TREM-1 Protects HIV-1-Infected Macrophages from Apoptosis through Maintenance of Mitochondrial Function

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ABSTRACT Macrophages are a reservoir for latent human immunodeficiency type 1 (HIV) infection and a barrier to HIV eradication. In contrast to CD4+ T cells, macrophages are resistant to the cytopathic effects of acute HIV infection. Emerging data suggest a role for TREM1 (triggering receptor expressed on myeloid cells 1) in this resistance to HIV-mediated cytopathogenesis. Here, we show that upon HIV infection, macrophages increase the expression of BCL2, BCLXL, TREM1, mitofusin 1 (MFN1), and MFN2 and the translocation of BCL2L11 (BIM) to the mitochondria and decrease the expression of BCL2-associated agonist of cell death (BAD) and BAX while maintaining a 95% survival rate over 28 days. The HIV proteins Tat and gp120 and the GU-rich single-stranded RNA (ssRNA) (RNA40) from the HIV long terminal repeat region (and a natural Toll-like receptor 8 [TLR8] agonist) induced similar effects. TREM1 silencing in HIV-infected macrophages led to decreased expression of BCL2, BCLXL, MFN1, and MFN2 and increased expression of BAD and BAX. This correlated with a significant increase in apoptosis mediated by a disruption of the mitochondrial membrane potential (Δψm), leading to the release of cytochrome c and caspase 9 cleavage. Exposure of TREM1-silenced macrophages to Tat, gp120, or RNA40 similarly resulted in the disruption of Δψm, cytochrome c release, caspase 9 cleavage, and apoptosis. Thus, our findings identify a mechanism whereby HIV promotes macrophage survival through TREM1-dependent upregulation of BCL2 family proteins and mitofusins that inhibits BCL2L11-mediated disruption of Δψm and subsequent apoptosis. These findings indicate that TREM1 can be a useful target for elimination of the HIV reservoir in macrophages.

IMPORTANCE The major challenge to human immunodeficiency virus (HIV) treatment is the development of strategies that lead to viral eradication. A roadblock to accomplishing this goal is the lack of an approach that would safely eliminate HIV from all resting/latent reservoirs, including macrophages. Macrophages are a key part of the innate immune system and are responsible for recognizing invading microbes and sending appropriate signals to other immune cells. Here, we found that HIV induces the upregulation of the protein TREM1 (triggering receptor expressed on myeloid cells 1), which signals an increase in the expression of antiapoptotic proteins, thus promoting survival of HIV-infected macrophages.

KEYWORDS BCL2 family, TREM-1, apoptosis, human immunodeficiency virus, macrophages, mitochondria, mitofusin
the absence of CD4+ T cells (4, 5), and contain lower intracellular concentrations of antiretrovirals than CD4+ T cells, resulting in ongoing HIV replication (6). Latently infected macrophages also harbor and transmit replication-competent virus to nearby CD4+ T cells through virologic synapses that are resistant to antibody-mediated neutralization (7). Additionally, they survive for months to years and are resistant both to the cytopathic effects of HIV infection and to CD8+ T cell-mediated killing (8–10).

Notwithstanding recent advances defining cell death signaling pathways, much is unknown about the mechanism(s) by which macrophages escape apoptosis during HIV infection. Despite this, studies suggest that HIV signals through multiple pathways to reprogram the transcriptome and proteome of human macrophages to render them resistant to HIV-mediated apoptosis. HIV Nef induces the phosphorylation and inactivation of proapoptotic BCL2-associated agonist of cell death (BAD) (11), and HIV Tat and gp120 induce the transcription of apoptosis regulatory proteins, including colony-stimulating factor 1 (CSF1), caspase 8 (CASP8) and Fas associated via death domain (FADD)-like apoptosis regulator (CFLAR), BCL2 family members BCL2 and BCLXL, and inhibitor of apoptosis proteins (IAP) X-linked inhibitor of apoptosis (XIAP), baculoviral IAP repeat-containing 2 (BIIRC2), and BIIRC3 (12–14). Additionally, acute exposure to HIV and to HIV gp41 peptides upregulates triggering receptor expressed on myeloid cells 1 (TREM1) in peripheral blood mononuclear cells (PBMC) and macrophages (15–17).

TREM1 is a 30-kDa glycoprotein expressed on macrophages that belongs to the immunoglobulin variable (IgV) domain superfamily of proteins and consists of a positive-charged transmembrane domain and a short cytoplasmic domain that lacks any signaling motif. Thus, TREM1 requires TYRO protein tyrosine kinase binding protein (TYROBP) for signaling (18, 19). Although the precise ligands for TREM1 are unknown, CD177, peptidoglycan recognition protein 1 (PGLYRP1), high-mobility group box 1 protein (HMGB1), and some damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) activate TREM1 signaling (20–24). TREM1 ligation leads to an increase in reactive oxygen species and the secretion of proinflammatory cytokines through the activation of NF-κB. Additionally, TREM1 ligation synergistically amplifies Toll-like receptor (TLR)-mediated and Nod-like receptor (NLR)-mediated proinflammatory responses (19). TREM1 is also an antiapoptotic molecule that prolongs macrophage survival. Specific ligation or overexpression of TREM1 induces BCL2 expression and the subsequent depletion of caspase 3, preventing the cleavage of PARP1 (25). In addition, both ligation and overexpression of TREM1 lead to an increase in mitofusin expression levels, suggesting that TREM1 contributes to the maintenance of mitochondrial integrity, thus favoring cell survival (25). The involvement of and mechanisms by which TREM1 may alter macrophage survival during HIV infection are unknown. Therefore, we investigated whether TREM1 affects cell survival during HIV infection of human primary macrophages.

RESULTS

HIV infection of macrophages results in persistent infection and prolongs cell survival. We designed our initial experiments to determine whether HIV infection results in increased apoptosis of infected macrophages. We infected macrophages with HIV for 28 days, during which time we assessed HIV replication kinetics and apoptosis. HIV p24 antigen levels increased in culture supernatants between day 3 and day 18 followed by a decline after 18 days (Fig. 1A). Uninfected macrophages showed low levels of apoptosis, with an observed maximum of 13.5% of cells displaying formamide-sensitive single-stranded DNA (ssDNA; a specific indicator of apoptosis [26]) by day 28 (Fig. 1B). HIV infection of macrophages was associated with a decrease in cell death, reaching a high of just 5.5% at day 28 (± 0.3% standard error of the mean [SEM]; P = 0.017) (Fig. 1B). Culture supernatants assessed for lactate dehydrogenase (LDH) release demonstrated the same profile (Fig. 1C). Collectively, these results suggest that HIV infection prolongs survival of macrophages.

