Statins Could Be Used to Control Replication of Some Viruses, Including HIV-1

ABSTRACT

Statins are mainly known for their plasma cholesterol-lowering properties and are widely used for the prevention of cardiovascular diseases. They however also exert pleiotropic effects through a variety of mechanisms, among which several immunosuppressive effects that are unrelated to their cholesterol-lowering activity. Interestingly, there has been recent evidence of antiviral effects, including preliminary studies on the efficacy of statins against HIV-1. This paper more particularly focuses on the specific inhibition of the binding of leukocyte function-associated antigen-1 (LFA-1) to intercellular adhesion molecule (ICAM-1) by statins, independently of the inhibition of HMG-CoA reductase. Targeting the statin-binding site within LFA-1 or regulating LFA-1 affinity by inhibiting prenylation of the small GTPases could prove useful to treat inflammatory, autoimmune diseases and possibly viral infections, including HIV-1.

INTRODUCTION

INDIVIDUALS INFECTED with human immunodeficiency virus type-1 (HIV-1) undergo treatment with highly active antiretroviral therapy (HAART) in most industrialized nations. This very expensive therapy is effective in reducing the viral load and re-constituting CD4 counts, but the latent virus reservoirs persist and the immune system is nonetheless destroyed. Emergence of drug-resistant strains and the appearance of many undesirable secondary effects of HAART prompt the scientific community to search for new therapeutic approaches.

Statins constitute today the most prescribed lipid-lowering drugs in many countries. They are considered very safe, provided they are not taken in combination with other drugs sharing the same metabolizing pathways. This review article presents intriguing new possibilities offered by statins. Indeed, recent observations indicate that statins display some anti-HIV-1 properties. Up to now, however, very limited clinical data are available on this topic, and thus this paper will focus on more fundamental and speculative aspects of statins with regard to their potential use to control HIV-1 replication.

We will first briefly describe the statins and the sparse clinical data on their putative antiviral potency, and then explore the multiple aspects of HIV-1 life cycle that could be targeted by the pleiotropic effects of these compounds. The capacity of statins to control replication of other viruses will also be discussed.

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STATINS

Statins are competitive analogues of the substrate of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (42,130). This enzyme catalyzes the rate-limiting step in the cholesterol (chol) biosynthetic pathway, that is, the conversion of hydroxymethylglutarate into mevalonic acid, a precursor for biosynthesis of chol and isoprenoids (geranylgeranyl-PP and farnesyl-PP) (Fig. 1). The first statins, mevastatin (compactin) and lovastatin (mevinolin), were originally obtained from yeast fermentation, whereas more recently developed statins such as simvastatin and pravastatin are semisynthetic compounds (69). A number of new compounds belonging to this family have since been designed and synthesized in the laboratory, among which are fluvastatin, atorvastatin, and cerivastatin. The family is likely to expand as additional members are currently synthesized and tested. The majority of these compounds are metabolized by the cytochrome pathway as indicated in Table 1.

Clinical use. The statins are primarily used as hypolipidemic agents, for reducing plasma chol levels and, consequently, for preventing cardiovascular diseases. These products are also administered in the treatment of dyslipidemia associated with AIDS and with HAART, and of lipodystrophy brought about by HAART, especially by protease inhibitors. However, the beneficial effects on the cardiovascular diseases also depend on mechanisms other than chol reduction (52). For example, statins have been demonstrated to improve endothelial function, reduce blood thrombogenicity (8,33,34,126,137), modulate inflammatory responses (16,87,102,150,154), and exert recently described immunomodulatory actions (16,32,46,84,113,159). These pleiotropic effects of statins that are unrelated to their chol-lowering properties could also explain certain positive clinical and laboratory observations, especially in cardiac (52,82,149) and kidney transplantations (76,143), ischemic strokes (43, 150), murine inflammatory arthritis (87), rat allergic asthma (102), protective effects against cancer (58), and different models of virus infection (37,55,57,67,100,123).

