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REVIEW ARTICLE

Influenza virus and cholesterol: touch points and potential consequences for the host cell

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ABSTRACT

This review considers the observations concerning the interactions of influenza virus with cholesterol and consequences of these interactions for the host cell. There are at least two crucial "touch points", when influenza virus deals with cholesterol. First, cholesterol is required for influenza virus entry into the cell. Second, during budding, when new viral particles form their envelopes from plasma membrane of the infected cell, selectively acquiring "raft lipids", cholesterol and sphingolipids. This may involve significant losses of lipids and especially cholesterol in the host cell membrane, which may cause dysfunction and death of the infected cell. The interaction of viral proteins with cell membrane cholesterol may be mediated by cholesterol-binding motifs in viral proteins. There is experimental evidence indicating that a peptide derived from influenza virus protein M1 and containing cholesterol-binding motif is indeed cytotoxic, and that lowering the membrane cholesterol concentration considerably reduces the concentration of the peptide´s cytotoxic effect. This may call into question the appropriateness of using cholesterol-lowering agents to reduce influenza virus infectivity and productivity. In the conditions of reduced cholesterol, any infection with enveloped virus sequestering cholesterol from cell membranes may be detrimental, as further lowering cholesterol level in cell membrane during virus budding may lead to cell damage. Perhaps to minimize the virus infectivity and the consequences of the massive virus budding, advantageous can be alternative approaches, such as a search for and design of agents that would selectively interfere with cholesterol-dependent virus–cell interactions and inhibit or prevent them. Understanding the mechanisms and consequences of these interactions should be useful in the developing of new antiviral drugs.

Keywords: influenza virus, protein M1, cholesterol, raft lipids, cell membranes, statins.

Introducution

Influenza epidemics can occur several times a year, causing severe complications and deaths. Vaccination is regarded to be one of the most effective means of preventing influenza. However, the antigenic variability of influenza viruses makes it necessary to periodically review the composition of influenza vaccines. WHO publishes annual recommendations on influenza vaccine formulations and provides training through the OpenWHO influenza prevention and control course (see https://www.who.int/publications/i/item/9789240058224 as well as https://www.cdc.gov/flu/professionals/vaccination/vax-summary.htm)[1].

The need for annual vaccination and changes in vaccine formulations, and increased vulnerability of people before the first vaccination, as well as the potential for severe complications from vaccination in some groups of people necessitates the search for additional ways to prevent the infection and relieve the virus-induced diseases induced by influenza virus, as well as by other enveloped viruses such as SARS-CoV-2. This review proposes to consider how influenza virus interacts with host cell cholesterol and what the consequences of this interaction are for the infected cell.

The influenza virus life cycle includes the following major steps: virus entry into the cell, replication, assembly and exit from the cell by budding. This is also characteristic of many other enveloped viruses[2-5]. The entry step comprises virus binding to a target receptor on a cell followed by fusion of the viral envelope with the host cell membrane and release of the viral nucleic acids into the cytoplasm. At the replication step, the nucleic acid is replicated in cytoplasmic 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.

Some interactions of various enveloped viruses with the cell during penetration, assembly, budding, and exit are known to depend on the presence of cholesterol and lipid rafts in the membranes of the host cells[6-8]. This has been shown for coronaviruses[9-15], immunodeficiency viruses[16-21], herpes[22], Newcastle disease virus[23], rotavirus[24], as well as hepatitis C virus[25–27] and some other viruses of the Flaviviridae family[28,29] (Yellow fever virus, Zika virus, Dengue virus, West Nile virus). The cholesterol dependence of virus–cell interactions has also been demonstrated for influenza virus[2,30–35].

There are at least two crucial “touch points”, when influenza virus deals with cholesterol: it is first required for influenza virus entry into the cell[7,30,36] and then, during budding, when new viral particles form their envelopes from plasma membrane of the infected cell, selectively acquiring “raft lipids”, cholesterol and sphingolipids[2,31,32], similar to other enveloped viruses[37-40]. This review addresses two questions regarding influenza virus-cholesterol interactions: what are the mechanisms of cholesterol recognition and binding by viruses, and what are the consequences of these virus-cholesterol interactions for the host cell. Answers to these questions can be worthwhile for the development of new antiviral drugs that control virus-cholesterol interactions and prevent virus entry and virus-induced host cell damage.