HIV influences pro- and antiapoptotic protein expression in macrophages. To determine why there is an absence of apoptosis in HIV-infected macrophages, we
analyzed the induction of proapoptotic (BAD and BAX) and antiapoptotic (BCLXL and BCL2) proteins in HIV-infected macrophages (Fig. 2A). At day 10 postinfection, the expression levels of two antiapoptotic factors, BCL2 and BCLXL, were increased \( (P < 0.01) \) whereas those of the proapoptotic proteins BAX and BAD were decreased.
Next, we determined if the HIV antigens gp120, Tat, and RNA40 (a 20-mer synthetic single-stranded RNA oligoribonucleotide derived from the HIV long-terminal repeat and TLR8 ligand) influenced the expression of these pro- and antiapoptotic proteins in macrophages. Both gp120 and Tat increased the expression levels of BCL2 and BCLXL in a dose-dependent manner while simultaneously decreasing those of both BAD and BAX (see Fig. S1 in the supplemental material). In contrast, while RNA40 also dose dependently increased the expression of both BCL2 and BCLXL, it also increased the expression of BAD while having no effect on BAX expression levels (Fig. S1).

A key event in apoptosis is the translocation of BCL2L11 from the cytoskeleton to the mitochondrial membrane (27). In healthy cells, BCL2L11 is inactivated by its interaction with dynein light chain LC8-type 1 (DYNLL1), which recruits it to the microtubule-based dynein motor complex (28). During HIV infection of CD4+ T cells, HIV Tat binds tubulin through a four-amino-acid subdomain of its conserved core region, altering microtubule dynamics (29, 30) and leading to the mitogen-activated protein kinase 8 (MAPK8)-mediated phosphorylation of BCL2L11 and dissociation from DYNLL1 and the dynein motor complex. Phosphorylated BCL2L11 then translocates to the mitochondrial membrane, where it directly activates the BAX/BAK-dependent mitochondrial pathway of apoptosis and cell death (27, 31, 32). Therefore, we examined whether HIV infection also induces the translocation of BCL2L11 to mitochondria in macrophages despite the absence of HIV-mediated cytopathogenesis. Using cell fractionation, we determined that BCL2L11 translocates to mitochondria, suggesting that HIV is inhibiting the mitochondrial pathway of apoptosis (Fig. 2C).

HIV infection increases the expression of TREM1 in macrophages. As HIV infection resulted in increased expression of BCL2 and as overexpression of TREM1 can induce the expression of BCL2 (25), we examined whether macrophages productively infected with HIV express higher levels of TREM1. At 10 days after infection, HIV-infected macrophages displayed increased levels of TREM1 ($P = 0.02$; Fig. 3A). We then investigated the mechanism by which HIV induces TREM1 expression. As NF-κB transcriptionally regulates TREM1 in macrophages in response to LPS (33), we utilized RNA interference (RNAi) for the NF-κB subunit RELA to investigate whether NF-κB regulates TREM1 expression in response to HIV infection. RELA silencing resulted in the almost complete ablation of TREM1 expression in response to HIV (Fig. 3B), suggesting that NF-κB regulates TREM1 expression in HIV-infected macrophages. To confirm the sequence-specific interaction of RELA with the TREM1 promoter, we analyzed the promoter region of TREM1 using Genomatix (version 3.11; Intrexon) and found consensus binding sites for NF-κB. We performed an electrophoretic mobility shift assay using a biotinylated probe that encompassed the predicted binding site. This probe showed evidence of strong binding to nuclear proteins and was supershifted by antibodies against RELA and NFKB1 but not by antibodies against REL, NFKB2, RELB, or BCL3 (Fig. 3C). To investigate whether HIV triggers the classical NF-κB signaling pathway, we analyzed the ability of RELA to physically interact with NFKB1 p50 and to translocate to the nucleus using coimmunoprecipitation. NFKB1 p50/RELA complexes were detected in both the cytoplasm and the nucleus, whereas no NFKB1 p50/RELB complexes were detected (Fig. 3D). Interestingly, although uninfected cells transfected with siRELA showed apoptosis levels similar to the levels seen with those transfected with control RNAi, RELA-silenced HIV-infected macrophages showed an increased number of apoptotic cells (Fig. 3E; $P < 0.001$).

We next investigated whether HIV gp120, Tat, and RNA40 induce TREM1 expression in human macrophages. All three antigens induced a dose-dependent increase in TREM1 expression; HIV Tat and gp120 induced 18-fold (±3.9 SEM; $P = 0.0005$) and 7.5-fold (±2.9 SEM; $P = 0.0075$) increases, respectively, at 10 ng ml$^{-1}$, and HIV RNA40 induced a 42-fold increase (±4.6 SEM; $P < 0.0001$) at 5 μg ml$^{-1}$ (Fig. 4A). Similarly to HIV-infected macrophages, RELA silencing of uninfected macrophages (Fig. 4B and C) significantly increased cell death in response to the presence of HIV Tat, gp120, or
RNA40 (Fig. 4D), while it also attenuated TREM1 expression (Fig. 4E). Electrophoretic mobility shift assay (EMSA) demonstrated that HIV Tat, gp120, and RNA40 rapidly induced NF-κB DNA binding, with supershift analysis demonstrating that the NF-κB complex consisted primarily of NFKB1 and RELA subunits (Fig. S2A). We then investigated whether these antigens trigger the classical NF-κB signaling pathway. HIV Tat, gp120, and RNA40 all triggered the rapid processing of NFKB1 p105 to p50, the dimerization of NFKB1 p50 with RELA, and the subsequent nuclear translocation (Fig. S2B and C).