Possible mechanisms of action. Three mechanisms are known to explain the various effects of statins. The first two are based on the inhibition of HMG-CoA reductase, an enzyme catalyzing the transformation of HMG-CoA into mevalonic acid (Fig. 1). The mevalonic pathway produces chol and non-sterol isoprenoid products. Obviously, the first mode of action of statins is the very extensively studied chol reduction. The second is the inhibition of the formation of isoprenoids, in particular geranylgeranyl-pyrophosphate and farnesyl-pyrophosphate, two donors of protein prenylation. This type of post-translational modification targets a variety of proteins involved in intracellular signalling. Once prenylated, these proteins can be inserted into membranes. The best-known examples are the small GTPases Rho and Ras, and inhibition of the prenylation of Rho affects its GTPase activity and, consequently, actin cytoskeleton rearrangement. The third mechanism through which statins might exert their effects is unrelated to inhibition of HMG-CoA reductase and might explain the efficiency of statins for the treatment of inflammatory and/or autoimmune disorders (16,87,102,125). Random screening of chemical libraries has recently revealed that lovastatin specifically inhibits the binding of leukocyte function-associated antigen-1 (LFA-1) to intercellular adhesion molecule (ICAM-1) (72,156). This selective inhibition is mediated by binding to a novel allosteric site within LFA-1, the lovastatin site (L-site). Involvement of this site in the conformational change of another LFA-1 domain, the I-domain, has been shown by mutagenesis experiments (157). Among statins tested, only pravastatin was shown not to block the L-domain of LFA-1 (157) (Table 1). On the other hand, LFA703, a statin-like synthetic compound has been shown to abolish LFA-1-dependent leukocyte adhesion in ischemia and reperfusion (154). This novel statin-derived compound does so with an IC50 10 times lower than the original compound (i.e., lovastatin), without affecting the HMG-CoA reductase activity. Development of drugs targeting the statin-binding L-site of LFA-1 could be useful in the treatment of inflammatory or immunologic disorders that are due to recruitment of leukocytes mediated by the LFA-1/ICAM-1 interaction.
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such as arthritis, atherosclerosis, rejection of transplanted organs, ischemia, and inflammatory damages resulting from reperfusion. Moreover, recent findings suggest that such compounds might also be useful for the treatment of HIV-1–infected patients (see below).

**CLINICAL DATA**

**Effect of statins on HIV-1.** Although a wealth of clinical data are available on lipid-lowering effects of statins and their interactions with protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), very few clinical studies have addressed specifically the anti-HIV-1 activity of statins. However, the results of a proof-of-concept small-scale clinical study were published at the end of 2004 (37). Six A1 stage HIV-1 patients not receiving HAART were given lovastatin for a month as their only medication. This short-term statin treatment clearly reduced serum viral RNA loads in all patients and in general increased their CD4+ T cell counts. Discontinuation of treatment was followed by a rebound in viral load. Unlike the study by del Real et al., which addressed the anti–HIV-1 potency of statins, several studies have been conducted with the aim of assessing their lipid-lowering effects when associated with HAART (14,17,38). Interestingly, some specific parameters of interest such as CD4+ T cell counts and viral RNA were recorded in some of these studies. For example, an observational study conducted by Bonnet et al. did not detect any significant changes with respect to these parameters for patients given various regimens of PIs and NNRTIs in combination with statins (17). It must be noted here that the well known potency of HAART in significantly reducing HIV-1 loads will most likely mask any statin-mediated inhibition of virus production, all the more so since viral load is generally already very low at the onset of the studies. Thus, the possible clinical efficacy of statins must be evaluated in individuals treated only with such HMG-CoA reductase inhibitors.

**Pharmacokinetic interactions.** Unlike their antiviral effects, pharmacokinetic interactions between statins and drugs used for HAART have been studied in great details (28,31,48,117). It is known that all PIs act as inhibitors of hepatic cytochrome CYP3A isozymes and can thus potentially increase the levels of statins and the risk of skeletal muscle toxicity. In fact, simvastatin and lovastatin are no longer recommended for HAART-treated patients. On the other hand, pravastatin, which is eliminated by the kidney, does not accumulate to toxic levels when administered with HAART (29,38). Thus, if valuable antiviral effects of statins are confirmed, drug interactions will have to be considered carefully, and candidate statin-like drugs selected on the basis of their metabolic pathways.

**MODES OF ACTION OF STATINS: EFFECT ON HOST CELL CHOLESTEROL**

As stated above, statins are best known for their cholesterol-lowering properties. We will thus first address the multiple ways in which chol participates in HIV-1 biology, as these actions could all possibly be affected by statin effects (Fig. 2A).

