions of cultured macrophages with 2-μm particles that mimic bacteria (phagocytic index). Of the three peptides, NNMDKAVKLYRKLK was most potent and stimulated the cell activity by 50–60% at 35 μM [108]. Peptide RTKLWEMLVELGNMDKAVKLWRKLKR obtained by combining two of these short peptides and containing two CRAC motifs produced much stronger and more complex effect: in a narrow range of low concentrations (1–5 μM) the peptide exerted a stimulatory effect and at 50 μM the peptide was cytotoxic [109]. Reducing the cholesterol content in the cells with methyl-β-cyclodextrin (mβCD) abolished the stimulatory component and lowered the peptide concentration required for the toxic effect. Substitution of the motif-forming amino acids abolished these effects [110]. The cytotoxic effect of the M1-derived peptide RTKLWEMLVELGNMDKAVKLWRKLKR can be explained by the binding (sequestration) of membrane cholesterol by the peptide; this can imitate removal of cholesterol from cell membranes, which occurs during the formation of the viral envelope.

S proteins of coronaviruses SARS-CoV and SARS-CoV2 also contain CRAC motifs [51, 109, 110]. For example, in the case of the S protein of coronavirus SARS-CoV, the CRAC motif YIKWPWYVW is located in the "aromatic" region of the transmembrane domain of the S-protein; this highly conserved region of the S-protein was shown to be necessary for the infection of cells with coronavirus [111, 112].

If the assumption about the essential role of sequestration and removal of membrane cholesterol by viral CRAC-containing proteins in the COVID-19 parthenogenesis is correct, then in order to prevent this destructive action for the cell, it is necessary to maintain a safe level of cholesterol in the plasma membrane. A significant decrease in total cholesterol and low-density lipoprotein (LDL) cholesterol levels in COVID-19 patients [13, 88] may be indicative of the critical loss of cholesterol by cells, and an efficient cholesterol delivery to the cholesterol-depleted cells may be helpful. Cyclodextrins are possible candidates as non-toxic

cholesterol transporters [113–116], which can redistribute cholesterol from endogenous and/or exogenous sources. The use of cyclodextrins increased the lifespan of NPC1$^{-/-}$ experimental mice [117] and improved the condition of patients with Nieman–Pick disease [118]. Another alternative is to prevent viral proteins from interacting with membrane cholesterol. At least some of the drugs that are currently tested – for example, polyphenolic substances like quercetin and saponin glycyrrhizin [119, 120] – can act at the protein/cholesterol interface and hinder cholesterol binding by the CRAC motif of the viral S protein and thus inhibit the assembly of new viral particles. Glycyrrhizin, an active component of liquorice roots, was shown to inhibit SARS-CV replication in Vero cells and replication of SARS-associated coronavirus [121, 122]. However, such agents are not very selective and can affect other cholesterol-dependent proteins and therefore cause side effects. Perhaps specially designed CRAC-peptides specifically blocking the interactions of S-protein with cholesterol will prevent the cellular cholesterol loss leading to permeabilization of membranes, oxidative stress, and cell death. The ability of CRAC-containing peptides to regulate cholesterol-dependent cell functions has been demonstrated in a number of works [17, 109, 122, 123], and studies of the antiviral activity of these peptides may be useful and promising.

5. Conclusions

The SARS-CoV2 pandemic has sparked a brainstorming session over the underlying mechanisms of viral diseases. Many assumptions have been made. This chapter considers possible consequences of cholesterol depletion in the membranes of infected cells due to the formation of cholesterol-rich viral envelopes. At a high viral load and high replication rate the reduction in the cholesterol level in the cell membranes can lead to their permeabilization and subsequent cell death, and this can be one of the factors in pathogenesis of diseases induced by SARS-CoV2. Cholesterol-recognition/interaction (CRAC) motifs in viral proteins may represent a mechanism for the binding of the viral protein with cholesterol. Substances preventing these interactions of viral proteins with cholesterol can suppress the formation of the viral envelope and therefore can be studied as possible antiviral drugs. Peptides containing CRAC motifs from viral proteins may be among these substances.

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Conflict of interest

The author declares that there is no conflict of interest.

