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Original article

Statin-induced inhibition of HIV-1 release from latently infected U1 cells reveals a critical role for protein prenylation in HIV-1 replication

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Abstract

Latent infection of human immunodeficiency virus type 1 (HIV-1) represents a major hurdle in the treatment of acquired immunodeficiency syndrome (AIDS) patients. Statins were recently reported to suppress acute HIV-1 infection and reduce infectious virion production, but the precise mechanism of inhibition has remained elusive. Here we demonstrate that lypophilic statins suppress HIV-1 virion release from tumor necrosis factor alpha-stimulated latently infected U1 cells through inhibition of protein geranylgeranylation, but not by cholesterol depletion. Indeed, this suppression was reversed by the addition of geranylgeranylpyrophosphate, and a geranylgeranyltransferase-1 inhibitor reduced HIV-1 production. Notably, silencing of the endogenous Rab11a GTPase expression in U1 cells by RNA interference destabilized Gag and reduced virion production both in vitro and in NOD/SCID/γc^{null} mice. Our findings thus suggest that small GTPase proteins play an important role in HIV-1 replication, and therefore could be attractive molecular targets for anti-HIV-1 therapy.

Keywords: Statins; Prenylation; HIV-1; Rab11a; Small GTPases

1. Introduction

Infection with human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), is characterized clinically by a long asymptomatic period of latency preceding the development of AIDS. Even during this period of latency, the virus is continuously replicating and causing *de novo* infection. Recent studies using combination anti-retroviral therapy have revealed a population of latently infected cells that are refractory to antiviral therapy, which is believed to be a leading cause of the persistence of infection [1]. Although patients treated successfully with the highly active anti-retroviral therapy (HAART) achieved undetectable levels of virus load, viremia recurred in almost every patient when the drug therapy was stopped, because latent virus in reservoir cells is not susceptible to this anti-retroviral therapy or host immune responses [2,3]. Thus, HIV-1 infection remains incurable and new therapeutic approaches need to be developed.

Recent studies have suggested that lypophilic statins have direct anti-HIV effects. del Real et al. showed that lovastatin reduced acute infection by HIV-1 NL4-3.Luc.R.E. pseudotyped with HIV-R5 or X4 envelopes, but not that by the virus pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) envelope. Lovastatin treatment of HEK 293T producer cells also reduced HIV-1-X4-enveloped infectious virus production, but not that of VSV-G-pseudotyped virus. The

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proposed mechanism was that statins targeted Rho GTPases and affected the actin cytoskeleton re-arrangement necessary for virus entry or budding [4,5]. It was also reported that statins suppressed virion-associated intercellular cell adhesion molecule 1–leukocyte function antigen 1 interactions that are required for viral entry [6]. Audoly et al., using inhibitory toxins, proposed that small GTP-binding proteins are involved in the assembly of HIV-1 Gag in their acute infection model [7]. Quite recently, Nabatov et al. reported that statins disrupt CCR5 and RANTES expression levels in CD4+ T lymphocytes in vitro and preferentially decrease infection of R5 versus X4 HIV-1 [8]. However, the effect of statins in chronically HIV-1-infected cells and its precise mechanism remain to be uncovered.

Statins, which are used to treat hypercholesterolemia, inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis in the liver catalyzing the conversion of HMG-CoA to mevalonic acid [9,10]. In addition to inhibiting cholesterol synthesis, statins also block the synthesis of isoprenoid intermediates such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGpp). Both FPP and GGpp serve as important lipid attachments for the post-translational modification of variety of proteins, including heterotrimeric G proteins and small GTP-binding proteins such as the Ras, Rho, Rap, and Rab GTPase family proteins [11,12]. This modification, called protein prenylation, is a common mechanism for membrane association of approximately 0.5% of all intracellular proteins. Prenylation consists of the covalent attachment, via thioether linkage, of a C15 (farnesyl) or C20 (geranylgeranyl) isoprenoid group to a C-terminal cysteine residue in the context of a 'prenylation motif'. Farnesyl and geranylgeranyl moieties can bind covalently to several low molecular weight GTPase proteins, and this binding is catalyzed by three prenyltransferases: farnesyltransferase (FTase), geranylgeranyltransferase-1 (GGTase-I) or geranylgeranyltransferase-2 (GGTase-2, also called Rab GGTase). Thus, inhibition of the mevalonate pathway or geranylgeranyltransferases leads to impairment of protein prenylation.