The importance of cholesterol and lipid rafts in HIV-1 life cycle. Numerous studies have shown that HIV-1 requires lipid rafts for several key stages of its replication cycle. Lipid rafts are also known as detergent-resistant membranes, detergent-insoluble complexes, detergent-insoluble glycolipid-enriched complexes, glycolipid-enriched membranes, detergent-insoluble lipid microdomains and Triton-insoluble floating fractions. They are microdomains in the plasma membrane and other intracellular vesicles that are highly enriched with laterally-associated sphingolipids, interspersed with chol. They move in the fluid lipid bilayer and serve as docking sites for specific proteins involved in membrane trafficking and as relay stations in cell signalling (20,136). Depletion of chol destroys the capacity of a cell to form lipid rafts and it has been demonstrated that chol is critical for the maintenance of HIV-1 infectivity.

**HIV-1 entry into host cells.** Viruses rely heavily on protein interactions for cell invasion and productive infection. Virus binding and entry are achieved through spatial and temporal tuning of these protein interactions at the plasma membrane level, which often leads to cell signalling events that favour pathogen infection (147). Depletion of plasma membrane chol has been shown to inhibit HIV-1 entry and infection in both cell lines and primary cells (89,97,105,118). During HIV-1 infection, CD4 acts as the primary cell surface receptor for the virus (95) and it induces T cell activation by recruiting p56lk and promoting clustering with the T-cell receptor and necessary chemokine coreceptors such as CXCR4 or CCR5 (15,93,106,159). Using CD4 mutants that do not retain raft partitioning, it has been shown that CD4 localisation in lipid rafts is necessary for p56lk activation and HIV-1 entry in a CD4+ cell line (36). Nevertheless, in peripheral blood mononuclear cells (PBMCs) and H9 leukemic T cells, the virus co-localizes with CXCR4 and CCR5 in non-raft regions (83,118). The latter two reports suggest that CD4 binding and chemokine co-receptor recruitment take place in different plasma membrane microdomains and that HIV-1 adhesion and entry occur in separate regions on the cell surface. Another report shows that CD4, CXCR4 and CCR5 co-localize with raft-resistant markers to a non-raft environment, and HIV-1 binds to lipid rafts in the required presence of chol and chol depletion.
inhibits productive virus infection (122). Chol has also been shown to be critical for CXCR4 and CCR5 conformation and function (110,111). Finally, a report has shown that statins reduce HIV-1 replication in the HT-29 human T lymphocyte cell line (100).

**Virus entry in other cell types.** For an infection to ensue, HIV-1 particles must first make it through epithelial cells of the genitourinary, anorectal, or gastrointestinal tract. During mucosal transmission, epithelial cells achieve HIV-1 translocation from their apical side to their basolateral side through non-degradative transcytosis. Lipid raft disruption has been shown to inhibit HIV-1 transcytosis in endometrial HEC-1 or intestinal HT-29 cells (2). In immature dendritic cells, HIV-1 binding and internalization have been shown to be dependent on lipid rafts through a chol-dependent pathway (61). Conflicting results have been obtained with brain microvascular endothelial cells (BMVECs) as to the requirement of chol and lipid rafts for cell entry (5,94).

**Fusion.** Fusion between the viral envelope and the plasma membrane of target cells is the step that follows HIV-1 attachment. Even though the mechanism of membrane fusion still remains to be fully understood, the chol present in the viral envelope has been shown to play an important role in this process (62). Removal of chol from PBMCs reduces CD4 and CXCR4 co-localization with actin and cell susceptibility to virus-induced membrane fusion (151). The capacity of HIV-1 to initiate fusion of membranes may rely heavily on the ability of gp41 to bind to chol and get positioned in lipid rafts (152). The transmembrane envelope gp41 possesses a signal sequence capable of targeting lipid domains thought to be important in gp41-dependent membrane fusion (81,108,129).