TREM1 is required for HIV-infected macrophage survival. To address the role of TREM1 in macrophage survival during HIV infection, we used TREM1 small interfering RNA (siRNA). We reasoned that if TREM1 is involved in promoting cell survival, then silencing TREM1 should increase cell death during HIV infection. Macrophages infected with HIV for 10 days were transfected with control scrambled siRNA (siNS) or TREM1 siRNA (siTREM1) for 48 h and then either assessed for apoptotic ssDNA damage or stained with annexin A5 (ANXA5) and propidium iodide (PI) (Fig. 5A). Uninfected cells transfected with siNS showed apoptosis levels similar to those transfected with siNS. In contrast, TREM1 siRNA-transfected HIV-infected macrophages showed an increased number of apoptotic cells (83.9% ± 6%) compared with HIV-infected macrophages transfected with siNS (4.2 ± 1.4%; P = 0.02). Because TREM1 silencing induced...
apoptosis in productively infected cells, we posited that \textit{TREM1} silencing altered the balance of anti- and proapoptotic proteins. Indeed, \textit{TREM1} silencing resulted in a significant reduction in HIV-induced BCLXL and BCL2 expression ($P < 0.0003$) while having a modest impact on BAD and BAX expression ($P > 0.06$; Fig. 5B).

Next, we analyzed the effect of HIV antigen-induced apoptosis on \textit{TREM1}-silenced cells (Fig. 6A) after exposure of the cells to HIV antigens for 24 h. Importantly, exposure of siNS-transfected cells to HIV Tat, gp120, or RNA40 did not result in increased
apoptosis \((P > 0.4; \text{Fig. 6B})\). In contrast, and similarly to HIV infection, \(TREM1\)-silenced macrophages treated with HIV antigens displayed an enhanced number of apoptotic cells compared with vehicle-treated cells (Fig. 6B). \(TREM1\)-silenced cells exposed to Tat exhibited the highest proportion of apoptotic cells (mean, 92.4 ± 0.4%; \(P < 0.0001\)) closely followed by RNA40 (mean, 84.7% ± 1.3%; \(P < 0.0001\)) and gp120 (mean, 73.5% ± 2.2%; \(P < 0.0001\)). Importantly, staurosporine exposure similarly induced apoptosis in both siNS-transfected and si\(TREM1\)-transfected cells (mean, 94.5% ± 1.4% versus 94.0% ± 0.4%; \(P = 0.4\) (Fig. 6B). We then evaluated the effect of \(TREM1\) silencing on HIV antigen-induced expression of antiapoptotic and proapoptotic proteins. \(TREM1\) silencing decreased BCLXL and BCL2 expression in uninfected macrophages by more than 86% \((P < 0.001)\) while having no noticeable effect on the expression of either BAX or BAD (Fig. 6C). Of note, neither HIV Tat nor gp120 nor RNA40 had a significant effect on the expression of BCLXL, BCL2, BAX, or BAD compared to the vehicle in these \(TREM1\)-silenced cells. Collectively, these data suggest that induction of \(TREM1\) by HIV inhibits apoptosis and prolongs survival of HIV-infected macrophages.
Next, we sought to define the expression of BCL2 in relation to specific ligation of TREM1 by the use of monoclonal antibodies (mTREM1). BCL2 expression was significantly increased in macrophages in response to mTREM1 compared with the isotype control antibody ($P < 0.0006$; Fig. 7A). We then sought to define the relationship between BCL2 expression and cell survival during HIV infection of macrophages using RNAi for BCL2. BCL2 silencing of HIV-infected macrophages led to an increased number of apoptotic cells ($86.3\%\pm5.4\%$; $P < 0.0001$) (Fig. 7B). Similarly, BCL2 silencing also significantly increased uninfected macrophage cell death in response to exposure to HIV Tat, gp120, or RNA40 ($P < 0.001$; Fig. 7C).

**TREM1 silencing leads to the induction of apoptosis through the intrinsic pathway.** The interactions between anti- and proapoptotic proteins exist in a delicate balance at the mitochondrial membrane that determines cell fate, with mitochondrial fragmentation a central mechanism of apoptosis. Loss of balance between pro- and antiapoptotic BCL2 proteins leads to outer membrane permeabilization of mitochondria, calcium influx, and mitochondrial fragmentation, leading to the subsequent activation of effector caspases. In contrast, a fused mitochondrial phenotype protects cells from programmed cell death. Therefore, we examined the effect of TREM1 silencing on the mitochondrial membrane potential ($\Delta \psi /m$) using DIOC1(5) ($1,1',3,3',3',3'-$hexamethyldiiodocarbocyanine iodide). Following TREM1 silencing, there was no difference in $\Delta \psi /m$ levels between siNS-transfected HIV-infected and uninfected macro-

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**Fig 6** Uninfected macrophages were transfected with siTREM1 or siNS for 48 h and then treated with vehicle, 10 ng ml$^{-1}$ Tat, 10 ng ml$^{-1}$ gp120, 5 $\mu$g ml$^{-1}$ RNA40, or 5 $\mu$g ml$^{-1}$ RNA41 for 24 h. (A) Cells were assessed for TREM1 expression. (Left) Representative Western blots of TREM1 with antibodies against TREM1 and ACTB. (Right) Densitometric analysis of blots, $n = 4$. (B) Cells were assessed for cell death. (Left) Cells were harvested and stained with annexin V (ANXA5) and PI. The frequency of ANXA5-positive or ANXA5/PI-positive labeling is illustrated as a plot. (Right) Quantification of the number of cells with apoptotic ssDNA using an ssDNA ELISA, $n = 4$. (C) Cells were lysed and subjected to Western blotting. (Left) Representative Western blots of BCLXL, BCL2, BAD, and BAX with antibodies against BCLXL, BCL2, BAD, BAX, and ACTB. (Right) Densitometric analysis of blots, $n = 4$. 

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phages, indicating that mitochondria are still functional during infection. Similarly, we observed no difference in Δ\(\psi\)m levels between uninfected cells transfected with siTREM1 and those transfected with siNS. In contrast, we observed a marked decrease in DiIC1(5) staining intensity in TREM1-silenced HIV-infected macrophages (Fig. 8A). These data suggest that TREM1 silencing results in the depolarization of the Δ\(\psi\)m in HIV-infected macrophages but not in uninfected macrophages.

A key event in apoptosis is the formation of the transition pore in the mitochondrial membrane and the release of mitochondrial factors such as cytochrome c (CYCS) into
To determine whether TREM1 silencing in HIV-infected macrophages leads to the mitochondrial release of CYCS into the cytoplasm, we performed cell fractionation. Our data indicate that mitochondria retain CYCS in siNS-transfected HIV-infected and uninfected cells (Fig. 8B). In contrast, TREM1 silencing induces the release of CYCS into the cytoplasm in HIV-infected macrophages but not in uninfected macrophages (P < 0.018; Fig. 8B). In the presence of ATP/dATP, CYCS released into the cytosol binds to and triggers the oligomerization of cytosolic apoptotic protease activating factor 1 (APAF1). The resultant complex recruits and activates multiple copies of the apical caspase in the mitochondrial pathway of apoptosis, caspase 9 (CASP9). TREM1 silencing resulted in the cleavage and activation of CASP9 in HIV-infected macrophages but had no effect on CASP9 activation in uninfected macrophages (Fig. 8C).