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References

[1] Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273 DOI: 10.1038/s41586-020-2012-7

[2] WHO Coronavirus Disease (COVID-19) Dashboard, Data last updated: 2021/1/7. Available from: https://covid19.who.int/ Accessed: 2021-01-07]

[3] Guan WJ, Liang WH, Zhao Y, et al. Comorbidity and its impact on 1590 patients with COVID-19 in China: A nationwide analysis. Eur Respir J. 2020;55:2000547. DOI: 10.1183/13993003.00547-2020

[4] Vinciguerra M, Romiti S, Fattouch K, De Bellis A, Greco E. Atherosclerosis as pathogenetic substrate for SARS-Cov2 cytokine storm. J Clin Med. 2020;9(7):2095. DOI:10.3390/jcm9072095

[5] Dodek P. Diabetes and other comorbidities were associated with a poor outcome in the severe acute respiratory syndrome. ACP J Club. 2004;140(1):19. DOI: 10.1001/jama.289.21.JOC30885

[6] Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. Nat Rev Immunol. 2020;20:442-447. DOI: 10.1038/s41577-020-0348-8

[7] Dudley JP, Lee NT. Disparities in age-specific morbidity and mortality from SARS-CoV-2 in China and the Republic of Korea. Clinical Infectious Diseases. 2020; 71(15):863-865. DOI: 10.1093/cid/ciaa354

[8] Potluri T, Fink AL, Sylvia KE, Dhakal S, Vermillion MS, Vom Steeg L, Deshpande S, Narasimhan H, Klein SL. Age-associated changes in the impact of sex steroids on influenza vaccine responses in males and females. NPJ Vaccines. 2019;4:29. DOI: 10.1038/s41541-019-0124-6. Erratum in: NPJ Vaccines. 2019;4:35.

[9] Huang C, Wang Y, Li X, Ren L, Zhao J., Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497-506. DOI: 10.1016/S0140-6736(20)30183-54

[10] Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B., Zhu N., et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet. 2020;395:565-574. DOI: 10.1016/S0140-6736(20)30251-8

[11] Wu SC. Progress and concept for COVID-19 vaccine development. Biotechnol J. 2020;15(6):e2000147. DOI:10.1002/biot.202000147

[12] Ledford H. Moderna COVID vaccine becomes second to get US authorization. Two RNA vaccines will be useful as US infections surge, but the speedy authorizations complicate clinical trials. Nature NEWS. 2020. 18 December 2020. Update 19 December 2020. Available from: https://doi.org/10.1038/d41586-020-03593-7 [Accessed: 2021-01-07]

[13] Radenkovic D, Chawla S, Pirro M, Sahebkar A, Banach M. Cholesterol in relation to COVID-19: Should we care about it? J Clin Med. 2020;9:1909. DOI: 10.3390/jcm9061909

[14] Kočar E, Režen T, Rozman D. Cholesterol, lipoproteins, and COVID-19: Basic concepts and clinical applications. Biochim Biophys Acta Mol Cell Biol Lipids. 2020;1866(2):158849. DOI: 10.1016/j.bbalip.2020.158849

[15] Abu-Farha M, Thanaraj TA, Qaddoumi MG, Hashem A, Abubaker J, Al-Mulla ad Fahd.The role of lipid metabolism in COVID-19 virus infection and as a drug target. Int J Mol Sci. 2020;21:3544. DOI:10.3390/ijms21103544 www.mdpi

[16] Li H, Papadopoulos V. Peripheral–type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern. Endocrinology. 1998;139:4991-4997. DOI: 10.1016/s0039-128x(96)00154-7

[17] Li H, Yao Z, Degenhardt B, Teper, G, Papadopoulos V. Cholesterol binding at the cholesterol recognition/interaction amino acid consensus (CRAC) of the peripheral–type benzodiazepine receptor and inhibition of steroidogenesis by an HIV TAT–CRAC peptide. Proc Natl Acad Sci USA. 2001;98:1267-1272. DOI: 10.1073/pnas.031461598

[18] Jones JE, Le Sage V., Lakdawala SS. Viral and host heterogeneity and their effects on the viral life cycle. Nat Rev Microbiol. 2020;Oct 6:1-11. DOI: 10.1038/s41579-020-00449-9

[19] V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: Implications for SARS-CoV-2. Nat Rev Microbiol. 2020;28:1-16. DOI: 10.1038/s41579-020-00468-6

[20] de Haan CAM, Rottier PJM. Molecular interactions in the assembly of coronaviruses. Adv Virus Res. 2005;64:165-230. DOI: 10.1016/S0065-3527(05)64006-7

[21] Chazal N, Gerlier D. Virus entry, assembly, budding, and membrane rafts. Microbiol Mol Biol Rev. 2003;67:226-237. DOI: 10.1128/mmbr.67.2.226-237.2003

[22] Nayak DP, Hui EK. The role of lipid microdomains in virus biology. Subcell Biochem. 2004;37:443-491. DOI: 10.1007/978-1-4757-5806-1_14

[23] Rawat SS, Viard M, Gallo SA, Rein A, Blumenthal R, Puri A. Modulation of entry of enveloped viruses by cholesterol and sphingolipids (Review). Mol Membr Biol. 2003;20(3):243-254. DOI: 10.1080/0968768031000104944