**HIV-1 expression and budding.** Chol-dependent events have been identified as also playing a prominent role during later steps in HIV-1 life cycle such as assembly and budding as well as in the maintenance of virus morphology and infectivity (22). In fact, chol is in itself an integral constituent of the virus membrane, and thus participates to its structure; the virions being formed must acquire it from the host cell. Chol depletion of HIV-1-infected cells results in a significant diminution of virus release, and virions released from these cells show little infectious potential (90). Interestingly, endogenous chol has been shown to be more important than exogenous chol for viral assembly (36). Furthermore, it has been shown that HIV-1 Pr55Gag and Gag-Pol precursors assemble in cytoplasmic detergent-resistant complexes prior to their transport to the plasma membrane (86). The large precursor polyprotein Pr55Gag contains a signal sequence that promotes interaction with the cellular endocytic machinery, and the site of viral particle release is regulated by the sub-cellular distribution of chol (92). Previous works have revealed that Pr55Gag specifically associates with lipid rafts and depletion of chol reduces HIV-1 production (66,112). It is noteworthy that HIV-1 particles can incorporate the adhesion molecule ICAM-1 due to an association between the cytoplasmic domain of ICAM-1 and Pr55Gag and the physical presence of host-derived ICAM-1 is known to increase virus infectivity (12,27,49). As for the newly synthesized viral glycoprotein gp41, it binds chol, which in turn allows it to be incorporated into lipid rafts (152). As discussed earlier, the complementary roles of chol and gp41 in membrane fusion may play a pivotal role in HIV-1 entry and exit from target cells. When emerging from their host cell, newly formed viral entities must once more interact with the plasma membrane of the host cell. Interestingly, lipid rafts have again been shown to be specifically involved in viral budding (3,66,109).

**Effect on Nef.** The viral regulatory protein Nef has been shown to bind chol, transport newly synthesized chol to the site of viral budding, enrich lipid rafts with newly synthesized chol, increase the formation of lipid rafts and promote the biosynthesis of viruses (161). Nef expression has been shown to correlate with high viral titers and disease progression (35,79). It has been shown to associate with p56Lck, to then depress p56Lck kinase activity and impair p56Lck signalling (30,132,133). Lipid raft-associated Nef has however been shown to prime T cell activation through IL-2 secretion resulting from CD3 or CD28 stimulation (155). This in turn promotes HIV-1 replication and virus spread.

**HIV-1 and the cell cycle.** Chol has been shown to play a major role in the cell cycle and, since HIV-1 replication relies heavily on the activation status of the host cell, this is of considerable importance in regard to virus gene expression. For example, it has been demonstrated that chol regulates cyclin-dependent kinases (e.g., cdk2, cdk4, and cdk6), which are essential for the progression of the cell into the S-phase. Furthermore, chol increases the rigidity of the nuclear matrix during the S-phase, a process that favors a weaker dispersion of duplicating DNA (1). Moreover, chol seems to be necessary for mitosis and cytokinesis (47).

Altogether these studies indicate that HIV-1 has evolved to rely on the host cell lipid rafts and more particularly on chol to support its propagation during multiple stages of the virus replication cycle.

**IMPORTANCE OF THE LFA-1/ICAM-1 INTERACTION IN HIV-1 REPLICATION**

Having examined the major role of chol in HIV-1 biology, let us now consider the contribution of the LFA-1/ICAM-1 interaction given that it is affected by certain statins as well (Fig. 2B).
STATINS AND VIRUSES

FIG. 2. Possible targets of statins in HIV-1 life cycle. (A) Statins through their effect on chol biosynthesis can affect virus entry, virus gene expression through signal transduction events and modulation of the cell cycle, and virion egress. (B) Statins can either mask the LFA-1 L-site or lead to inhibition of isoprenoid biosynthesis, which will in turn affect the interaction between LFA-1 and ICAM-1. An eventual block of the ICAM-1/LFA-1 interaction can reduce primary infection through the mucosa, virus infection of permissive cells (e.g., CD4+ T cells and macrophages), HIV-1 capture, and transmission from non-permissive cells (e.g., DC and FDC) to more natural target cells and transcytosis and/or infection of less permissive cells such as BMVECs, epithelial cells, and trophoblasts.
General biological functions of the LFA-1/ICAM-1 interaction. LFA-1 (αβ2, CD11a/CD18) is a member of the β2 integrin family (68). This group of transmembrane heterodimeric adhesion molecules plays important roles in wound healing, immune system functions and organ development. Recent studies indicate that adhesion of LFA-1 to its cognate ligands—that is, intercellular adhesion molecules ICAM-1, ICAM-2, and ICAM-3, is not constitutive but is dynamically regulated by intracellular signal transduction pathways to transiently induce affinity/avidity changes (124,148). The small GTPases Rho and Rap1 and their effector molecules, ROCK (Rho-associated kinase) and RAPL (Rap1 effector), have been demonstrated to control these changes in LFA-1 affinity/avidity states and, consequently, the adhesion process (75,80,128). The efficiency of these small GTPases can be controlled by isoprenylation, a mechanism that can be affected by statins (54). Transient affinity/avidity states of LFA-1 in leukocytes may fulfill important roles in the interrelated phenomena of locomotion, initial cell-cell adhesion and cell-cell death (41). In leukocytes, the interaction between LFA-1 and ICAM-1 help guide the cells to sites of inflammation (70,71); enhance antigen presentation (107,153), and amplify cytotoxic cell functions (138). Moreover, this interaction promotes virological (121) and immunological synapses (39,40,99).