We next assessed the effect of HIV Tat, gp120, and RNA40 on ΔΨm, CYCS release, and subsequent CASP9 activation in uninfected macrophages. There was no significant difference in ΔΨm after Tat, gp120, and RNA40 exposure in siNS-transfected macrophages, indicating that Tat, gp120, and RNA40 have little effect on ΔΨm in the presence of TREM1 (Fig. S3A). In contrast, we observed a marked decrease in DiIC1(5) staining intensity in TREM1-silenced macrophages after Tat, gp120, and RNA40 treatment, suggesting that TREM1 is essential for maintaining ΔΨm after exposure to HIV antigens. Similarly, treatment of siNS-transfected macrophages with HIV Tat, gp120, or RNA40 did not induce the release of CYCS into the cytosol or induce CASP9 cleavage (Fig. S3B). In contrast, HIV Tat, gp120, and RNA40 exposure of TREM1-silenced cells resulted in CYCS release from the mitochondria and subsequent CASP9 cleavage. Together, these results indicate that TREM1 is essential for macrophage survival of acute HIV infection.

**TREM1 enhances mitochondrial integrity by inducing mitofusin expression.** At the molecular level, mitochondrial fusion is a two-step process requiring the coordinated fusion of outer and inner mitochondrial membranes by separable sequential events. In humans, this process depends on distinct mitochondrial sublocalization of three fusogenic proteins: mitofusins 1 and 2 (MFN1 and MFN2) (embedded within the outer mitochondrial membrane) and optic atrophy 1 (located at the inner mitochondrial membrane). In addition to the well-characterized role of the profission dynamin-
1-like protein in mediating mitochondrial fission in cells undergoing cell death, inhibition of mitochondrial fusion is also an established consequence of apoptosis induction and occurs around the time of BAX activation. During oxygen glucose deprivation, MAPK1/2 phosphorylates MFN1, inhibiting its profusion function, and triggers its association with and oligomerization of BAK, facilitating the subsequent release of CYCS from mitochondria through BCL2L11 (34). Similarly, in response to cellular stresses, MAPK8 phosphorylates MFN2, leading to its ubiquitin-dependent proteasomal degradation and subsequently to mitochondrial fragmentation and enhanced apoptotic cell death (35). Therefore, we assessed mitofusin expression in HIV-infected macrophages. We found that at day 10 postinfection, the expression levels of both MFN1 and MFN2 were increased (P < 0.007; Fig. 9A). We next assessed the effect of HIV Tat, gp120, and RNA40 on mitofusin expression. All three antigens induced dose-dependent increases in both MFN1 and MFN2 (Fig. S4A).

We next assessed the effect of TREM1 silencing on mitochondrial integrity in HIV-infected cells by determining mitofusin expression post-TREM1 RNAi treatment. TREM1 silencing resulted in a significant reduction in HIV-induced MFN1 and MFN2 to levels similar to those seen with uninfected cells (P > 0.3; Fig. 9B). When we exposed TREM1-silenced uninfected macrophages to HIV Tat, gp120, or RNA40, each still increased MFN1 expression, although the increase was appreciably less than in the siNS cells (Fig. S4B). In contrast, TREM1 silencing reduced MFN2 expression in all treatments, including the vehicle treatment. Notably, this decrease was significantly enhanced post-HIV Tat treatment (mean, 87% ± 1.5% reduction; P < 0.0001), gp120 treatment (mean, 89% ± 0.5% reduction; P = 0.0001), and RNA40 treatment (mean, 85% ± 1.5% reduction; P = 0.0007) compared with the vehicle. These data suggest that TREM1 expression is required for the HIV-mediated upregulation of mitofusins.

**DISCUSSION**

Efforts to purge the latent HIV reservoir predominantly involve reactivation of viral production from latently infected CD4+ T cells followed by clearance of these cells through a combination of viral and cell-mediated cytoxicity while ART prevents subsequent rounds of infection. Although this strategy has shown promise in cell lines, it has been ineffective on primary resting CD4+ T cells from patients on suppressive ART and in *in vivo* studies (36–40). This approach is even less effective in macrophages, as HIV-infected macrophages are particularly resistant to HIV-mediated apoptosis and CD8+ T cell-mediated killing (8–10). Thus, we require an improved understanding of the precise mechanisms underlying resistance of macrophage and microglia to cytopathic effects of HIV in order to develop approaches capable of efficiently and effectively eliminating infected cells. Depleting HIV-infected macrophages may result in the decline of cytokine and chemokine production and hence suppress the excessive inflammation and aberrant immune cell recruitment that persists despite suppressive ART and which has been associated with poor prognosis and ongoing viral replication. The present study demonstrated that HIV upregulates the expression of TREM1 in...
macrophages and that expression of TREM1 is essential in protecting HIV-infected macrophages from HIV-induced apoptosis.

Infected macrophages display an increase in the levels of expression and recruitment of the proapoptotic protein BCL2L11 into mitochondria. Proapoptotic BH3-only proteins such as BCL2L11 bind to antiapoptotic proteins, allowing the proapoptotic multidomain proteins BAX and BAK1 to oligomerize and form channels on the mitochondrial membrane and leading to CYCS release and apoptosis. It is possible that the observed upregulation and sequestration of BCL2L11 into the mitochondria of HIV-infected macrophages are early steps in the apoptotic response to a viral infection, a response that HIV subsequently blocks downstream as we do not observe the release of CYCS from mitochondria nor an increase in apoptosis once infection is established. However, when we silenced TREM1, the expression levels of BCL2, BCLXL, and mitofusins all decreased, while the expression levels of BAX and BAD remained unchanged. This coincided with the release of CYCS from mitochondria and an increase in apoptosis.