[24] Sezgin E, Levental I, Mayor S, Eggeling C. The mystery of membrane organization: Composition, regulation and roles of lipid rafts. Nat Rev Mol Cell Biol. 2017;18:361-374. DOI: 10.1038/nrm.2017.16

[25] Brown DA, London E. Structure and origin of ordered lipid domains in biological membranes. J Membr Biol. 1998;164:103-114. DOI: 10.1007/s002329900397

[26] Harder T, Scheiffele P, Verkade P, Simons K. Lipid domain structure of the plasma membrane revealed by patching of membrane components. J Cell Biol. 1998;141:929-942. DOI: 10.1083/jcb.141.4.929

[27] Carter GC, Bernstone L, Sangani D, Bee JW, Harder T, James W. HIV entry in macrophages is dependent on intact lipid rafts. Virology. 2009;386(1):192-202. DOI: 10.1016/j.virol.2008.12.031

[28] Campbell SM, Crowe SM, Mak J. Virion-associated cholesterol is critical for the maintenance of HIV-1 structure and infectivity. AIDS. 2002;16:2253-2261. DOI: 10.1097/00002030-200211220-00004

[29] Graham DR, Chertova E, Hilburn JM, Arthur LO, Hildreth JE. Cholesterol depletion of human immunodeficiency virus type 1 and simian immunodeficiency virus with b-cyclodextrin inactivates and permeabilizes the virions: evidence for virion-associated lipid rafts. J Virol. 2003;77:8237-8248. DOI: 10.1128/jvi.77.15.8237-8248.2003

[30] Sviridov D, Mukhamedova N, Makarov AA, Adzhubei A, Bukrinsky M. Comorbidities of HIV infection: Role of Nef-induced impairment of cholesterol metabolism and lipid raft functionality. AIDS. 2020;34:1-13. DOI: 10.1097/QAD.0000000000002385

[31] Sung-Tae Y, Kiessling V, Simmons JA, et al. HIV gp41-mediated membrane fusion occurs at edges of cholesterol-rich lipid domains. Nat Chem Biol. 2015;11:424-431. DOI: 10.1038/nchembio.1800

[32] Keller P, Simons K. Cholesterol is required for surface transport of influenza virus hemagglutinin. J Cell Biol. 1998;140(6):1357-1367. DOI: 10.1083/jcb.140.6.1357

[33] Gerl MJ, Sampaio JL, Urban S, Kalvodova L, Verbavatz JM, Binnington B, Lindemann D, Lingwood CA, Shevchenko A, Schroeder C, Simons K. Quantitative analysis of the lipidomes of the influenza virus envelope and MDCK cell apical membrane. J Cell Biol. 2012;196(2):213-221. DOI: 10.1083/jcb.201108175

[34] Nayak DP, Hui EK-W, Barman S. Assembly and budding of influenza virus. Virus Res. 2004;106:147-165. DOI: 10.1016/j.virusres.2004.08.012

[35] Zhang J, Pekosz A, Lamb RA. Influenza virus assembly and lipid raft microdomains: A role for the cytoplasmic tails of the spike glycoproteins. J Virol. 2000;74:4634-4644. DOI: 10.1128/jvi.74.10.4634-4644.2000

[36] Veit M, Thaa B. Association of influenza virus proteins with membrane rafts. Adv Virol. 2011;2011:Article ID 370606. DOI: 10.1155/2011/370606

[37] Ali A, Avalos RT, Ponimaskin E, Nayak DP. Influenza virus assembly: Effect of influenza virus glycoproteins on the membrane association of M1 protein. J Virol. 2000;74:8709-8719. DOI: 10.1128/jvi.74.18.8709-8719.2000

[38] Bender FC, Whitbeck JC, Ponce de Leon M, Lou H, Eisenberg RJ, Cohen GH. Specific association of glycoprotein B with lipid rafts during herpes simplex virus entry. J Virol. 2003;77:9542-9552. DOI: 10.1128/jvi.77.17.9542-9552.2003

[39] Laliberte JP, McGinnes LW, Morrison TG, Incorporation of functional HN-F glycoprotein-containing complexes into Newcastle disease virus is dependent on cholesterol and membrane lipid integrity. J Virol. 2007;81:10636-10648. DOI: 10.1128/JVI.01119-07

[40] Dou X, Li Y, Han J, Zarlenga DS, Zhu W, Ren X, Dong N, Li X, Li G. Cholesterol of lipid rafts is a key determinant for entry and post-entry control of porcine rotavirus infection. BMC Vet Res. 2018;14(1):45. DOI: 10.1186/s12917-018-1366-7