Effect on HIV-1 pathogenesis. LFA-1, ICAM-1, ICAM-2, and ICAM-3 are all expressed on HIV-1–infected cells (21), and they are also found embedded onto virions themselves (10,11,144). The expression levels of these adhesion molecules are increased as the disease is progressing (115), as compared to the asymptomatic phase (4). It has been shown that the cell-to-cell contact area is largely expanded by the virus (25,104,116,120,131,134,158). Therefore, the pathogenesis of HIV-1 infection can be modulated by the ICAM-1/LFA-1 interaction through modulatory effects on cell-to-cell transmission of HIV-1 (25,51,101,131,145), virus replication (45,65,73), virus-mediated syncytium formation (9,63,64), depletion of CD4+ T cells, and destruction of the architecture of secondary lymphoid organs (63,114).

Effect on the initial infection. The pivotal role played by immature dendritic cells in the establishment of the initial infection is well accepted. Dendritic cells (DCs) capture virus particles in the mucosa and subsequently transport them to the draining lymph nodes, where viruses are presented to CD4+ T cells. Immunological synapse between DCs and T cells is crucial for virus transmission (7,85,101), which is increased by co-stimulatory molecules such as ICAM-1 and LFA-1, as well as LFA-3 and CD2 (101,131,145). The transfer is known to be mainly mediated by the DC-specific surface molecule DC-SIGN that can bind to the virus-encoded external envelope glycoprotein gp120 (7,53). An increased transmission of HIV-1 by Th1-promoting effector DCs (also called DC1) has been correlated with a higher expression of ICAM-1 in these cells (131). Interestingly, LFA-1–bearing virions were found to be more infectious in immature DCs than isogenic virions lacking LFA-1 (unpublished data).

Effect on lymph nodes and syncytium formation. Altering interactions between LFA-1 and its natural counter-receptors can have important consequences on two cell types present in lymph nodes and involved in evolution of the disease, that is, CD4+ T lymphocytes and follicular dendritic cells (FDCs). First, an enhanced ICAM-1/LFA-1–mediated adhesion phenotype of CD4+ T cells was observed for samples from HIV-1–infected individuals as well as altered lymphocyte subpopulations in the HIV-1–infected lymph nodes (141). Moreover, the efficient capture of HIV-1 particles by FDCs was strongly inhibited by the presence of anti-ICAM-1 and anti-LFA-1 monoclonal antibodies, therefore suggesting that adhesion molecules play an important role in the interaction between HIV-1 and FDCs (51,119). A follicular hyperplasia leading to an enhancement of the FDCs network has been observed in lymph nodes during the early stage of HIV-1 infection (119). In vitro, spontaneous formation of small cellular aggregates of FDCs with neoplastic lymphocytes has been shown to be dependent on LFA-1 adhesion to ICAM-1 (119).

One of the best-described types of HIV-1–induced cell modification is syncytium formation, a phenomenon that is characterized by a high rate of cell-to-cell fusion events between uninfected and virus-infected cells. It was reported that LFA-1 and ICAMs actively participate in HIV-1–mediated syncytium formation even under conditions where virus expression is low (60). Moreover, it has been observed that a lack of LFA-1 expression or a treatment with an anti-LFA-1 antibody decreases virus-dependent cell fusion and syncytium formation (19,45,60,63,146). A high proportion of activated T cells express LFA-1 under an activated state in lymph nodes, a process that promotes cell fusion and syncytium formation (9,10,50), and might possibly contribute to the depletion of HIV-1–infected CD4+ T lymphocytes and destruction of FDCs (114).