Although TREM1 activity was initially described only in the context of bacterial or fungal infections, a role for TREM1 during viral infections is emerging. Early studies focused on and demonstrated that TREM1 signaling modulates pattern recognition receptor virus-associated inflammation and that TLR signaling upregulates TREM1 expression. TL3, TL7, TL8, and TL9 are all common TLRs that respond to viral PAMPS. For example, TREM1-activated murine bone marrow-derived dendritic cells display increased tumor necrosis factor (TNF) expression after treatment using CpG DNA (a TL9 ligand) (41). Similarly, TREM1-activated PBMC display enhanced production of TNF following stimulation with either CpG DNA or the TL3 ligand poly(I:C) (42); poly(I:C) induces transcription of TREM1 in human primary monocytes (43). In neutrophils, TREM1 activation acts synergistically with TL7 and TL8 ligand-mediated activation with regard to effector mechanisms but does so antagonistically for survival (44).

In the present study, we demonstrated that RNA40, a TL8 ligand, upregulates TREM1 expression and that TREM1 is required for macrophage survival. With regard to viral infections, West Nile virus (WNV) increases TREM1 expression in murine macrophages and dendritic cells (45) and enhances TREM1 signaling in murine livers (46). Consistent with this, exogenous activation of TREM1 increases expression levels of the interferon alpha 2 gene (IFNA2), TNF, and the interleukin-6 gene (IL-6) in WNV-infected murine embryonic fibroblasts, while antagonizing TREM1 leads to a reduction in their expression, suggesting that TREM1 plays a role in modulating the inflammatory response to WNV (45). Similarly, Zika virus also upregulates TREM1 signaling (47). Furthermore, the Filoviridae Marburg virus and Ebola virus both upregulate the expression and activation of TREM1 on neutrophils following internalization (20). Although Ebola virus does not replicate in these cells, it is possible that Ebola virus glycoprotein, which interacts with neutrophils, acts as a ligand for TREM1 and contributes to the cytokine storm associated with the terminal stage of Ebola virus infection (20, 48, 49). While we showed that HIV infection of macrophages increases TREM1 expression and is essential for cell survival, the role of TREM1 in the inflammatory response to HIV infection remains to be elucidated.

Cells also shed soluble TREM1 (sTREM1) from cell membranes. Although the role of sTREM1 in the context of viral pathogenesis is unknown, sTREM1 levels are increased during infection with a number of viruses, including Crimean-Congo hemorrhagic fever orthonairovirus (50), hepatitis B virus (51), WNV (45), dengue virus (52), and hepatitis C virus (51). It is possible that such increases in the levels of sTREM1 could represent a virally induced compensatory mechanism to counteract inflammatory processes. Alternatively, such increases could represent a host-induced mechanism to control tissue damage by attenuating downstream inflammatory signals. Studies are required to elucidate this function and to identify the potential of sTREM1 as a marker of disease severity in acute infections by such viruses as influenza virus and dengue virus or in chronic infections such as HIV.

Evading apoptosis by upregulation of antiapoptotic or downregulation of proapo-
ptotic proteins is an important step in altering cell survival. Importantly, the implementation of macrophage apoptosis may contribute to viral latency and persistence during HIV infection (10, 53). In summary, this study demonstrated an important role and a mechanism for TREM1 in prolonging survival of HIV-infected macrophages. Our report also highlights TREM1 as a therapeutic target to clear the macrophage HIV reservoir and suggests that current HIV reservoir paradigms include the targeting of TREM1 in any effort to achieve HIV eradication.

MATERIALS AND METHODS

Macrophages. Venous blood was drawn from human subjects using protocols approved by the Human Research Protections Program of the University of California, San Diego, in accordance with the requirements of the Code of Federal Regulations (CFR) on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56). All volunteers gave written informed consent prior to their participation. PBMC were isolated from whole blood by density gradient centrifugation over Ficoll-Paque Plus (GE Healthcare). Macrophages were prepared by incubating $6 \times 10^5$ PBMC ml$^{-1}$ in macrophage media (RPMI 1640 [Gibco] supplemented with 10% [vol/vol] heat-inactivated fetal bovine serum [FBS; Sigma]; 2 mM l-glutamine, 0.1 mg ml$^{-1}$ streptomycin, and 100 U ml$^{-1}$ penicillin [all Gibco]; and 10 ng ml$^{-1}$ CSF1 (Peprotech), after which nonadherent cells were removed by aspiration and washed with Dulbecco’s phosphate-buffered saline (Gibco). Adherent cells were further incubated in macrophage media for 10 days at 37°C and 5% CO$_2$, with medium changes performed every 2 days, before use. This protocol results in a high proportion of CD14$^+$: sialic acid binding Ig-like lectin 1 (SIGLEC1)$^+$ macrophages that are permissive to HIV infection (54, 55).

HIV. HIV Ba-L was obtained through the NIH AIDS Reagent Program from Suzanne Gartner, Mikulas Popovic, and Robert Gallo (56, 57). Virus stocks were prepared as previously described (58). HIV infectivity levels were calculated as the 50% tissue culture infectious doses (TCID$_{50}$) as described previously (59) and multiplicity of infection (MOI) levels confirmed using TZM-bl cells (from John C. Kappes, Xiaoyun Wu, and Tranzyme Inc.) (60). Macrophages were infected with HIV at an MOI of 0.04 unless otherwise stated.

Chemicals. HIV$_{R5}$ gp120 (AAA44191.1) was obtained through the NIH AIDS Reagent Program. LyoVec precomplexed RNA40 (catalog no. tfl-lrna40) and LyoVec precomplexed RNA41 (a derivative of RNA40 in which adenosine replaced all uracil nucleotides; catalog no. tfl-lrna41) were purchased from InvivoGen. Full-length HIV Tat (B.FR.83.HXB2; K03455 [61]) was prepared on 0.5mmol 4(hydroxy-ethyl)phenoxymethyl-copolystyrene-1% divinylbenzene-resin (Applied Biosystems) using an Applied Biosystems 433A peptide synthesizer with FastMoc Chemistry as previously described (62). Peptides were deprotected and cleaved from the support using trifluoroacetic acid supplemented with 5% (vol/vol) H$_2$O, 10% (vol/vol) (methylsulfanyl)benzene, and 5% (vol/vol) ethane-1,2-dithiol. Crude product was precipitated in 2-methoxy-2-methylpropane at –20°C, washed several times, filtered, and dried under vacuum. Tat was purified by reverse-phase high-performance liquid chromatography (HPLC) as previously described (29). HPLC analysis was performed as previously described (62), amino acid analysis was performed on a Beckman model 6300 amino acid analyzer, mass spectrometry was carried out using an Ettan matrix-assisted laser desorption ionization—time of flight apparatus (Amersham Biosciences), and concentrations were determined using a Beckman DU640 UV light-visible light spectrometer and an extinction coefficient of 8,480 M$^{-1}$ cm$^{-1}$ (63). Stauroporine (Sigma) was used at 1 μM. Monoclonal TREM1 antibody was purchased from R&D Systems (catalog no. MAB1278; RRID: AB_2208452).