Cholesterol and rafts are required for influenza virus entry

Virus entry is a process resulting in the release of the viral gene material into the cytoplasm of the cell. This process involves virus attachment and recruitment to the cell surface followed by either fusion of viral envelope with plasma membrane or by endocytic uptake of the virus. In the latter case, the internalized viruses are delivered to endosomes or other intracellular organelles, where fusion of viral envelope and endosome membrane occurs and viral material is released into the cytoplasm[3,4,36,41-45]. Influenza virus employs the endocytic entry mechanism[2,36,43,45]. Influenza virus particles, bound to cell surface sialic acid, are internalized by receptor-mediated endocytosis and viruses possessing cleaved hemagglutinin (HA) undergo fusion with the endosomal membrane at low pH (pH ~5.0).

It has been shown earlier in many works that influenza virus requires cholesterol and sphingolipids in the target membrane for fusion pore expansion and that the influenza virus hemagglutinin transmembrane domain associates with membrane microdomains enriched with cholesterol and sphingolipids (rafts), or detergent-resistant membrane domains (DRM)[2,7,8,46-50]. As shown[46], hemagglutinin-expressing insect cells with naturally low levels of cholesterol could fuse with labelled erythrocytes, and cholesterol enrichment of the hemagglutinin-expressing virus-mimicking cells promoted both lipid mixing and fusion pore expansion. The authors hypothesized that cholesterol may promote the formation and growth of contact sites between membranes and then enhance fusion efficiency by promoting fusion pore expansion.

The involvement of cholesterol and rafts at the endocytic stage of the influenza virus entry was also demonstrated in[47]. The authors used ganglioside 1 (GM1)-cholera toxin-based lipid raft staining and biochemical raft isolation and showed that Influenza A viruses select host membrane rafts for polyvalent host binding. By applying methyl-β-cyclodextrin (mβCD) to extract cell cholesterol leading to raft disruption, the authors demonstrated a significant reduction in attachment of viruses in cholesterol-depleted cells. Besides, mβCD inhibited raft-dependent endocytosis, which the authors identified as an additional internalization route for influenza virus.

Cholesterol and rafts are also critically required for the cell entry of as many other viruses[51-56], and cholesterol-rich rafts participate both in virus attachment to the cell surface and in the endocytic and non-endocytic mechanisms of virus entry to the host cells. It was generally agreed that virus binding with a cell and consequent fusion with cell membrane occurs in these membrane microdomains, where cell receptors could concentrate, increase their local density, and enhance interactions of virus with the host cell, promoting productive viral entry[28,30,35,43,47,57]. The issues concerning the importance of the host cell membrane cholesterol and rafts at different stages of the life cycle of influenza and many other enveloped viruses have been addressed in many experimental works and reviews[3,8,15,19,21,29-33,58-65]. An example is coronavirus: Glende et al[14] showed that removal of cholesterol from cell membranes using mβCD reduces the efficiency of infection of cells with the SARS-CoV. The authors also reported that the cellular receptor of the SARS-CoV virus, angiotensin-converting enzyme (ACE2), is colocalized with Flotilin2 and LAMP2, the protein markers of rafts.

Thus, the process of influenza virus entry into the cell, from virus attachment to fusion of viral envelop with host cell membrane and release of the viral content into the cell cytoplasm, depends on cholesterol and rafts. This is true for many other enveloped viruses, and the mechanism of this dependence deserves further study.

Formation of viral envelope, cholesterol recruitment, and what it costs to cells

To leave the cell, the influenza virus employs the budding mechanism, during which viral particles acquire their envelopes from cell plasma membrane. In the process of viral morphogenesis, viral core (ribonucleocapsid, vRNP) and matrix protein M1 are brought to the assembly site, i.e., the cytoplasmic surface of the apical plasma membrane in polarized epithelial cells[2,8,31,66]. Transmembrane viral proteins are also transported to the assembly site on the plasma membrane. Both hemagglutinin and neuraminidase possess apical sorting signals and use lipid rafts for cell surface transport and apical sorting. There the viral envelope surrounding the viral core is formed from the host plasma membrane as a bud, and virus particles released with the closure of buds. Viral lipids, derived from the host plasma membrane, are selectively enriched in "raft" lipids – cholesterol and glycosphingolipids.