Infection of permissive and non-permissive cells. Several authors have reported that the LFA-1/ICAM-1 interaction seems to play a dominant role in productive infection of permissive cells such as CD4+ T cells (19,45,60,63,146), monocytes (73), and macrophages (142). It must be noted that depending on the blocking antibody used and the incubation time (56), virus replication is not perfectly correlated with syncytium formation (which is the measured parameter) and thus these results must be considered with some caution. However,
using a specific peptide against ICAM-1 and blocking the expression of LFA-1 by a pre-treatment of permissive cells with the alpha-glucosidase 1 inhibitor 6-O-butanoyl castanospermine (MDL 28574) have resulted in inhibition of HIV-1 replication in the MT-2 T cell line (45) and chronically infected T cells (19), respectively, thus confirming a role for the LFA-1/ICAM-1 interaction in infection of permissive cells.

These adhesion molecules may also facilitate early stages of HIV-1 infection both in cis and in trans in non-permissive cells such as epithelial cells (26), trophoblasts (6), and BMVECs (94). For example, it has been shown that the adhesion molecules ICAM-2 and ICAM-3 are overexpressed on epithelial cells under proinflammatory conditions. When these conditions are brought about by damaged genital tissue, the transmigration of infected monocytic cells to the apical membrane of epithelial cells was shown to be dependent on an initial interaction between LFA-1 and ICAM-2 or ICAM-3 (26). Furthermore, expression of ICAM-1 is also increased on HIV-1–infected placental trophoblasts. These cells could be infected by HIV-1 by a mechanism involving contact between T cells and placental cells. Treatment of cells with an antibody against LFA-1 might contribute to the passing of the virus through the placental barrier during in utero HIV-1 vertical transmission (6). Interaction between LFA-1 and ICAM-1 also promotes the passing of the virus through the encephalic barrier. In fact, ICAM-1 is involved in HIV-1 entry into BMVECs (94).

Role of LFA-1 and ICAM-1 once incorporated within HIV-1. It is known that HIV-1 emerges at the cell-to-cell contact zone, where both LFA-1 and ICAM-1 are found to colocalize (44). Thus, it is not surprising to find that both molecules are inserted within the virus envelope (103), an observation that was confirmed when testing various clinical isolates of HIV-1 that were expanded in primary human cells (10,18,23,24,98). Virus-anchored host ICAM-1 and LFA-1 have been demonstrated to remain functional on the surface of viral entities (10,11,49,140). In fact, there is now much convincing evidence that HIV-1 infectivity is increased following incorporation of host ICAM-1 (49,140) or LFA-1 within budding viruses (91). Additional studies indicate that virus-associated ICAM-1 molecules facilitate HIV-1 entry and favor the cytoplasmic delivery of virus material, a process that culminates in productive infection (140). The binding of HIV-1 to non-susceptible cells or immobilized adhesion ligands (e.g., LFA-1) can increase virus infectivity and play a role in in vivo dissemination and transmission of HIV-1 (91). Moreover, ICAM-1 incorporation renders HIV-1 particles less susceptible to neutralization by monoclonal antibodies directed against the viral envelope glycoproteins (127) or the fusion inhibitor T-20 (13). These observations provide new insights into how interactions other than those between gp120 and CD4–coreceptor complexes can affect infection of CD4+ T lymphocytes (140), non-permissive cells (91), and lymphoid tissues (18). Finally, neutralization of ICAM-1–bearing HIV-1 particles by a pre-treatment of target cells with lovastatin or simvastatin confirms the importance of these adhesion molecules in HIV-1 replication and also the efficiency of statins in blocking the LFA-1/ICAM-1 interaction (53).

