EBioMedicine 8 (2016) 230–236

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Accumulation of MxB/Mx2-resistant HIV-1 Capsid Variants During Expansion of the HIV-1 Epidemic in Human Populations

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ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 15 April 2016
Accepted 15 April 2016
Available online 17 April 2016

Keywords:
MxB
HIV-1
Capsid variants
Positive selection

ABSTRACT

Recent studies have identified human myxovirus resistance protein 2 (MxB or Mx2) as an interferon induced inhibitor of HIV-1 replication. However, whether HIV-1 can overcome MxB restriction without compromise of viral fitness has been undefined. Here, we have discovered that naturally occurring capsid (CA) variants can render HIV-1 resistant to the activity of MxB without losing viral infectivity or the ability to escape from interferon induction. Moreover, these MxB resistant HIV-1 variants do not lose MxB recognition. Surprisingly, MxB resistant CA variants are most commonly found in the Clade C HIV-1 that is the most rapidly expanding Clade throughout the world. Accumulation of MxB resistant mutations is also observed during HIV-1 spreading in human populations. These findings support a potential role for MxB as a selective force during HIV-1 transmission and evolution.

1. Introduction

Human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS), has been a major global public health issue for decades. Approximately 35.0 million people were infected with HIV at the end of 2013 globally (http://www.who.int/mediacentre/factsheets/fs360/en/). Although aggressive treatment regimens using highly active antiretroviral therapy (HAART) is successful in suppressing HIV viral replication and the progression of HIV disease, the persistence of virus within latently infected cells enables viral rebound to occur once antiretroviral treatment is terminated (Chun et al., 1995). The association of many of the currently available antiretroviral agents with adverse side-effects dictates the need for continuing efforts to identify other antiretroviral targets and therapies.

Interferon (IFN), a key component of human innate immunity against invading pathogens acts by inducing expression of hundreds of IFN-stimulated genes (ISGs). Several HIV-1 restriction factors such as APOBEC3G, BST-2 and SAMHD1 can be stimulated by IFN (Malim and Bieniasz, 2012) but none of them account for the strong IFN-mediated suppression of HIV-1. Recently, human myxovirus-resistance protein B (MxB) has been identified as a major inhibitor of HIV-1 replication induced by IFN (Kane et al., 2013, Goujon et al., 2013, Liu et al., 2013).

Like most mammals, humans carry two myxovirus resistance genes, MX1 and MX2, which have arisen by gene duplication (Horisberger et al., 1983, Aebi et al., 1989) and encode the interferon-inducible dynamin-like GTPases MxA and MxB (Aebi et al., 1989). Human MxA has been shown to suppress a wide range of pathogenic DNA and RNA viruses (Gordien et al., 2001). In contrast, human MxB does not suppress the viruses tested in previous studies (Melen et al. 1996). MxB inhibits HIV-1 replication at post-entry steps (Schulte et al., 2015, Buffone et al., 2015, Fackler and Keppler, 2013, Liu et al., 2013, Kane et al., 2013, Goujon et al., 2013, Goujon et al., 2015, Goujon et al., 2014). MxB binding to HIV-1 capsid (CA) has been shown to be important for viral restriction (Fribourgh et al., 2014, Fricke et al., 2014, Kong et al., 2014).

Unlike APOBEC3, BST-2 and SAMHD1, MxB have not been found to be antagonized by HIV or SIV encoded regulatory proteins. Whether MxB antiviral activity plays an important role in vivo and whether HIV-1 can develop mechanisms to escape MxB activity during viral

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http://dx.doi.org/10.1016/j.ebiom.2016.04.020

transmission has not been well characterized. In the present study, we identified a number of capsid variants that render the virus resistant to the activity of MxB. These MxB resistant capsid variants do not alter viral fitness and can still be recognized by MxB. Some of the identified variants are most commonly found in subtype C HIV-1 and are accumulating during expansion of the subtype C epidemic.

2. Materials and Methods

2.1. Ethics Statement

Whole blood was obtained from healthy donors that signed an informed consent. This study has been approved by the Ethics Committee of Institute of Virology and AIDS Research, First Hospital of Jilin University.

2.2. Plasmid Construction

The HIV-1-based expression vector pSCRPSY-MxB was a generous gift from Dr. Paul D. Bieniasz (The Rockefeller University). The MxB expression plasmid Retro-X-MxB and shRNA (TRCN0000056713) targeting MxB were generous gifts from Dr. Chen Liang (McGill University). To generate an expression vector encoding HA-tagged MxB fusion protein, the MxB-HA fragment was PCR amplified using Retro-X-MxB as the template, it was then digested with SalI and XbaI and cloned into VR1012 to generate pVR1012-MxB-HA. Plasmids were generated encoding MxB with an N-terminal truncation: MxB(Δ1–82). This truncated protein was fused with HIV-1 core binding motif sequences derived from PDZD8(MxB(Δ1–82)-sPDZD8), which was constructed by Generay Biotech (Shanghai). The HIV-1 GFP reporting vector (pNL4-3-ΔEnv-EGFP, Cat# 11,100) provided by Dr. Robert Siliciano (Johns Hopkins University) was obtained through the NIH AIDS Research and Reference Reagent Program, NIH. HIV-1 NL4-3 ΔEnv-GFP with capsid mutants was provided by Dr. Christopher Aiken (Vanderbilt University School of Medicine). The NL4-3 CA G116A infectious clone was generated by replacing the BssHII-SpeI DNA fragment from NL4-3 (Cat#114, provided by Dr. Malcolm Martin and obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH) with that from NL4-3 ΔEnv-GFP (G116A). The composition of the NL4-3 CA G116A construct was confirmed by DNA sequencing.