Quantitative analysis of the lipidomes of the influenza virus envelope budding from the host Madin–Darby canine kidney (MDCK) cells performed in[31] showed that the lipidome of the viral particle was significantly different from that of the cell apical membrane. The virus membrane exhibited higher levels of sphingolipids and cholesterol compared with the donor membrane at the expense of phosphatidylcholines. The sterol level in the viral particles was about 52 mol%, which exceeded the cholesterol level in the apical membranes of uninfected cells (ca. 45 mol%).

The ability to selectively withdraw raft lipids from host cell membranes is not a unique property of influenza virus but is inherent in many enveloped viruses such as togaviruses, rhabdoviruses, paramyxoviruses, orthomyxoviruses, and retroviruses, including immunodeficiency viruses (HIV)[3,5,16,43,67-70]. For example, HIV-1, like influenza, selectively buds from membrane domains enriched in cholesterol and sphingolipids (rafts), 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 notably higher than in the intracellular membranes[16-20]. This process also involves selective acquisition of raft lipids by viral envelope. Other enveloped viruses, instead of plasma membrane, use endoplasmic reticulum (coronaviruses[9-15] and flaviviruses[28,29]) or/and a Golgi complex (bunyaviruses) or have more complicated budding scenario (e.g., herpes simplex virus[22]), still use host membrane rafts for budding and concentrate cholesterol in the viral envelops. For example, in the 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[54]. Similarly, coronaviruses (SARS-CoV-2) are also assembled and make their cholesterol-rich envelopes in the ER[9-15].

What are possible consequences for the cell of such budding? According to estimates based on in vitro experiments on MDCK cells[71], one virus-infected cell produces on average about

10^4 viral particles (15,000). Although not all of them are infectious, all viral particles have the membrane envelope. Using the particle average diameter 100 nm and assuming a spherical shape, we can calculate the surface area using the formula for the surface area of a sphere (πd^2). This gives a particle surface area of about 3×10^4 nm^2 (31,400 square nanometers), so 10 thousand particles (10^4) will require 3×10^8 nm^2 of the membrane. Surface area of a spherical cell with diameter 20 μm (2×10^4 nm) is about 12×10^8 nm^2; this means that, potentially, 10 thousand viral particles can take away 25% of the cell membrane. And since the virus mainly takes up lipids of a certain type (particularly cholesterol), we can expect that unless the cell can quickly restore this loss, the lipid composition of the cell membrane will be significantly altered, and the cell may be damaged. This is in fact supported by the observations of Frensing et al[71]: Simultaneous to the decrease in the production of infectious particles, the adherent MDCK cells detached from the bottom of the cell culture flask and their viability decreased. Most of the cells were found in the supernatant at 28 h after infection, when the production of total virus particles also declined. It is conceivable that one of the reasons for the decreased production of viral particles is the loss of cholesterol by membranes; the same circumstance may explain the decreased susceptibility to infection of cells that have lost cholesterol.

Thus, the formation of the viral envelopes in many viruses involves lipid sorting and accumulation in the viral envelope of raft lipids, namely cholesterol and sphingolipids that are withdrawn from the host cell membranes. What does the virus-induced cholesterol depletion mean for a host cell?

Membrane-protective role of cholesterol

The dependence of the viral life cycle on cellular cholesterol, as well as the effects of viruses on cellular lipids and, in particular, on cholesterol, may provide a basis for the development of new antiviral drugs that will help in the pre-vaccination and disease progression phases[19,20,60,61]. And indeed, in some works, cholesterol-lowering treatments are considered as possible antiviral prophylactic or preventive measures[27,72]. However, alterations in the cell lipid status produced by viruses impose more complex requirements on potential medicines.