ANTIVIRAL EFFECTS OF STATINS ON OTHER VIRUSES

Interestingly, a literature search yielded reports of antiviral activities of statins on four other virus species: cytopathic envelope (103), an observation that was confirmed when testing various clinical isolates of HIV-1 that were expanded in primary human cells (10,18,23,24,98). Virus-anchored host ICAM-1 and LFA-1 have been demonstrated to remain functional on the surface of viral entities (10,11,49,140). In fact, there is now much convincing evidence that HIV-1 infectivity is increased following incorporation of host ICAM-1 (49,140) or LFA-1 within budding viruses (91). Additional studies indicate that virus-associated ICAM-1 molecules facilitate HIV-1 entry and favor the cytoplasmic delivery of virus material, a process that culminates in productive infection (140). The binding of HIV-1 to non-susceptible cells or immobilized adhesion ligands (e.g., LFA-1) can increase virus infectivity and play a role in in vivo dissemination and transmission of HIV-1 (91). Moreover, ICAM-1 incorporation renders HIV-1 particles less susceptible to neutralization by monoclonal antibodies directed against the viral envelope glycoproteins (127) or the fusion inhibitor T-20 (13). These observations provide new insights into how interactions other than those between gp120 and CD4–coreceptor complexes can affect infection of CD4+ T lymphocytes (140), non-permissive cells (91), and lymphoid tissues (18). Finally, neutralization of ICAM-1–bearing HIV-1 particles by a pre-treatment of target cells with lovastatin or simvastatin confirms the importance of these adhesion molecules in HIV-1 replication and also the efficiency of statins in blocking the LFA-1/ICAM-1 interaction (53).

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DISCUSSION

From the previously listed information, it is clear that almost every aspect of the life cycle of HIV-1 relies on choline. In the absence of this sterol, HIV-1 attachment to its host cell is severely impaired because clustering of receptors in lipid rafts is hindered and conformational state and function of CXCR4 and CCR5 co-receptors are critically affected, virus-cell fusion is greatly diminished, virus transcytosis is inhibited, virus production and budding are reduced, and cell signalling is altered. The effects of choline depletion on the cell cycle are also likely to be the most detrimental to HIV-1 biology, bringing virus gene expression to a virtual stop in CD4+ T cells. The control of choline through statins is thus likely to interfere with several key steps of HIV-1 replication, which offers the possibility of new therapeutic strategies to the current arsenal of antiviral drugs. It would be of high interest to assess the effectiveness of different statins to control virus replication based on their ability to affect choline biosynthesis.

In other respects, it is now clear that interactions between LFA-1 and its various ligands play a determining role in immune regulation and also in different steps of HIV-1 life cycle. Blocking the L binding site on LFA-1 with new derivatives of statins such as LFA703 might represent a new form of safe treatment (157). In addition, inhibition of the prenylation of Rho and Rap can also regulate the affinity state of LFA-1 and attenuate several aspects of the immune response by modifying the intracellular signalling pathway. Inhibition of prenylation of small GTPases by lovastatin appears to be promising in the treatment of HIV-1-infected patients (37). There are strong indications that these compounds could be useful and safe for treatment of inflammatory and immunological disorders (16,52,59,87,102,125). However, discrimination between the two statin effects—inhibition of the LFA-1/ICAM interaction or perturbation of signalling pathways via isoprenoid synthesis inhibition—will necessitate the testing of new-generation statins, such as GGTI-298 (88), LFA703, LFA-1 antagonists such as BIRT-337 (78), or specific prenylation inhibitors such as GGTI-298 (88), GGTI-286 (37), and L-778,123 (135).

It is known that the hyper-activation status of the immune system plays a pivotal role in the evolution of the disease in HIV-1-infected persons. In order to reduce viral load, virostatic drugs have been proposed as alternative strategies. More specifically, Kelly and colleagues have suggested compounds such as rapamycin, leflunomide, hydroxyurea and mycophenolic acid to control HIV-1 replication by reducing an excessive immune activation (77). In this context, statins could also be considered as virostatic agents. At the present time, a very limited number of in vitro and in vivo studies support the possible anti-HIV-1 activity displayed by statins (37,55,100). On the other hand, large amounts of data in the literature describe the anti-inflammatory and immunomodulatory effects of statins in various experimental model systems (16,87,102,125).

In summary, as depicted in Fig. 2, statins could possibly act on three distinct levels for controlling HIV-1 replication: (i) by lowering cholesterol, they could slow down the cell cycle, modify several signalling pathways and diminish viral entry and budding, (ii) by inhibiting the prenylation of small GTPases, they could hinder cell movement and decrease expression or affinity level of immune molecules such as LFA-1 and ICAM-1, and (iii) by blocking LFA-1/ICAMs interaction, they could attenuate several components of inflammatory, immunologic and virological responses. The statins could thus eventually prove to be efficient drugs against HIV-1.

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