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HIV-1 and HIV-2 infections induce autophagy in Jurkat and CD4$^+$ T cells

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ABSTRACT

Autophagy plays important roles during innate and adaptive immune responses to pathogens, including virus infection. Viruses develop ways to subvert the pathway for their own benefit in order to escape restriction by autophagy, leading to increased viral replication and/or control over apoptosis of their host cells. The effects of HIV infection on the autophagic pathway in host cells have been little documented. Using the susceptible Jurkat cell line and CD4$^+$ T cells, we studied the relationship of HIV-1 and -2 infections with autophagy. We found that HIV infections significantly increase transcription of ULK1, a member of the autophagy-initiated complex. Two ubiquitin-like conjugation systems, the Atg12 conjugation system and the microtubule-associated protein L chain 3 (LC3) conjugation system that control the elongation of the autophore to form the autophagosome, were activated after HIV infection, with upregulation of Atg12–Atg5 complex and increased transcription of LC3, and formed more autophagosome in infected cells detected using an EM assay. We also found that HIV-1 induced more autophagic death in Jurkat cells relative to HIV-2, and the inhibition of autophagy with 3MA and Beclin-1 knockdown decreased HIV-1 replication significantly. The results indicate that HIV is able to induce the autophagic signaling pathway in HIV-infected host cells, which may be required for HIV infection-mediated apoptotic cell death.

1. Introduction

HIV-1 infection causes a progressive decline in the function and number of CD4 T lymphocytes, and high levels of viremia resulting in the development of AIDS. HIV is able to kill infected CD4-expressing primary cells directly, while bystander cell killing is achieved through cells exposed to proteins associated with HIV infection [1]. Both infected and uninfected CD4 T cells have been shown to undergo cell death with apoptosis being a major death pathway to achieve cell death [2]. However, recent reports have shown that HIV-1 infection may also lead to cell death through the autophagic pathway [3–5].

Autophagy is a degradative lysosomal pathway involving the sequestration of cytoplasmic constituents (including organelles) into double-membrane-bound vesicles or autophagosomes, which eventually fuse with lysosomes for degradation. Autophagy-related (Atg) proteins modulate the formation of autophagosomes, which have been highly conserved in all eukaryotic organisms, from yeast to humans [6]. To date, 33 different autophagy-related genes have been identified in yeast [7], and most of the corresponding homologous molecules have been confirmed in mammalian cells [8]. The Atg12 and microtubule-associated protein L chain 3 (LC3; Atg8 in yeast) conjugation systems have been shown to control the elongation of the autophore to form the autophagosome, which are known as ubiquitin-like conjugation systems. Atg7 and Atg10 catalyze Atg12 and Atg5 to form an Atg12–Atg5–Atg16L complex [9]. LC3 is cleaved by the protease Atg4 to generate the cytosolic LC3-I; Atg3 and Atg7 catalyze the conjugation of LC3-I to generate the membrane-bound lipid form, LC3-phosphatidylethanolamine, which is also called LC3-II [8].

Currently, there are only a few reports of autophagic signaling pathways including autophagic cell death in HIV infection. In 2006, it was reported that in HIV infection, envelope glycoprotein (Env) is associated with autophagy in bystander CD4 T lymphocytes [3]. HIV-1-infected cells with Env expression display autophagy and accumulation of Beclin-1, an important Atg protein, in bystander CD4 T cells independent of HIV-1 replication [3,5], which may be necessary for both apoptotic and nonapoptotic cell death required to trigger CD4 T cell apoptosis [10]. HIV-1 tat is able to induce autophagy in neuroblastoma cells [11], and autophagy is increased in postmortem brains of persons with HIV-1-associated encephalitis [12]. Alternatively, some reports demonstrated that HIV-1 infection inhibits starvation or rapamycin-induced autophagy in T cells [13] and dendritic

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cells [14], and in bystander macrophage/monocytic cells [15]. It has not been demonstrated whether autophagy is involved in direct killing of CD4-expressing infected cells, especially in the early stage of its infection. Here, we used a susceptible Jurkat cell line and primary CD4 T cells to study the effects of HIV infection on autophagy, and found that HIV infection increased autophagy in CD4 T cells and induced Jurkat cell death through the autophagic pathway with involvement of both Atg12 conjugation system and the microtubule-associated LC3 conjugation system. We also compared differences in autophagic pathway molecules induced by HIV-1 and HIV-2.

2. Materials and methods

2.1. Chemicals and reagents

3-Methyladenine (3MA) and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Rabbit polyclonal antibodies against Beclin-1, Atg5 and Atg12, and siRNA (control or Beclin-1) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-p62 antibody was purchased from MBL (Medical and Biological Laboratories CO (Woburn, MA)).

2.2. Cell culture

The human Jurkat T cell line (clone JE6.1) was obtained from American Type Culture Collection (Manassas, VA), and cultured at 37 °C in 5% CO_2 in RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, 50 μg/ml penicillin, and 50 μg/ml streptomycin. Cell viability was determined by trypan blue exclusion analysis (Life Technologies).