As follows from the previous sections, virus entry to a cell can barely alter the cholesterol content in the plasma membrane, except that during endocytosis-dependent raft internalization some plasma membrane cholesterol ends up in the intracellular compartments. In contrast, during assembly and budding of viral particles, cholesterol is lost from cell membranes, since the envelope of many viruses, including influenza virus, consists predominantly of raft lipids. This may have adverse effects on host cells, as cholesterol plays a vitally important role of in the organization and functions of cell membranes (reviewed in[73-77]). Cholesterol affects many physicochemical properties of phospholipid bilayers and is essential for self-organization of lipid membranes, forming cholesterol-enriched detergent-resistant membrane microdomains, or lipid rafts[78-81]. Rafts are not only used by many viruses, as was mentioned in previous sections, but are involved in functioning of a normal healthy cell. Certain membrane proteins can accumulate in these lipid microdomains, and the vicinity of these

proteins may accelerate and optimize their interactions required for cell metabolism, signalling, or other processes. The importance of rafts in carrying out a variety of biological functions has been demonstrated in many works[78-81].

Cells maintain the sterol content of various membranes at very different levels[73-84]. The highest content of cholesterol is in plasma membrane (25–50 mol%) and the lowest, in the nuclear membrane and endoplasmic reticulum (1–10 ml%)[77]. Deviations from the proper distribution, transport, and metabolism of cholesterol at the cell and whole-body level lead to various pathological conditions[75,77-79]. At the cell level, deviations from optimal level of cholesterol in different cell membrane compartments may considerably affect the functioning of membrane proteins, such as receptors, ion channels, enzymes, transporters, the activity of which critically depends on the presence of cholesterol ([74,77,78]; reviewed in[85]). Because of the cholesterol influences on physicochemical state of the membrane and on the protein functioning, many cellular processes are also known to be cholesterol-dependent: adhesion[86], locomotion[87], endocytosis[88,89], phagocytosis[90-92], synaptogenesis[93], and many others.

Considering the importance of cholesterol for normal cell functioning, one can expect that withdrawal of cell cholesterol by enveloped viruses will deteriorate the host cell cholesterol homeostasis. Indeed, ample evidence indicates that lowered membrane cholesterol is associated with altered mechanical properties and increased permeability of the membrane, and cholesterol supplementation prevents the leakage[94,95]. Interestingly, in[96] it was found that the presence of fusion peptide (FP) derived from influenza virus hemagglutinin induces fusion of liposomes, and the addition of cholesterol to liposomes decreases the lifetime of the fusion pore, thus hindering FP-induced fusion and indicating greater stability of the cholesterol-containing membrane. An illustrative example of a protective effect of cholesterol is also given in[97]: it was shown that West Nile virus modulates host cell cholesterol homeostasis by upregulating cholesterol biosynthesis and redistributing cholesterol to viral replication membranes. This virus-induced redistribution of cellular cholesterol downregulates the interferon-stimulated Jak-STAT antiviral signalling response to infection, and addition of exogenous cholesterol counteracts this effect.

As virus entry requires cholesterol, lowering cholesterol can indeed inhibit virus entry, and therefore cholesterol-lowering drugs such as statins can reduce the effectiveness of virus entry[98-104]. However, appropriateness of using statins to treat viral diseases, including influenza, remains a debated issue[101-104]. The results obtained so far are contradictory, and there is no certainty about the effectiveness of using statins in infectious diseases; more research is needed to understand the situation. However, a decrease in cholesterol content in cell membranes owing to the formation of viral envelopes can be one of the most harmful 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 (reviewed in[15]). Considering that the concentration of cholesterol in the ER is much lower than in the plasma membrane and ER is responsible for many vital functions (Ca^{2+} signalling and various syntheses, including cholesterol)[76-78,82-85], it can be expected that the loss of cholesterol