[41] Stoeck IK, Lee J-Y, Tabata K, Romero-Brey I, Paul D, Schult P, Lohmann V, Kaderali L, Bartenschlager R. Hepatitis C virus replication depends on endosomal cholesterol homeostasis. J Virol. 2018;92:e01196-17. DOI: 10.1128/JVI.01196-17

[42] Paul D, Hoppe S, Saher G, Krijnse-Locker J, Bartenschlager R. Morphological and biochemical characterization of the membranous hepatitis C virus replication compartment. J Virol. 2013;87(19):10612-10627. DOI:10.1128/JVI.01370-13

[43] Elgner F, Ren H, Medvedev R, Ploen D, Himmelsbach K, Boller K, Hildt E. The intracellular cholesterol transport inhibitor U18666A inhibits the exosome-dependent release of mature hepatitis C virus. J Virol. 2016;90:11181-11196. DOI: 10.1128/JVI.01053-16

[44] Leier HC, Messer WB, Tafesse FG. Lipids and pathogenic flaviviruses: An intimate union. PloS Pathogens. 2018;14(5):e1006952. DOI: 10.1371/journal.ppat. 1006952

[45] Osuna-Ramos JF, Reyes-Ruiz JM, Del Angel RM. The role of host cholesterol during flavivirus infection. Front Cell Infect Microbiol. 2018;8:388. DOI: 10.3389/fcimb.2018.00388

[46] Cervin M, Anderson R. Modulation of coronavirus-mediated cell fusion by homeostatic control of cholesterol and fatty acid metabolism. J Med Virol. 1991;35:142-149. DOI: 10.1002/jmv.1890350213

[47] Thorp EB, Gallagher TM. Requirements for CEACAMs and cholesterol during murine coronavirus cell entry. J Virol. 2004;78(6):2682-2692. DOI: 10.1128/jvi.78.6.2682-2692.2004

[48] Choi KS., Aizaki H, Lai MMC. Murine coronavirus requires lipid rafts for virus entry and cell-cell fusion but not for virus release. J Virol. 2005;79(15):9862-9871. DOI: 10.1128/JVI.79.15.9862-9871.2005

[49] Pratelli A, Colao V. Role of the lipid rafts in the life cycle of canine coronavirus. J Gen Virol. 2015;96(Pt 2):331-337. DOI: 10.1099/vir.0.070870-0

[50] Li G-M, Li Y-G, Yamate M, Li S-M, Ikuta K. Lipid rafts play an important role in the early stage of severe acute respiratory syndrome-coronavirus life cycle. Microbes and Infection. 2007;9:96-102. DOI: 10.1016/j.micinf.2006.10.015

[51] Glende J, Schwegmann-Wessels C, Al-Falah M, Pfefferle S, Qu X, Deng H, Drosten C, Naim HY, Herrler G. Importance of cholesterol-rich membrane microdomains in the interaction of the S protein of SARS-coronavirus with the cellular receptor angiotensin-converting enzyme 2. Virology. 2008;381(2):215-221. DOI: 10.1016/j.virol.2008.08.026

[52] Lee W, Ahn JH, Park HH, Kim HN, Kim H, Yoo Y, Shin H, Hong KS, Jang JG, Park CG, Choi EY, Bae J, Seo Y. COVID-19-activated SREBP2 disturbs cholesterol biosynthesis and leads to cytokine storm. Signal Transduct Target Ther. 2020;5. DOI: 10.1038/s41392-020-00292-7.

[53] Raulin J. Human immunodeficiency virus and host cell lipids. Interesting pathways in research for a new HIV therapy. Prog Lipid Res. 2002;41(1):27-65. DOI: 10.1016/s0163-7827(01)00019-4

[54] Wang S, Li W, Hui H, Tiwari SK, Zhang Q, Croker BA, Rawlings S, Smith D, Carlin AF, Rana TM. Cholesterol 25-Hydroxylase inhibits SARS-CoV-2 and other coronaviruses by depleting membrane cholesterol. EMBO J. 2020;39(21):e106057. DOI: 10.15252/embj.2020106057

[55] Veterinary Virology. Academic Press, 1987. Chapter 1, Structure and Composition of Viruses. DOI: https://doi.org/10.1016/C2013-0-10647-2

[56] Nguyen DH, Hildreth JE. Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. J Virol. 2000;74(7):3264-3272. DOI: 10.1128/jvi.74.7.3264-3272.2000

[57] Chazal N, Gerlier D. Virus entry, assembly, budding, and membrane rafts. Microbiol Mol Biol Rev. 2003;67(2):226-237. DOI: 10.1128/mmbr.67.2.226-237.2003

[58] Wilflingseder D, Stoiber H. Float on: Lipid rafts in the lifecycle of HIV. Front Biosci. 2007;12:2124-2135. DOI: 10.2741/2216.