Apoptosis assays. Cell death was estimated using Alexa Fluor 488-tagged ANX5 and PI staining on a FACSCalibur flow cytometer and analyzed using CellQuest Pro (both BD Biosciences). Apoptotic ssDNA damage measurements were made using an ssDNA apoptosis enzyme-linked immunosorbent assay (ELISA) kit (Millipore) as described previously (64). To assess the extent of necrotic cell death, the lactate dehydrogenase (LDH) activity of supernatants was measured using a mixture of diaphorase/NAD$^+$ and 3-(4-iodophenyl)-2-(4-nitrophenyl)-5-phenyl-2H-tetrazol-3-ium chloride/sodium 2-hydroxypropanoate and percent cytotoxicity calculated according to the protocol of the manufacturer (Roche). The mitochondrial membrane potential ($\Delta\psi_m$) gradient was measured by flow cytometry using 50 nM 1,1',3,3',5,5'-hexamethylindodicarbo-cyanine iodide [DiIC1(5); Molecular Probes].

Flow cytometry. Intracellular staining of HIV p17 was performed as previously described (65) using rabbit anti- mouse anti-gag-p17 (Abcam catalog no. ab9067; RRID: AB_306976) followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology catalog no. sc-2010; RRID: AB_631735).

siRNA transfection. Macrophages were transfected with Thermo Fisher Silencer Select BCL2 (identifier [ID] s1916), RELA (ID s11915), TREM1 (ID s28910), or control (catalog no. 4390846) siRNA (siNS) using Lipofectamine RNAiMAX transfection reagent (Thermo Fisher) in Opti-MEM (Gibco) according to the manufacturer’s instructions. At 48 h, cells were analyzed for target gene silencing and used in experiments. Transfection efficiency was assessed with BLOCK-iT Alexa Fluor red fluorescent control (Thermo Fisher) using flow cytometry.

Western blotting. The following antibodies were used: anti-ACTB from Sigma (catalog no. A2228; RRID: AB_476697); anti-LYM-1 from Abcam (catalog no. ab9080; RRID: AB_2050414); and anti-BAD (catalog no. 5233; RRID: AB_2744530), anti-BAX (catalog no. 5023; RRID: AB_2744530), anti-BCL2 (catalog no. 5071; RRID: AB_2744528), anti-BCL2L11 (catalog no. 2933; RRID: AB_1030947), anti-BCLXL (catalog no. 2764; RRID: AB_2288008), anti-CASP3 (catalog no. 9662; RRID: AB_331439), anti-CASP9 (catalog no. 9502; RRID: AB_2068621), anti-CYCS (catalog no. 9502; RRID: AB_2637071), anti-H3C1 (catalog no. 4499; RRID: AB_133439).
Whole-cell lysates were prepared using 20 mM HEPES (Gibco), 150 mM NaCl (Fisher), and 1 mM EDTA (Sigma) supplemented with 1% (vol/vol) Triton X-100 (Sigma) and 1% (vol/vol) Halt protease and phosphatase inhibitor cocktail (Thermo Scientific). Cytoplasmic fractions for parallel mitochondrion analysis were prepared using a mixture containing 220 mM d-mannitol, 68 mM sucrose, 50 mM HEPES/N-2-hydroxyethyl)piperazine-N'2-ethanesulfonic acid (HEPES)-KOH (pH 7.4), 50 mM KCl, 5 mM EGTA, 2 mM MgCl₂, 1 mM dithiothreitol (all Sigma), and 1% (vol/vol) Halt protease and phosphatase inhibitor cocktail. After 30 min on ice, cell homogenates were spun at 14,000 g for 15 min and supernatants harvested. For mitochondrion preparations, 1 × 10⁶ macrophages were scraped and pelleted by centrifugation at 800 × g for 10 min. Cells were resuspended in 1.2 ml cold mitolysis buffer (20 mM HEPES [pH 7.5], 10 mM KCl, 1.5 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride [all Sigma]) and incubated for 3 min on ice. Cells were homogenized with 10 strokes of a 27-gauge needle and centrifuged at 750 × g for 15 min at 4°C. The supernatant (cytosolic fraction) was removed, and the pellet containing mitochondria was resolved in 50 μl mitolysis buffer. Cytoplasmic and nuclear fractions were prepared for communoprecipitation and EMSA using NE-PER nuclear and cytoplasmic extraction reagent according to the protocol of the manufacturer (Thermo Fisher). For communoprecipitation, 50 μg anti-NFKB1 (Cell Signaling Technology catalog no. 12540; RRID:AB_2667614) was immobilized using a coupling gel and then 50-μg amounts of cell lysates were incubated with the antibody-immobilized coupling gel using a Thermo Scientific-Pierce communoprecipitation kit. Lysates were resolved using a 2-(bis(2-hydroxyethyl)amino)-2-(hydroxymethyl)propane-1,3-diol-buffered 12% polyacrylamide gel and transferred to 0.2-μm-pore-size nitrocellulose membranes (Thermo Scientific), followed by detection with primary antibodies, alkaline phosphatase-tagged secondary antibodies (Invitrogen), and 0.25 mM disodium 2-chloro-5-(4-methoxySpiro{1,2-dioxetane-3,2-(5-chloro)tricyclo[3.3.1.13,7]decane}-4)-yl)-1-phenyl phosphate supplemented with 5% (vol/vol) Nitro-Block II (both Applied Biosystems). The densities of the target bands were compared to that of the applicable reference, i.e., ACTB (for whole-cell lysates and cytoplasm fractions), COX41 (for mitochondrion fractions), or H3C1 (for nuclear fractions), and the data were calculated using ImageJ (NIH).