by ER membranes because of viral envelope formation (as in the case of SARS-CoV and -CoV-2[15]) may be more detrimental to cells than in the case of viruses budding from the plasma membrane. 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 SARS-CoV-2, a significant decrease (several fold) in total cholesterol and low-density lipoprotein (LDL) cholesterol levels was recorded[15,105-107]. Such a drop of the LDL cholesterol level in Covid-19 patients can reflect an enhanced recruitment of circulating cholesterol by the cells to compensate for its loss associated with virus reproduction. In these conditions, cholesterol-lowering treatments do not seem advisable for Covid-19 patients with life-threatening infection, at least until they recover from the infection[15,106]. Perhaps the clinical prognosis depends on the timely and successful delivery of cholesterol required for cell membrane repair. It seems possible that the more severe course of the Covid-19 disease (and possibly other viral diseases) in diabetic patients may be related to a reduced cholesterol content in cell membranes, which is apparently associated with diabetes[108-111].

Therefore, although in experiments in vitro lowering cell cholesterol reduces the efficiency of both cell infection and virus production and budding, but at the level of the whole organism decreasing cholesterol level cannot serve as a measure of antiviral defence. Cholesterol depletion may be not only unhelpful, but also dangerous, since a decrease in cholesterol content in cell membranes below the optimal level (specific for each cell type) leads to disturbances in the work of cholesterol-dependent proteins, destabilization of membranes and ultimately cell death. On the contrary, the delivery of exogenous cholesterol to sustain cells during infection and the formation of new viral particles may prevent massive death of cells that lose cholesterol.

This does not mean, however, that in order to increase cellular resistance to infection or during viral infection one should unrestrictedly and uncontrollably load cells with cholesterol beyond its optimal level. As was shown in[112], artificial increase of cholesterol content in cell membranes in vitro (cholesterol overload of endoplasmic reticulum in macrophages) activates apoptotic processes. Therein lies the difficulty – to keep membrane cholesterol content within an optimal range, and this should be carefully studied, so that the struggle to reduce "bad cholesterol" does not turn out to be deteriorating. We certainly need to know more about the level of cell membrane cholesterol, and blood plasma "lipid profile" may not be sufficiently informative in this regard.

So, besides preventing cell membrane cholesterol levels from getting too low, what are other antiviral tools based on the mechanisms of viruses' interaction with cholesterol? How can the cholesterol dependence of viruses be exploited for the development of antiviral drugs? One possible direction could be to search for agents that prevent viral proteins from interacting with cholesterol, and this search should be based on an understanding of the mechanisms of these interactions.

Cholesterol-binding motifs as one of possible mechanisms of interactions of viral proteins with cholesterol

One of possible mechanisms ensuring binding and concentration of cholesterol in the viral envelope is the presence of cholesterol-binding motifs in viral proteins. These motifs could be responsible for binding of cell membrane´s cholesterol by the viral protein. Cholesterol-binding motifs (original name, cholesterol-recognizing amino acid consensus, or CRAC motif[113-115]) are small regions with a specific set of amino acid residues involving a branched non-polar amino acid residue (valine (V), leucine (L), or isoleucine (I)), aromatic residue (tyrosine (Y), tryptophan (W), or phenylalanine (F)), and cationic amino acid residue (arginine (R) or lysine (K)). These motif-forming amino acids are separated by short segments of any 1–5 amino-acid residues. The general formula for these 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.[116,117] The presence of this motif in many proteins and its participation in the protein–cholesterol interactions has been confirmed by different methods[113-115].

Cholesterol-binding motifs are found in many viral proteins, and their role in cholesterol-dependent virus–cell interactions have been demonstrated. For example, such 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[118,119]. Cholesterol-binding motif is found in the α-helical domain of the hepatitis C virus non-structural protein NS5A, which is anchored at the cytoplasmic leaflet of the endoplasmic reticulum and is involved in replication hepatitis C virus[120]. S proteins of coronaviruses SARS-CoV and SARS-CoV2 also contain cholesterol-binding motifs that are located in the “aromatic” region of the S-protein transmembrane domain, which is necessary for the infection of cells with coronavirus[14, 121] (reviewed in[15]).