[59] Navaratnarajah CK, Warrier R, Kuhn RJ. Assembly of Viruses:

Enveloped Particles. Encyclopedia of Virology. 2008;193-200. DOI: 10.1016/B978-012374410-4.00667-1

[60] Miller S, Krijnse-Locker J. Modification of intracellular membrane structures for virus replication. Nat Rev Microbiol. 2008;6:363-374. DOI: 10.1038/nrmicro1890

[61] Lorizate M, Kräusslich HG. Role of lipids in virus replication. Cold Spring Harb Perspect Biol. 2011;3(10):a004820. DOI:10.1101/cshperspect.a004820.

[62] Welsch S, Müller B, Kräusslich HG. More than one door – Budding of enveloped viruses through cellular membranes. FEBS Lett. 2007;581(11):2089-2097. DOI: 10.1016/j.febslet.2007.03.060

[63] Pagliari F, Marafioti MG, Genard G, Candeloro P, Viglietto G, Seco J, Tirinato L. ssRNA virus and host lipid rearrangements: Is there a role for lipid droplets in SARS-CoV-2 infection? Front Mol Biosci. 2020;7:578964. DOI: 10.3389/fmolb.2020.578964

[64] Sung-Tae Y, Kiessling V, Simmons JA, et al. HIV gp41-mediated membrane fusion occurs at edges of cholesterol-rich lipid domains. Nat Chem Biol. 2015;11:424-431. DOI: 10.1038/nchembio.1800

[65] Zhang Z, He G, Filipowicz NA, Randall G, Belov GA, Kopek BG, Wang X. Host lipids in positive-strand RNA virus genome replication. Front Microbiol. 2019;10:286. DOI: 10.3389/fmicb.2019.00286

[66] Bender SJ, Phillips JM, Scott EP, Weiss SR. Murine coronavirus receptors are differentially expressed in the central nervous system and play virus strain-dependent roles in neuronal spread. J Virology. 2010;84(21):11030-11044; DOI: 10.1128/JVI.02688-09

[67] Lu YE, Cassese T, Kielian M. The cholesterol requirement for Sindbis virus entry and exit and characterization of a spike protein region involved in cholesterol dependence. J Virology. 1999;73(5):4272-4278. DOI: 10.1128/JVI.73.5.4272-4278.1999

[68] DeTulleo L, Kirchhausen T. The clathrin endocytic pathway in viral infection. EMBO J. 1998;17(16):4585-4593. DOI:10.1093/emboj/17.16.4585

[69] Callens N, Brügger B, Bonnafous P, Drobecq H, Gerl MJ, Krey T, et al. Morphology and molecular composition of purified bovine viral diarrhea virus envelope. PLoS Pathog. 2016;12(3):e1005476. DOI: 10.1371/journal.ppat.1005476

[70] Laliberte JP, McGinnes LW, Morrison TG. Incorporation of functional HN-F glycoprotein-containing complexes into Newcastle disease virus is dependent on cholesterol and membrane lipid integrity. J Virol. 2007;81:10636-10648. DOI: 10.1128/JVI.01119-07

[71] Wattenberg BW, Silbert DF. Sterol partitioning among intracellular membranes. Testing a model for cellular sterol distribution. J Biol Chem. 1983;258(4):2284-2289.

[72] Kaplan MR, Simoni RD. Transport of cholesterol from the endoplasmic reticulum to the plasma membrane. J Cell Biol. 1985;101(2):446-453. DOI: 10.1083/jcb.101.2.446

[73] Lange Y, Swaisgood MH, Ramos BV, Steck TL. Plasma membranes contain half the phospholipid and 90% of the cholesterol and sphingomyelin in cultured human fibroblasts. J Biol Chem. 1989;264(7):3786-3793.

[74] Litvinov DY, Savushkin EV, & Dergunov AD. Intracellular and plasma membrane events in

cholesterol transport and homeostasis. J Lipids. 2018;2018:3965054. DOI: 10.1155/2018/3965054

[75] van't Wout AB, Swain JV, Schindler M, Rao U, Pathmajeyan MS, Mullins JI, Kirchhoff F. Nef induces multiple genes involved in cholesterol synthesis and uptake in human immunodeficiency virus type 1-infected T cells. J Virology. 2005;79(15):10053-10058. DOI: 10.1128/JVI.79.15.10053-10058.2005

[76] Jennelle L, Hunegnaw R, Dubrovsky L, Pushkarsky T, Fitzgerald ML, Sviridov D, Popratiloff A, Brichacek B, Bukrinsky M. HIV-1 protein Nef inhibits activity of ATP-binding cassette transporter A1 by targeting endoplasmic reticulum chaperone calnexin. J Biol Chem. 2014;289(42):28870-28884. DOI: 10.1074/jbc.M114.583591