**Electrophoretic mobility shift assay.** EMSA was conducted using a LightShift chemiluminescent EMSA kit (Thermo Fisher) according to the manufacturer's instructions using the following biotin end-labeled duplex DNA oligonucleotide: 5’-AGGTGTTCACCTCTGGATCTTATCCTTCAAAGGTCCACTTTTAA-3’. For supershift experiments, nuclear extracts were preincubated with one of the following antibodies: anti-NFKB1 (catalog no. 13586; RRID:AB_2665516), anti-NFKB2 (catalog no. 3017; RRID:AB_10697356), anti-RELA (catalog no. 8242; RRID:AB_10544537), anti-MFN1 (catalog no. 14739; RRID:AB_2744531), anti-MFN2 (catalog no. 9482; RRID:AB_2546259), anti-NFKB1 (catalog no. 13586; RRID:AB_2665516), and anti-RELA (catalog no. 8242; RRID:AB_10859369) from Cell Signaling Technology.

**Supplemental material**

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.02638-19.

**FIG S1**, TIF file, 2.9 MB.

**FIG S2**, TIF file, 2.5 MB.

**FIG S3**, TIF file, 2.9 MB.

**FIG S4**, TIF file, 2.9 MB.

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REFERENCES

1. Igarashi T, Brown CR, Endo Y, Buckler-White A, Plishka R, Bischoffger N, Hirsh V, Martin MA. 2001. Macrophage are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): implications for HIV-1 infections of humans. Proc Natl Acad Sci U S A 98:6568–663. https://doi.org/10.1073/pnas.021551798.

2. Abbas W, Tariq M, Iqbal M, Kumar A, Herbein G. 2015. Eradication of HIV-1 from the macrophage reservoir: an uncertain goal? Viruses 7:1578–1598. https://doi.org/10.3390/v7101578.

3. Ganor Y, Reif A, Sennepin A, Dutertre CA, Prevedel L, Xu L, Tudor D, Charmetateau B, Couedel-Courretelle A, Marion S, Zenak AR, Jourdain JP, Zhou H, Schmitt A, Capron C, Eugenin EA, Cheynier R, Revel M, Cristofori S, Hosmalin A, Bomsel M. 2019. HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. Nat Microbiol 4:1644. https://doi.org/10.1038/s41564-018-0335-z.

4. Honeycutt JB, Wahl A, Baker CE, Spagnuolo RA, Foster J, Zakharova O, Russell RA, Franklin JB, Wahl A, Baker CE, Spagnuolo RA, Foster J, Zakharova O, Russell RA, Franklin JB. 2014. HIV-1 infection and neutralizing antibody evasion mediated by the macrophage-T cell virological synapse. J Virol 88:2025–2034. https://doi.org/10.1128/JVI.03243-15.

5. Abbas W, Herbein G. 2014. Plasma membrane signaling in HIV-1 infection. Biochim Biophys Acta 1838:1132–1142. https://doi.org/10.1016/j.bbamem.2013.06.020.

6. Clayton KL, Collins DR, Lengieza J, Ghebremichael M, Dotiwala F, Lieber MA, Torres-González R, Estrada-García I, López-Macías C, Isibasi A. 2006. Triggering receptor expressed on myeloid cells (TREM-1) is regulated by pro-inflammatory cytokines and neutralizing antibody evasion mediated by the macrophage-T cell virological synapse. J Virol 80:7235–7244. https://doi.org/10.1128/JVI.00434-06.

7. Wong-Baeza I, González-Roldán N, Ferat-Osorio E, Esquivel-Callejas N, Aduna-Vicente R, Arriaga-Pizzo L, Astudillo-de la Vega H, Villasen-Keeve TA, Torres-Rodríguez R, Estrada-García I, López-Macías C, Isibasi A. 2006. Triggering receptor expressed on myeloid cells (TREM-1) is regulated post-transcriptionally and its ligand is present in the sera of some septic patients. Clin Exp Immunol 145:438–445. https://doi.org/10.1111/j.1365-2249.2006.03158.x.

8. Haselmaier P, Grosse-Hovest L, von Landenberg P, Schich H, Radak MP. 2007. TREM-1 ligand expression on platelets enhances neutrophil activation. Blood 110:1029–1035. https://doi.org/10.1182/blood-2007-01-069195.

9. El Mezayen R, El Gazzar M, Seeds MC, McCall CE, Dreskin SC, Nicoll MS. 2007. Endogenous signals released from necrotic cells augment inflammatory responses to bacterial endotoxin. Immunol Lett 111:36–44. https://doi.org/10.1016/j.imlet.2007.04.011.

10. Pelham CJ, Pandya AN, Agrawal DK. 2014. Triggering receptor expressed on myeloid cells receptor family modulators: a patent review. Expert Opin Ther Pat 24:1338–1395. https://doi.org/10.1517/13547767.2014.977865.

11. Yuan Z, Syed MA, Panchal D, Joo M, Colonna M, Brantly M, Sadikot RT. 2014. Triggering receptor expressed on myeloid cells 1 (TREM-1)-mediated Bcl-2 induction prolongs macrophage survival. J Biol Chem 289:15118–15129. https://doi.org/10.1074/jbc.M113.536490.

12. Frankfurt OS, Krishan A. 2001. Enzyme-linked immunosorbent assay (ELISA) for the specific detection of apoptotic cells and its application to rapid drug screening. J Immunol Methods 253:133–144. https://doi.org/10.1016/s0022-1759(00)00387-8.

13. Castellano P, Prevedel L, Eugenin EA. 2017. HIV-infected macrophages and microglia that survive acute infection become viral reservoirs by a mechanism involving Bim. Sci Rep 7:12866. https://doi.org/10.1038/s41598-017-12758-w.

14. Puthalakath H, Huang DC, O'Reilly LA, King SM, Strasser A. 1999. The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynin motor complex. Mol Cell 3:287–296. https://doi.org/10.1016/s1097-2765(00)80456-6.

15. Campbell GR, Pasquier E, Watkins J, Bourgarel-Rey V, Peyrot V, Esquivié D, Barbir P, de Mareuil J, Brauger D, Kaleebu P, Yrrell DL, Loret EP. 2004. The glutamine-rich region of the HIV-1 Tat protein is involved in T-cell apoptosis. J Biol Chem 279:48197–48202. https://doi.org/10.1074/jbc.M406195200.

