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Hepatitis C virus core protein enhances HIV-1 replication in human macrophages through TLR2, JNK, and MEK1/2-dependent upregulation of TNF-α and IL-6

Gokul Swaminathan [a,b,1], Daniel Pascual [b,c,1], Germaine Rival [b,d], Renzo Perales-Linares [a,b], Julio Martin-Garcia [b,e], Sonia Navas-Martin [b,e,*]

[a] Microbiology and Immunology Graduate Program, Drexel University College of Medicine, Philadelphia, PA 19102, USA
[b] Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102, USA
[c] Master of Science in Forensic Science Program, Professional Studies in the Health Sciences, Drexel University College of Medicine, Philadelphia, PA 19102, USA
[d] Master of Interdisciplinary Health Sciences Program, Professional Studies in the Health Sciences, Drexel University College of Medicine, Philadelphia, PA 19102, USA
[e] Center for Molecular Virology and Translational Neuroscience, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19102, USA

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ABSTRACT

Despite their differential cell tropisms, HIV-1 and HCV dramatically influence disease progression in coinfected patients. Macrophages are important target cells of HIV-1. We hypothesized that secreted HCV core protein might modulate HIV-1 replication. We demonstrate that HCV core significantly enhances HIV-1 replication in human macrophages by upregulating TNF-α and IL-6 via TLR2-, JNK-, and MEK1/2-dependent pathways. Furthermore, we show that TNF-α and IL-6 secreted from HCV core-treated macrophages reactivates monocytic U1 cells latently infected with HIV-1. Our studies reveal a previously unrecognized role of HCV core by enhancing HIV-1 infection in macrophages.

1. Introduction

Increased progression rates to acquired immunodeficiency syndrome (AIDS) and liver disease have been reported in individuals coinfected with human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) [1,2]. Strikingly, activation of $CD4^+$ T cells, macrophages, and dendritic cells enhances HIV-1 viral replication [3–6]. In HIV-1-infected individuals, higher viral loads and faster progression to AIDS correlate with systemic markers of immune activation [7]. Furthermore, cells latently infected by HIV-1 serve as long-lasting viral reservoirs and hinder the eradication of HIV-1 from infected patients with antiretroviral treatment.

HCV infection causes chronic inflammation of the liver and affects 3% of the human population worldwide [8]. The molecular mechanism(s) underlying uncontrolled, chronic liver inflammation remain poorly understood but may include direct viral effects and immune activation and dysregulation.

Combined immune dysregulation, immune deficiency, and immune activation have been proposed to worsen disease progression in HIV-1/HCV-coinfected patients [9]. The influence of HCV on HIV-1 infection remains poorly understood [10,11]. HIV-1 infection of macrophages plays a key role in viral pathogenesis, allowing accumulating replication-competent HIV-1, even in patients

Abbreviations: APCs, antigen-presenting cells; ERK, extracellular signal-regulated protein kinase 1/2; HKLM, heat killed Listeria monocytogenes; IL-6, interleukin 6; JNK, c-Jun amino-terminal kinase; MDMs, primary monocyte derived macrophages; p38, p38 MAP kinase; PD98059, 2′-amino-3′-methoxyflavone; PMA, phorbol myristate acetate; SB203580, [4-(4′-fluorophenyl)-2-(4′-methylsulfinylphenyl)-5-(4′-pyridyl)-imidazole]; SP600125, c-Jun N-terminal kinase inhibitor; TLR, Toll-like receptor; TNF-α, tumor necrosis factor alpha; TRAFs, TNF receptor-associated factors; U0126, 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio] butadiene

* Corresponding author at: Department of Microbiology and Immunology, Drexel University College of Medicine, 245 N. 15th Street, Philadelphia, PA 10102, USA. Fax: +1 215 762 7284.
E-mail address: sonia.navas-martin@drexelmed.edu (S. Navas-Martin).
[1] These authors contributed equally to this work.

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receiving antiretroviral treatment [12]. Although there is evidence supporting HCV infection of macrophages in vivo [13,14], the ability of macrophages to support productive HCV infection remains controversial ([15], and recently reviewed in [16]). In addition, HIV has been shown to facilitate HCV infection of native human macrophages in vitro [17]. Macrophage phagocytic uptake of HCV triggers immune activation [18], suggesting their contribution to chronic inflammation in infected individuals. Even though these viruses infect distinct cell types, a potential mechanism of interaction between HIV-1 and HCV is through the action of viral secreted proteins that takes place during viral replication.

HCV core is a secreted viral protein that has been detected in the circulation of HCV-infected patients [19]. Although several HCV proteins exhibit immunomodulatory activity, HCV core is unique in its pleiotropic effects [20]. In addition to its structural role in the encapsidation of the viral RNA, HCV core exhibits multiple regulatory functions, including the induction of tumorigenesis, regulation of viral and cellular gene expression, modulation of apoptosis, and suppression of host immunity [21–24]. The potential contribution of HCV core to HIV pathogenesis remains poorly understood. HCV core has been shown to mediate repression of the HIV-1 protein Tat-mediated transactivation of HIV-1 long terminal repeat (LTR) in a hepatoma cell line [25]. In contrast, Khan et al [26] have shown that HCV core and HIV-1 protein Nef upregulated HIV-1 LTR-driven luciferase expression in a transiently-transfected human monocytic cell line. Strikingly, the potential role of HCV core on HIV-1 infectivity in the context of infection, rather than plasmid-based transcription analysis, remains unknown.

Tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) are key proinflammatory cytokines known to enhance HIV-1 replication [27–30]. Interestingly, stimulation of naïve, uninfected human macrophages with HCV core induces TNF-α and IL-6 production through a Toll-like receptor 2 (TLR2)-mediated pathway [31].

Because HCV core has been shown to both inhibit and enhance HIV-1 transcription [25,26,32], and the impact of HCV core on HIV-1 infection in macrophages is not well known, we aimed to determine the effect of HCV core stimulation in HIV-1 infection of THP-1 promonocytic cells, primary monocyte-derived macrophages (MDMs), and in the HIV-1 latently-infected U1 monocytic cell line. We found that HCV core mediates enhancement of HIV-1 infection in macrophages through a TLR2-, JNK-, and MEK1/2-mediated pathway that results in upregulation of TNF-α and IL-6 as final molecular effectors. Our study identifies a novel role of HCV core-TLR2 interaction in augmenting HIV-1 infection in macrophages and reactivation of HIV-1-infected latent reservoirs.

2. Materials and methods

2.1. Ethics statement

De-identified human monocytes from healthy blood donors were obtained from the University of Pennsylvania's Human Immunology Core (operating under the supervision of the University of Pennsylvania's Institutional Review Board). We did not have any interaction with human subjects or protected information, and therefore no informed consent was required. All studies were approved and supervised by Drexel University's Institutional Review Board.

2.2. Cell culture

A promonocytic cell line (THP-1) was differentiated in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (hiFBS) certified for low endotoxin and hemoglobin levels (BenchMark FBS, Gemini Bio-Products, West Sacramento, CA), 100 ng/ml of phorbol 12-myristate 13-acetate (PMA), antibiotics, and L-glutamine for 3 days. Cells rested in media containing no PMA for 3 more days to obtain macrophages (referred to as THP-1 macrophages). Primary MDMs were generated by culturing human monocytes in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% hiFBS, antibiotics, and 16 U/ml macrophage colony-stimulating factor (eBioscience, San Diego, CA) for 7 days. Human embryonic kidney (HEK) 293T cells were cultured in DMEM supplemented with 10% DFBS and antibiotics. The HIV-1 latently-infected U1 monocytic cell line was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH from Dr. Thomas Folks. These cells were cultured in RPMI 1640 medium containing 10% hiFBS and L-glutamine. All experiments involving U1 cells were performed in a biosafety level 3 laboratory under standard operating procedures. All cells were maintained at 37 °C with 5% CO_2.

2.3. Single-cycle HIV-1 BaL infection assay

Single-round infectious, luciferase-reporter HIV-1 BAL pseudotyped virions were produced by cotransfecting HEK 293T cells and normalized by p24 as previously described [33]. Infectivity was measured by luciferase activity in cell lysates using a microplate luminometer (GloMax, Promega, Madison, WI).

2.4. Stimulation of macrophages with recombinant HCV core and β-galactosidase proteins

THP-1 macrophages and MDMs were treated with either 5 μg/ml of recombinant HCV core protein genotype 1b fused to β-galactosidase (Virogen, Watertown, MA), or 5 μg/ml of β-galactosidase (β-Gal) control protein (Virogen). THP-1 and MDMs were first infected with HIV-1 BAL pseudovirus and treated with either β-Gal or HCV core recombinant proteins at 14 h after infection. HIV-1-infected, HCV core-stimulated macrophages were incubated at 37 °C for 48 h; supernatants were collected for assessing cytokine production and cells were lysed to determine HIV-1 infectivity by luciferase activity (Promega).

2.5. Pharmacological inhibition of MAP kinases

THP-1 macrophages and human primary MDMs were treated with JNK [50 μM SP600125 (Invivogen, San Diego, CA)], p38 [20 μM SB203580 (Invivogen)], and MEK1/2 [50 μM PD98059 and 10 μM U0126 (both from Cell Signaling, Danvers, MA)] kinase inhibitors. To evaluate effects of MAP kinase inhibitors on HCV core-mediated induction of HIV-1 infectivity, macrophages were prestimulated with the inhibitors as above, then infected with HIV-1 BAL for 12 h (in the presence or absence of these inhibitors) and treated with β-Gal or HCV core proteins, and finally lysed to determine luciferase activity 48 h after infection. To define the effects of the MAP kinase inhibitors on HCV core-mediated induction of TNF-α and IL-6 in uninfected macrophages, cells were stimulated with MAP kinase inhibitors for 1 h before exposure to HCV core or β-Gal, and during stimulation for 14 h. Supernatants were collected and assayed by ELISA to determine cytokine induction.

2.6. Neutralization of TLR2, TNF-α, and IL-6

To determine the role of TLR2 in HCV core-mediated stimulation of uninfected macrophages, cells were prestimulated with 10 μg/ml of a TLR2 neutralizing antibody (nAb) (Invivogen) for 1 h and then treated with 5 μg/ml of HCV core and 5 μg/ml of β-Gal as a negative control, in the continuous presence of the nAb. To determine the role of TLR2 in HCV core-mediated enhancement of HIV-1 infectivity in THP-1 macrophages and primary