[77] Stertz S, Reichelt M, Spiegel M, Kuri T, Martínez-Sobrido L, García-Sastre A, Weber F, Kochs G. The intracellular sites of early replication and budding of SARS-coronavirus. Virology. 2007;361:304-315. DOI: 10.1016/j.virol.2006.11.027

[78] Wang S, Li W, Hui H, Tiwari SK, Zhang Q, Croker BA, Rawlings S, Smith D, Carlin AF, Rana TM. Cholesterol 25-Hydroxylase inhibits SARS-CoV-2 and other coronaviruses by depleting membrane cholesterol. EMBO J. 2020;39(21):e106057. DOI: 10.15252/embj.2020106057

[79] Olsen BN, Schlesinger PH, Ory DS, Baker NA. 25-Hydroxycholesterol increases the availability of cholesterol in phospholipid membranes. Biophys J. 2011;100(4):948-956. DOI: 10.1016/j.bpj.2010.12.3728

[80] Ikonen E. Cellular cholesterol trafficking and compartmentalization. Nat. Rev. Mol. Cell Biol. 2008;9(2):125-138. DOI: 10.1038/nrm2336

[81] Johansson AC, Appelqvist H, Nilsson C, Kågedal K, Roberg K, Ollinger K. Regulation of apoptosis-associated lysosomal membrane permeabilization. Apoptosis. 2010;15(5):527-540. DOI: 10.1007/s10495-009-0452-5

[82] Khatibzadeh N, Spector AA, Brownell WE, Anvari B. Effects of plasma membrane cholesterol level and cytoskeleton F-actin on cell protrusion mechanics. PLoS ONE. 2013;8(2): e57147. DOI: 10.1371/journal.pone.0057147

[83] Biswas A, Kashyap P, Datta S, Sengupta T, Sinha B. Cholesterol depletion by mβCD enhances cell membrane tension and its variations-reducing integrity. Biophys J. 2019;116(8):1456-1468. DOI: 10.1016/j.bpj.2019.03.016

[84] Appelqvist H, Nilsson C, Garner B, Brown AJ, Kågedal K, Ollinger K. Attenuation of the lysosomal death pathway by lysosomal cholesterol accumulation. Am J Pathol. 2011;178(2):629-639. DOI: 10.1016/j.ajpath.2010.10.030

[85] Zhang L, Kim SB, Luitel K, Shay JW. Cholesterol depletion by TASIN-1 induces apoptotic cell death through the ER stress/ROS/JNK signaling in colon cancer cells. Mol Cancer Ther. 2018;17(5):943-951. DOI: 10.1158/1535-7163

[86] Hsu SPC, Kuo JS, Chiang HC, Wang HE, Wang YS, Huang CC, Huang YC, Chi MS, Mehta MP, Chi KH. Temozolomide, sirolimus and chloroquine is a new therapeutic combination that synergizes to disrupt lysosomal function and cholesterol homeostasis in GBM cells. Oncotarget. 2018;9(6):6883-6896. DOI: 10.18632/oncotarget.23855

[87] King MA, Ganley IG, Flemington V. Inhibition of cholesterol metabolism underlies synergy between mTOR

pathway inhibition and chloroquine in bladder cancer cells. Oncogene. 2016;35(34):4518-4528. DOI: 10.1038/onc.2015.511

[88] Hu X, Chen D, Wu L, He G, Ye W. Declined serum high density lipoprotein cholesterol is associated with the severity of COVID-19 infection. Clin Chim Acta. 2020;510:105-110. DOI: 10.1016/j.cca.2020.07.015

[89] Coronavirus breakthrough: Dexamethasone is first drug shown to save lives. Nature. 2020;582:469. DOI: 10.1038/d41586-020-01824-5

[90] RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, et al. Effect of dexamethasone in hospitalized patients with COVID-19. Preliminary report. N Engl J Med. 2020 Jul 17:NEJMoa2021436. DOI: 10.1056/NEJMoa2021436

[91] Gozzo L, Longo L, Vitale DC, Drago F. Dexamethasone treatment for Covid-19, a curious precedent highlighting a regulatory gap. Frontiers in Pharmacol. 2020;11:621934. DOI: https://doi.org/10.3389/fphar.2020.621934

[92] Weir EK, Thenappan T, Bhargava M, Chen Y. Does vitamin D deficiency increase the severity of COVID-19? Clin Med (Lond). 2020;20(4):e107-e108. DOI: 10.7861/clinmed.2020-0301

[93] Jamin N, Neumann JM, Ostuni MA, Vu TK, Yao ZX, Murail S, Robert JC, Giatzakis C, Papadopoulos V, Lacapère JJ. Characterization of the cholesterol recognition amino acid consensus sequence of the peripheral-type benzodiazepine receptor. Mol Endocrinol. 2005;19(3):588-594. DOI: 10.1210/me.2004-0308