CD4 T cells were isolated with CD4 T cell isolation kit (Invitrogen Carlsbad, CA), from peripheral blood mononuclear cells (PBMCs) from healthy blood donors who are seronegative for HIV-1 and HIV-2, HTLV, HBV and HCV were provided by the Department of Transfusion Medicine, National Institutes of Health (NIH) (Bethesda, MD). Cells suspensions contained >95% CD4 T cells stimulated with 2 mg/ml PHA for 72 h. Activated CD4 T cells were then infected with HIV as indicated below.

2.3. HIV infection

Jurkat cells were seeded at 2×10^5 cells/ml for 24 h, and infected with known amounts (10^9 copies per 10^6 cells) of HIV-1 (MN) and HIV-2 (Rod) and cultured for different days indicated. CD4 T cells infected with known amounts (10^9 copies per 10^6 cells) of a primary HIV-1 subtype B virus were isolated from a US blood donor.

2.4. Electron microscopy (EM) assays

Cell cultures were fixed in 2% paraformaldehyde–2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 2 h. The cells were washed with PBS buffer and then postfixed in 1% osmium tetroxide, washed with distilled water three times, dehydrated in 50%, 70%, 95% and 100% ethyl alcohol, embedded in Epon 12. Thin sections (60 nm) were cut, stained with uranyl acetate and lead citrate, and examined on a Zeiss 912 transmission electron microscope.

2.5. LC3 staining

Cell cultures were washed with cold PBS twice and fixed in an acetone/methanol (vol/vol) mixture for 10 minutes at −20 °C. Rabbit antihuman LC3 polyclonal antibodies and FITC-conjugated goat anti-rabbit polyclonal antibody were used for detecting autophagy in the cells, by measuring fluorescence using Zeiss Cell Observer SD Confocal Microscope system.

2.6. Autophagy PCR array

Total RNA was isolated from Jurkat cells using TRIzol® LS Reagent (InvitrogenTM, Carlsbad, CA). mRNA was isolated from total RNA using Qiagen mRNA isolation kit (Qiagen Inc., Valencia, CA). Equal amounts of mRNA per sample (0.3 μg) were reverse transcribed using RT^2 First Strand kit from SuperArray Biosciences. Comparison of the relative expression of autophagy-related genes was performed using RT^2 profiler™ PCR array PAHS-084 (human autophagy array; SABiosciences™, Frederick, MD) on a TaqMan 7500 Analyzer using RT2 Real-Time™ SYBR Green PCR master mix PA-012. Hypoxanthine phosphoribosyltransferase-1 (HPRT1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β-actin (ACTB) "housekeeping" genes were used for normalization.

2.7. Western blot analysis

Proteins were isolated from cultured Jurkat cells with RIPA buffer (1 × PBS, 1% (v/v) NP-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 0.1 mg/ml PMSF, 30 μl/ml aprotinin, and 1 mM sodium orthovanadate). For SDS–PAGE, samples containing equal amounts of protein were boiled in loading buffer (100 mM Tris–HCl, 200 mM DTT, 4% SDS, 0.2% bromphenol blue, 20% glycerol) and separated on SDS–PAGE, followed by transfer to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat milk and stained with primary antibodies for 2 h at the optimal concentrations. After five washes in PBS with 0.2% Tween 20, the horseradish peroxidase-conjugated secondary antibody was applied and the blot was developed with ECL reagents (Amersham Biosciences, Piscataway, NJ, USA).

2.8. siRNA transfection

A small interfering RNA (siRNA) transfection kit corresponding to Beclin-1 (Human) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Jurkat cells were transfected with Beclin-1 siRNA or the control siRNA for 48 h according to the manufacturer's protocol.

2.9. Real-time PCR

Quantitative real-time reverse-transcriptase (RT) PCR was used for measuring virus production. Viral RNA was isolated from 140 μl of culture supernatant by using the QIAamp Viral RNA Mini Kit (Valencia, CA 91355) according to the manufacturer's protocol. Primers and a TaqMan probe were designed in the *gag* p24 region of the HIV-1 subtype B isolate sequences according to the sequences in the GenBank database. The forward primer was 5′-GACATCAAGCAGCCATGCAA-3′, corresponding to nucleotides 1367–1386, and the reverse primer was 5′-CTATCC CATTCTGCAGCTTCCT-3′, corresponding to nucleotides 1430–1409. The TaqMan probe was oligonucleotide 5′-ATTGATGGTCTCTTTTAACA-3′, corresponding to nucleotides 1488–1507, coupled with a reporter dye [6-carboxy fluorescein] (FAM) at the 5′ end and a non-fluorescent quencher and a minor groove binder (MGB), which is a Tm enhancer, at the 3′ end. Nucleic acids were amplified and detected in an automated TaqMan 7500 Analyzer by using QuantiTect™ Probe RT-PCR kit (Qiagen Inc., Valencia, CA). The 25-μl PCR mixture consisted of 100 nM primers and 100 nM probe. Following three thermal steps at 55 °C for 5 min, at 50 °C for 30 min and at 95 °C for 10 min, 45 cycles of two-step PCR at 95 °C for 15 s and at 60 °C for 1 min were performed. Results were obtained from at least 3 independent experiments.

2.10. Statistical analysis

The unpaired Student's *t* test was used for data analyses as indicated, and a value of $p<0.05$ (*) was considered significant and $p<0.01$ (**) very significant.