2.3. Cells

HEK293T cells (AIDS Research and Reference Reagents Program, Cat #3522) and TZM-bl cells (AIDS Research and Reference Reagents Program, Cat#8129) were maintained in Dulbecco's modified Eagle's medium (DMEM, Life Technology) with 10% fetal bovine serum (FBS) and penicillin-streptomycin. The cell lines U937 and K562 (ATCC) were provided by Dr. Donald Small (Johns Hopkins University) and cultured in RPMI medium with 10% FBS. SupT1 cells were grown in RPMI medium supplemented with 10%FBS. Peripheral blood mononuclear cells (PBMC) were obtained from buffy-coat by Ficoll density gradient centrifugation. Monocytes enriched from PBMC by positive selection using CD14 MicroBeads were differentiated into macrophages by culturing in RPMI supplemented with 10% FBS, 50 ng/ml human GM-CSF (R&D Systems) 100 U/ml penicillin, and 100 μg/ml streptomycin. shRNA-silenced cell lines were generated according to the manufacturer's instructions (Open biosystem) and selected for resistance to puromycin. All cultured cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.4. Transfection and Immunoblotting

DNA transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. HEK293T cells were harvested at 48 h after transfection, washed twice with cold PBS, and lysed in lysis buffer (150 mM Tris, pH 7.5, with 150 mM NaCl, 1% Triton X-100, and complete protease inhibitor cocktail tablets [Roche]) at 4 °C for 30 min, then centrifuged at 10,000*g* for 30 min. The materials were then analyzed by SDS-PAGE and immunoblotting with the appropriate antibodies as previously described (Wei et al., 2012). The following antibodies were used: anti-MxB (Novus Biologicals, NBP1-81018), anti-Cyclophilin A (Santa Cruz Biotechology, sc-134,310), anti-HA (Covance, MMS-101R-1000), and anti-β-actin MAb (Sigma, A3853). Monoclonal antibody anti-CAp24 (Cat #1513) was obtained from the AIDS Research and Reference Reagents Program.

2.5. Viruses and Infection

For HIV-1 GFP viral production, HEK293T cells were transfected with 9 μg of HIV-1ΔENV-GFP and 3 μg of pCMV-VSV-G encoding plasmids; for shRNA production, HEK293T cells were transfected with 5 μg of the shRNA construct, 5 μg of the packaging plasmid pΔ8.91, and 2 μg of the VSV-G encoding plasmid. Medium was harvested 48 h later, then filtered at 0.45 μm. Supernatants were then purified, and lentivirus was concentrated by ultracentrifigation at 28 K for 1.5 h with a 20% sucrose cushion. The virus pellet was then dissolved in PBS and stored at −80 °C. For single infections with the HIV-1-GFP virus, U937, K562, or 293T cells were plated in 24-well plates 12 h before infection, and the efficiency of productive infection was analyzed 48 h later by flow cytometry (FACSCalibur, BD Biosciences). For Transmitted/founder (T/F) of HIV-1 (ZM249M) infection, viruses were generated from transfected HEK293T cells. SupT1 or MxB-SupT1 cells were exposed to equal amount of ZM249M HIV-1 for 2 h and the supernatants were replaced by fresh media. Two days later, the amount of virus that was released into the culture supernatant was determined by infecting the TZM-bl indicator cells. All results are representative of more than three independent experiments.

2.6. Viral Replication Assay

Primary PBMC were separated from whole blood of healthy donors using the Ficoll-Hypaque method. $CD4^+$ cells were isolated using CD4 MicroBeads (Miltenyi, Bergisch Gladbach, Germany). Cells were then cultured in RPMI 1640 medium containing 5 μg/ml PHA and 20 IU/ml human recombinant IL2 for 3 days. HIV-1 NL4-3 and NL4-3 CA G116A viruses were generated by transfecting HEK293T cells. Virus stocks were assessed for CAp24 concentration using an HIV-1 p24 ELISA Assay kit (XpressBio, Frederick, MD). Equal amounts of virus were used to infect activated T cells and infected cells were washed 6 h after infection. Quantitation of newly produced virus in the supernatants of infected cultures was determined by CAp24 ELISA and a viral infectivity assay using TZM-bl cells. Viruses in the supernatants were harvested at 2, 4, and 8 days post infection and determined using the CAp24 ELISA assay. The infectivity of newly produced viruses was assessed by using the same volume of supernatants to infect the indicator TZM-bl cells. Briefly, 2×10^4 TZM-bl cells were placed in 96-well culture plates and cultured overnight. The cells were infected with 50 μl supernatants containing NL4-3 or NL4-3 CA G116A viruses. After 48 h incubation at 37 °C with 5% CO_2, luciferase activity in cell lysates was measured using the Luciferase Assay kit (Promega, Madison, WI).

2.7. Quantitative Real-time PCR

Cells were collected at 24 h post-infection and washed with PBS. Total RNA was extracted from cells by TRIzol (Invitrogen) and cDNA samples were generated by using the High Capacity cDNA Reverse Transcription Kit (Invitrogen) according to the manufacturer's instruction. Samples were then analyzed by quantitative PCR using FastStart Universal SYBR Green Master Mix (Roche) and the ABI 7000 sequence detection system (Applied Biosystems). The sequences of DNA primers for real-time PCR are as follows: Human IFN-β, sense: AGGACAGGATGA