[94] Listowski MA, Leluk J, Kraszewski S, Sikorski AF. Cholesterol interaction with the MAGUK protein family member, MPP1, via CRAC and CRAC-like motifs: An in silico docking analysis. PLoS One. 2015;10(7):e0133141. DOI:10.1371/journal. Pone.0133141

[95] Fantini J, Epand RM, Barrantes FJ. Cholesterol-recognition motifs in membrane proteins. In: Direct mechanisms in cholesterol modulation of protein function. Eds Rosenhouse-Dantsker A., Bukiya A.N. Series Advances in experimental medicine and biology, 2019. vol. 1135. Cham: Springer, p. 3-25. DOI: 10.1007/978-3-030-14265-0_1

[96] Epand RM. Cholesterol and the interaction of proteins with membrane domains. Prog Lipid Res. 2006;45:279-294. DOI: 10.1016/j.plipres.2006.02.001

[97] Palmer M. Cholesterol and the activity of bacterial toxins. FEMS Microbiol Lett. 2004;238:281-289. DOI: 10.1016/j.femsle.2004.07.059

[98] Epand RF, Thomas A., Brasseur R., Vishwanathan SA, Hunter E, Epand RM. Juxtamembrane protein segments that contribute to recruitment of cholesterol into domains. Biochemistry. 2006;45:6105-6114. DOI: 10.1021/bi060245+

[99] Miller CM, Brown AC, Mittal J. Disorder in cholesterol-binding functionality of CRAC peptides: A molecular dynamics study. J Phys Chem B. 2014;118:13169-13174. DOI: 10.1021/jp5106423

[100] Song Y, Kenworthy AK, Sanders CR. Cholesterol as a co-solvent and a ligand for membrane proteins. Protein Science. 2014;23:1-22. DOI: 10.1002/pro.2385

[101] He W, Mazzuca P, Yuan W, Varney K, Bugatti A, Cagnotto A, Giagulli C, Rusnati M, Marsico S, Diomede L, Salmona M, Caruso A, Lu W, Caccuri F. Identification of amino acid residues critical for the B cell

growth-promoting activity of HIV-1 matrix protein p17 variants. Biochim. Biophys Acta Gen Subj. 2019;1863(1):13-24. DOI: 10.1016/j.bbagen.2018.09.016

[102] Hanson JM, Gettel DL, Tabaei SR, Jackman J, Kim MC, Sasaki DY, Groves JT, Liedberg B, Cho N-J, Parikh AN. Cholesterol-enriched domain formation induced by viral-encoded, membrane-active amphipathic peptide. Biophys J. 2016;110:176-187. DOI: 10.1016/j.bpj.2015.11.032

[103] Cheng G, Montero A, Gastaminza P, Whitten-Bauer C, Wieland SF, Isogawa M, Fredericksen B, Selvarajah S, Gallay PA, Ghadiri MR, Chisari FV. A virocidal amphipathic α-helical peptide that inhibits hepatitis C virus infection in vitro. Proc Natl Acad Sci USA. 2008;105:3088-3093. DOI: 10.1073/pnas.0712380105

[104] Gallay PA, Chatterji U, Kirchhoff A, Gandarilla A, Pyles RB, Baum MM, Moss JA. Protection efficacy of C5A against vaginal and rectal HIV challenges in humanized mice. Open Virol J. 2018;12:1-13. DOI: 10.2174/1874357901812010001

[105] Schroeder C. Cholesterol-binding viral proteins in virus entry and morphogenesis. Subcell Biochem. 2010;51:77-108. DOI: 10.1007/978-90-481-8622-8_3

[106] Tsfasman T, Kost V, Markushin S, Lotte V, Koptiaeva I, Bogacheva E, Baratova L, Radyukhin V. Amphipathic alpha–helices and putative cholesterol binding domains of the influenza virus matrix M1 protein are crucial for virion structure organization. Virus Res. 2015;210:114-118. DOI: 10.1016/j.virusres.2015.07.017

[107] Radyukhin VA, Dadinova LA, Orlov IA, Baratova LA. Amphipathic secondary structure elements and putative cholesterol recognizing amino acid consensus (CRAC) motifs as governing factors of highly specific matrix protein interactions with raft–type membranes in enveloped viruses. J Biomol Str Dynam. 2018;36:1351-1359. DOI: 10.1080/07391102.2017.1323012