Cholesterol-binding motifs were also found in alpha-helices of matrix protein M1 of influenza A virus[122-124]. M1 is regarded the major driving force of influenza virus budding, since in the absence of M1 viral particles are not formed[125,126]. M1 is believed to be the key protein in recruiting, concentrating, and assembling viral and host components required for budding at the assembly site of the plasma membrane[127,128]. The influence of the cholesterol-binding motifs in protein M1 on the formation of new viral particles was shown in[124,125]. Further studies revealed that M1-derived peptide RTKLWEMLVELGNMDKAVKLWRKLKR (P4) containing two cholesterol-binding motifs from M1 protein in the micromolar range of concentrations stimulated cholesterol-dependent interactions of cultured macrophages with 2-μm particles and at 50 μM the peptide was cytotoxic[129]. Reducing the cholesterol content in the cells with methyl-β-cyclodextrin (mβCD) abolished the stimulatory component and significantly lowered the peptide concentration required for the toxic effect. Substitution of the motif-forming amino acids abolished these effects[130]. The cytotoxic effect of the M1-derived peptide RTKLWEMLVELGNMDKAVKLWRKLKR can be explained by the binding (sequestration) of membrane cholesterol by the peptide. This toxic effect of the M1-derived peptide with cholesterol-binding motifs can reproduce the consequences of the cholesterol removal from

cell membranes, which occurs during the formation of the viral envelope.

This deteriorating effect of viral cholesterol-binding proteins may be counteracted by agents that prevent membrane cholesterol from binding to such viral proteins and thereby prevent virus entry or/and compensate for depletion of membrane cholesterol resulting from the formation of viral particles. At least some of low-molecular weight substances, such as polyphenolic substances like quercetin and glycyrrhizin[131-133], can act at the protein-cholesterol interface and hinder cholesterol binding and thus inhibit virus entry and the assembly of new viral particles. Saponin glycyrrhizin, an active component of liquorice roots, is well known as an antiviral drug[132,133]. However, such agents are not very selective and can affect other cholesterol-dependent proteins and therefore cause side effects. Perhaps specially designed cholesterol-binding peptides specifically blocking the interactions of viral proteins with cholesterol will prevent the cellular cholesterol loss leading to permeabilization of membranes, oxidative stress, and cell death.

The ability of peptides containing cholesterol-binding motifs to regulate cholesterol-dependent cell functions has been demonstrated in[134,135,136], and further studies of the antiviral activity of these peptides can be useful and promising. For example, peptide C5A derived from α-helical domain of the hepatitis A virus nonstructural protein NS5A exhibited a broad-spectrum anti-viral activity[136]. In the antiviral activity of cholesterol-conjugated peptides derived from the influenza hemagglutinin was also reported[137]. The authors hypothesized that the cholesterol moiety, by localizing the peptides to the target cell membrane, allows the peptides to follow the virus to the intracellular site of fusion. It cannot be excluded though that the cholesterol molecule also contributed to the inhibition of fusion, consistent with[96]. Clearly, further research is needed to find or develop substances that, prior to vaccination, would selectively prevent the virus from entering the cell, depleting its resources, and further spread of viruses.

Conclusion

To summarise, understanding the role of cholesterol in the virus life cycle is crucial in devising novel preventive and therapeutic strategies for virus-induced diseases. Interfering with cholesterol-dependent influenza virus-cell interactions can inhibit both virus entry into the cell and the creation of a viral envelope from the cell membrane. Cholesterol-binding (CRAC) motifs found in the M1 protein of the influenza virus could play a significant role in virus-cell interactions that depend on cholesterol, both during the virus entry and when the cholesterol-rich viral envelope is formed. Binding of cell membrane cholesterol and subsequent removal of cholesterol from plasma membrane by newly formed viral particles can disturb normal functioning of cellular cholesterol-dependent proteins (receptors, ion channels, enzymes, etc.) and cause destabilization and permeabilization of cell membranes leading to cell death. This deteriorating effect of viral proteins with cholesterol-binding motifs can be mitigated by substances that either inhibit the binding of membrane cholesterol to viral proteins or counterbalance the depletion of membrane cholesterol caused by the virus. There's a possibility that specially engineered peptides

containing cholesterol-binding motifs could effectively impede the interactions between viral proteins and cholesterol, thereby broadening the arsenal of antiviral agents.

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