16. de Mareuil J, Carre M, Barbir P, Campbell GR, Lancelot S, Opi S, Esquivié

17. https://doi.org/10.1371/journal.pone.0055199.
47. Campbell et al. 2018. SMAC induces apoptotic cell death in host tissuedelete due to Zika virus (ZIKV) infection in retinal pigment epithelium. Sci Rep 8:11209. https://doi.org/10.1038/s41598-018-29329-2.
48. Yang Z, Delgado R, Xu L, Todd RF, Nabel EG, Sanchez A, Nabel GJ. 1998. Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. Science 279:1034–1037. https://doi.org/10.1126/science.279.5353.1034.
49. Wauquier N, Becqquart P, Pauwels C, Baze S, Leroy EM. 2010. Human fatal foie gras disease in France: a zoonosis with an aberrant innate immune response and with massive lymphocyte apoptosis. PLoS Negl Trop Dis 4:e837. https://doi.org/10.1371/journal.pntd.0000837.
50. Altay FA, Elaldi N, Şentürk Ğ, Altın N, Gözel MG, Albayrak Y, Şenchin I. 2016. Serum STREM-1 level is quite higher in Crimean Congo hemorrhagic fever, a viral infection. J Med Virol 88:1473–1478. https://doi.org/10.1002/jmv.24496.
51. Koizum JH, Trautmann T, Carambia A, Preti M, Lutgehetmann M, Krech T, Wiegard C, Heeren J, Herkel J. 2016. Attenuated viral neuropathogenesis in Tcm1−/− mice is associated with reduced inflammatory activity of neutrophils. Sci Rep 6:28556. https://doi.org/10.1038/srep28556.
52. Ruiz-Pacheco JA, Vivanco-Cid H, Izaguirre-Hernández IY, Estrada-García I, Arriaga-Pízano L, Chacón-Salinas R, Fonseca-Coronado S, Vaughan G, Tovar KR, Rivera-Osorio MP, Escobar-Gutiérrez A. 2014. TREM-1 modulation during early stages of dengue virus infection. Immunol Lett 158:183–188. https://doi.org/10.1016/j.imlet.2014.01.003.
53. Abbas W, Khan KA, Aman U, Tripathy MK, Dichamp I, Keita M, Maehlehnert U, Rohr O, Herbein G. 2014. Blockade of BFA-mediated apoptosis in macrophages by the HIV-1 Nef protein. Cell Death Dis 5:e1080. https://doi.org/10.1038/cddis.2014.16.
54. Jobe O, Trinh HV, Kim J, Alsalmi W, Tovanabutra S, Ehrenberg PK, Peachman KK, Gao G, Thomas R, Kim JH, Michael NL, Alving CR, Rao VB, Rao M. 2016. Effect of cytokines on Siglec-1 and HIV-1 entry in monocyte-derived macrophages: the importance of HIV-1 envelope V1V2 region. J Leukoc Biol 99:1089–1106. https://doi.org/10.1189/jlb .20A0815-361R.
55. Jobe O, Kim J, Tycksen E, Onkar S, Michael NL, Alving CR, Rao M. 2017. Human primary macrophages derived in vitro from circulating monocytes comprise adherent and non-adherent subsets with differential expression of Siglec-1 and CD4 and permissiveness to HIV-1 infection. Front Immunol 8:1352. https://doi.org/10.3389/fimmu.2017.01352.
56. Gartner S, Markovits P, Markovitz DM, Kaplan MH, Gallo RC, Popovic M. 1986. The role of mononuclear phagocytes in HTLV-I/IIIa/LAV infection. Science 233:215–219. https://doi.org/10.1126/science.3014648.
57. Popovic M, Gartner S, Read-Conomol E, Beaver B, Reitz M. 1988. Cell tropism and expression of human immunodeficiency virus type 1 isolates in natural targets. J Virol 64:21–27. In Girard M, Valette L (ed), Retroviruses of human AIDS and related animal diseases, Colloque des Cent Gardes, vol 3. Pasteur Vaccins, Marnes-La-Coquette, France.
58. Campbell GR, Bruckman RS, Trout RN, Spector SA. 2016. SMAC mimetics induce autoantibody-dependent apoptosis of HIV-1-infected resting memory CD4+ T cells. Cell Host Microbe 24:689–702.e7. https://doi.org/10.1016/j.chom.2018.09.007.
59. Jaapur AJ, Meyers DL, Johnson VA, Kurtzkes DR, Beckett LA, Arduino JM, Lane J, Black RJ, Reichelderfer PS, Alaquer CS, The RV-43 Study Group, The AIDS Clinical Trials Group Virology Committee Resis-tance Working Group. 1993. Standardized peripheral blood mononuclear cell culture assay for determination of drug susceptibilities of clinical human immunodeficiency virus type 1 isolates. The RV-43 Study Group, the AIDS Clinical Trials Group Virology Committee Resistance Working Group. Antimicrob Agents Chemother 37:1095–1101. https://doi.org/10.1128/AAC.37.5.1095.
60. Wei X, Decker JM, Liu H, Zhang Z, Arani RB, Kilby JM, Saag MS, Wu X, Shaw GM, Kappes JC. 2002. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob Agents Chemother 46:1996–1995. https://doi.org/10.1128/AAC.46.10.1996-19952002.
61. Ratner L, Hasegetine W, Patarca R, Livak KJ, Starcich B, Josephs SF, Doran ER, Rafalski JA, Whitehorn EA, Baumgauer K. 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature 313:277–284. https://doi.org/10.1038/313277a0.
62. Campbell GR, Watkins JD, Esquieu D, Pasquier E, Loret EP, Specter SA. 2003. The C terminus of HIV-1 Tat modulates the extent of CD4+T-cell apoptosis in monocytes. J Biol Chem 280:38373–38382. https://doi.org/10.1074/jbc.M500632000.

Gasteiger E, Hoogland C, Attigaler A, Duvaud S, Wilkins MR, Appel RD,}

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Bairoch A. 2005. Protein identification and analysis tools on the ExPASy server, p 571–607. In Walker JM (ed), The proteomics protocols handbook. Humana Press, Totowa, NJ.

64. Campbell GR, Watkins JD, Loret EP, Spector SA. 2011. Differential induction of rat neuronal excitotoxic cell death by human immunodeficiency virus type 1 clade B and C Tat proteins. AIDS Res Hum Retroviruses 27:647–654. https://doi.org/10.1089/AID.2010.0192.

65. Campbell GR, Spector SA. 2012. Vitamin D inhibits human immunodeficiency virus type 1 and Mycobacterium tuberculosis infection in macrophages through the induction of autophagy. PLoS Pathog 8:e1002689. https://doi.org/10.1371/journal.ppat.1002689.

66. Bergemann TL, Wilson J. 2011. Proportion statistics to detect differentially expressed genes: a comparison with log-ratio statistics. BMC Bioinformatics 12:228. https://doi.org/10.1186/1471-2105-12-228.