[108] Dunina-Barkovskaya AY, Vishnyakova KS, Golovko AO, Arutyunyan AM, Baratova LA, Batishchev OV, Radyukhin VA. Amphipathic CRAC-containing peptides derived from the influenza virus A M1 protein modulate cholesterol-dependent activity of cultured IC-21 macrophages. Biochemistry (Moscow). 2018;83(8):982-991. DOI: https://doi.org/10.1134/S0006297918080096

[109] Dunina-Barkovskaya AY, Vishnyakova KS, Baratova LA, Radyukhin VA. Modulation of cholesterol-dependent activity of macrophages IC-21 by a peptide containing two CRAC-motifs from protein M1 of influenza virus. Biochemistry (Moscow) Suppl Series A Membr Cell Biol. 2019;13(3): 268-276. DOI: https://doi.org/10.1134/S1990747819030139

[110] Dunina-Barkovskaya AY, Vishnyakova KS. Modulation of the cholesterol-dependent activity of macrophages IC-21 by CRAC peptides with substituted motif-forming amino acids. Biochemistry (Moscow) Suppl Series A Membr Cell Biol. 2020;14(4):331-343. DOI: https://doi.org/10.1134/S1990747820040054

[111] Corver J, Broer R, van Kasteren P, Spaan W. Mutagenesis of the transmembrane domain of the SARS coronavirus spike glycoprotein: Refinement of the requirements for SARS coronavirus cell entry. Virol J. 2009;6:230. DOI: 10.1186/1743-422X- 6-230

[112] Liu Z, Xiao X, Wei X, et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential

intermediate hosts of SARS-CoV-2. J Med Virol. 2020;1-7. DOI: 10.1002/jmv.25726

[113] Baglivo M, Baronio M, Natalini G, Beccari T, Chiurazzi P, Fulcheri E, Petralia PP, Michelini S, Fiorentini G, Miggiano GA, Morresi A, Tonini G, Bertelli M. Natural small molecules as inhibitors of coronavirus lipid-dependent attachment to host cells: a possible strategy for reducing SARS-COV-2 infectivity? Acta Biomed. 2020;91(1):161-164. DOI: 10.23750/abm.v91i1.9402.

[114] Zidovetzki R, Levitan I. Use of cyclodextrins to manipulate plasma membrane cholesterol content: Evidence, misconceptions and control strategies. Biochim Biophys Acta. 2007;1768:1311-1324. DOI: 10.1016/j.bbamem.2007.03.026

[115] Kurkov SV, Loftsson T. Cyclodextrins. Int J Pharm. 2013;453(1):167-80. DOI: 10.1016/j.ijpharm.2012.06.055

[116] Leclercq L. 2016. Interactions between cyclodextrins and cellular components: Towards greener medical applications? Beilstein J Org Chem. 12, 2644-2662.DOI: 10.3762/bjoc.12.261

[117] Taylor A M, Liu B, Mari Y, Liu B, Repa JJ. Cyclodextrin mediates rapid changes in lipid balance in Npc1−/− mice without carrying cholesterol through the bloodstream. J Lipid Res. 2012;53(11):2331-2342. DOI: 10.1194/jlr.M028241

[118] Matsuo M, Togawa M, Hirabaru K, Mochinaga S, Narita A, Adachi M, Egashira M, Irie T, Ohno K. Effects of cyclodextrin in two patients with Niemann-Pick Type C disease. Mol Genet Metab. 2013;108(1):76-81. DOI: 10.1016/j.ymgme.2012.11.005.

[119] Glinsky GV. Tripartite combination of candidate pandemic mitigation agents: Vitamin D, quercetin, and estradiol manifest properties of medicinal agents for targeted mitigation of the COVID-19 pandemic defined by genomics-guided tracing of SARS-CoV-2 targets in human cells. Biomedicines. 2020;8(5):129. DOI: 10.3390/biomedicines8050129

[120] Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet. 2003;361:2045-2046. DOI: 10.1016/s0140-6736(03)13615-x

[121] Bailly C, Vergoten G. Glycyrrhizin: An alternative drug for the treatment of COVID-19 infection and the associated respiratory syndrome? Pharmacol Therapeutics. 2020;214:107618. DOI: 10.1016/j.pharmthera.2020.107618

[122] Brown AC, Koufos E, Balashova NV, Boesze-Battaglia K, Lally ET. Inhibition of LtxA toxicity by blocking cholesterol binding with peptides. Molecular Oral Microbiology. 2016;31(1):94-105. DOI: 10.1111/omi.12133

[123] Lecanu L, Yao ZX, McCourty A, Sidahmed el-K, Orellana ME, Burnier MN, Papadopoulos V. Control of hypercholesterolemia and atherosclerosis using the cholesterol recognition/interaction amino acid sequence of the translocator protein TSPO. Steroids. 2013;78(2):137-146. DOI: 10.1016/j.steroids.2012.10.018