Protein prenylation is critical for intracellular localization and function of small GTPase proteins. In general, modification with FPP is necessary for proper localization of Ras family proteins, whereas GGpp is required for Rho, Rab, and Rap family proteins. Among them, Rab GTPase proteins form the largest family within the Ras-like GTPase superfamily [13,14]. More than 50 Rab proteins have been identified in mammalian cells. Each Rab is believed to be localized to a specific subcellular compartment, reflecting the complexity and variety of trafficking events found in mammalian cells. Rab proteins, unlike other small GTPases, exhibit a variety of prenylation motifs at their C-termini, containing either one or more frequently, two cysteine residues, both of which are modified by geranylgeranyl groups [15]. It was recently reported that siRNA-mediated silencing of Rab9 expression in JC53 HeLa-derived indicator cells inhibited HIV replication, as did silencing expression of other genes that facilitate the late-endosome-to-*trans*-Golgi vesicular transport [16]. Interestingly, acute HIV-1 replication in JC53 cells was also affected, although less profoundly, by silencing expression of Rab11a. It has been well documented that Rab11a is mainly located on pericentriolar recycling endosomes and plays a key role in regulating vesicle trafficking through recycling endosomes to the plasma membrane as well as in exocytosis [17,18].

Here we investigated the effect of statins on virus production in chronically HIV-1-infected promonocytic U1 cells, and showed a critical role for protein prenylation in the late phase of HIV-1 replication.

2. Materials and methods

2.1. Reagents and cells

Simvastatin and lovastatin were purchased from LKT Laboratories, Inc. (MN, USA), and activated by dissolving in ethanol and treatment with 0.1 M NaOH. The pH was then adjusted to 7.0 with HCl. GGTI-298 and FTI-277 were purchased from Calbiochem (Darmstadt, Germany). Anti-Rab11a monoclonal antibody was purchased from BD transduction laboratories (Japan). The serum derived from an HIV-1-infected patient was described previously [19]. Anti-mouse IgG (H&L), anti-human rabbit HRP-linked antibody was obtained from American Qualex manufactures (CA, USA). DMRIE-C reagent for transfection was purchased from Invitrogen (CA, USA). All other reagents including anti-tubulin (T-9026) monoclonal antibody, squalene, GGpp, cycloheximide, TNF-α and phorbol-12-myristate-13 acetate (PMA) were purchased from Sigma (MO, USA). U1 and HEK 293T cells were grown in RPMI 1640 and DMEM, respectively, supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml of penicillin and streptomycin at 37 °C.

2.2. Treatment and stimulation of cells

Cells were treated with or without simvastatin or lovastatin for 2 days and equivalent numbers of viable cells were stimulated with TNF-α or PMA for additional 2 days in the presence or absence of statins, and then intracellular and extracellular Gag (p24 and p55) antigen was quantified. More than 80% of cells were found viable after treatment with 1 μM of simvastatin, and we normalized the levels of Gag protein (p24 and p55) based on the number of viable cells in each sample. The amount of Gag per viable cell was calculated by dividing the Gag value with the number of viable cells. In some of the experiments, GGpp (1 μM), squalene (50 μg/ml) or GGTI-298 (1 μM) was added during the entire course of the experiment.

2.3. HIV-1 Gag quantification

Culture supernatant was collected after centrifugation and subjected to quantification of the HIV-1 Gag (p55 and p24) antigen by automated enzyme-linked immunosorbent assay (ELISA) (Fuji Rebio Inc., Tokyo, Japan). Cell pellets were