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SHORT COMMUNICATION

Depressed neurofilament expression associates with apolipoprotein E3/E4 genotype in maturing human fetal neurons exposed to HIV-1

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Abstract Exposure of differentiating human neural progenitor cells (NEP) to HIV-1 results in a neuronal "failure to thrive" phenotype characterized by a relative decrease in neurofilament-light (NF-L) expression. However, when NEP were segregated by their apolipoprotein E genotype, differentiating apolipoprotein E3/E4 cells showed reduced NF-L expression upon HIV-1 exposure, but differentiating apolipoprotein E3/E3 or apolipoprotein E4/E4 cells did not. These data suggest that apolipoprotein E genotype is a host factor that could affect the development of neurocognitive dysfunction in HIV-1 infected individuals.

Keywords HIV · Neuroepithelial progenitor cells · Neurofilament protein-light · Apolipoprotein E

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Introduction

Brain injury resulting from HIV invasion of the central nervous system (CNS) runs a gamut from severe encephalitis through a spectrum of neurocognitive impairments known as HIV-1-associated neurocognitive disorder (HAND). The latter is particularly prevalent in patients treated with antiretroviral therapy, who tend to live longer lives. Productive infection of microglia or restricted infection of astrocytes or neuroepithelial progenitor cells (NEP) (Lawrence et al. 2004; Mattson et al. 2005; Schwartz et al. 2007) can initiate the release of pro-inflammatory factors and oxidative stress; these are mechanisms that may drive the neurocognitive decline of infected individuals. These mechanisms may be compounded by impaired neurogenesis, as exemplified by a 75% reduction of proliferating NEP seen in hippocampal tissue specimens from HIV-associated dementia (HAD) patients when compared to those without dementia (Krathwohl and Kaiser 2004). The NEP are believed to be important for the regeneration of glia (astrocytes, oligodendrocytes) and neurons in the brain, reviewed in several studies (Horner and Gage 2000; Kempermann et al. 2004; Schaffer and Gage 2004). Localized stores of multipotent NEP can proliferate and then differentiate into glia and neurons in response to soluble factors or cell surface contact influences. Thus, dynamic genesis of differentiated cells from progenitor cell stores in the brain is particularly important to facilitate brain injury repair and renewal throughout life.

Host-determined factors are likely to influence susceptibility to brain injury and the effectiveness of repair mechanisms. Apolipoprotein E (apoE) is increasingly recognized as a genetic factor influencing susceptibility to oxidative stress or

neuronal injury in Alzheimer's disease (Christen 2000), and may influence susceptibility to neuroinflammatory and neurodegenerative disease (Bu 2009; Christen 2000). ApoE is a small, multifunctional, secreted protein involved in complex lipid and cholesterol homeostasis (Mahley 1988; Mahley and Rall 2000). ApoE is synthesized in the CNS mainly by astrocytes (Boyles et al. 1985), but is taken up by neurons to participate in the mobilization and distribution of cholesterol and phospholipids during neuronal membrane events such as synaptogenesis or neurite extension and branching (Qiu et al. 2004). Human apoE has three common isoforms, apoE2, apoE3, and apoE4, distinguished by specific amino acids at positions 112 and 158 of the polypeptide. Their allelic frequency in human populations is 77% for E3, 15% for E4, and 8% for E2 (Mahley 1988). In vitro studies suggest the apoE3 isoform can have neurotrophic effects on neurite elaboration (Bellosta et al. 1995; Nathan et al. 2002). The role of apoE in influencing susceptibility to HIV-1-associated neurological illness is still emerging. In the HIV-1-infected population, the apoE ε4 allele has been associated with an increased risk of dementia or peripheral neuropathy (Corder et al. 1998), or poorer performance on neurocognitive tests (Andres et al. 2011). In addition, HIV-infected individuals with the E4/E4 genotypes reportedly experience a faster progression of disease (Burt et al. 2008).

Our previous study, investigating the effect of HIV-1 exposure on differentiating human NEP in vitro, indicated that differentiation per se was not inhibited by exposure to HIV-1, but differentiating neurons showed decreased neurofilament antigen (NF-L) expression (McCarthy et al. 2006). Exposure to HIV or to the viral glycoprotein gp120 was sufficient for the observed effect and viral replication was not required. Given the reported correlations between apoE genotype and susceptibility to neurodegenerative diseases, we further examined neurofilament protein-light (NF-L) expression in differentiating NEP of known apoE genotype and exposed to a dual-tropic strain of HIV-1, SF2.

Methods

NEP proliferation and differentiation

Multipotent human NEP were derived from mechanically dissociated human fetal rostral CNS of 53–115 days gestation. NEP were selectively cultured in suspension as proliferating cell clusters ("neurospheres"), prepared as previously reported in detail (McCarthy et al. 2006). Human fetal CNS specimens were obtained from the Birth Defects Laboratory of the Human Embryology program, University of Washington Medical School, Seattle, WA, through a tissue distribution program supported by the National Institutes of Health (NIH). The procurement of tissues was monitored by the Institutional Review Board of the University of Washington. The use of human fetal CNS tissue for this study was reviewed by the Institutional Review Board of the Miller School of Medicine, University of Miami. Cells derived from distinct fetal specimens are always cultured separately so that independent cultures represent distinct specimens. To initiate directed differentiation and start a synchronized differentiation timeline at $t=0$, neurospheres were adhered onto substrate (poly-D,L-ornithine plus fibronectin-(PON-FN)-coated glass or plastic) for 1 h, then incubated in differentiation medium for 21–22 days ($t=21$ or 22). Differentiation medium contained DMEM/F12 supplemented with 1% (w/v) bovine serum albumin (BSA), glutamine, N2 supplement, and 2.5% (v/v) heat-inactivated fetal bovine serum (FBS; Gibco Invitrogen, Carlsbad, CA). This differentiation medium generated a mixed population of astrocytes and neurons, with post-mitotic neuronal markers Hu (Okano and Darnell 1997) and neurofilament proteins increasing through $t=22$ (McCarthy et al. 2006).

Preparation of viral and control supernatants and exposure of NEP to virus

This study used the HIV-1 strain SF2, characterized as a syncytia-inducing strain that can use CXCR4 or CCR5 as co-receptor (Trkola et al. 1998). Virus stocks were grown in cultures of human peripheral blood mononuclear cells (PBMCs) that were mitogenically activated for 24 h with phytohemagglutinin (PHA) and IL-2, and continued in IL-2 during viral infection. Parallel, matched cultures of PBMCs were similarly activated and mock-infected as controls during virus stock preparation (McCarthy et al. 1998, 2006). Viral-infected or mock-infected supernatants from PBMC cultures were collected and clarified by centrifugation at $800\times g$ for 10 min. Replicate NEP cultures were seeded for differentiation and appropriate PBMC supernatant aliquots were diluted to a final p24 concentration of 11 ng/ml into differentiation medium at $t=0$, the start of differentiation. Cultures were incubated 22 days, with change of medium and replenishment of PBMC supernatant aliquots every 8 days, followed by harvest for data analysis.

ApoE genotyping of cell cultures

ApoE genotyping was performed essentially as described previously (Hixson and Vernier 1990) and is based on amplification of apoE cellular DNA corresponding to amino acids 112 through 158. Restriction mapping of the amplified DNA with specific enzymes allows the discrimination of the different isoforms. DNA from 1.0×10^5 to 5.0×10^5 cells was isolated using a Qiagen QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and then quantified. DNA with a 260/280 absorbance ratio between 1.5 and 2.0 was used. Two micrograms of DNA was amplified by polymerase chain reaction

(PCR) using a GoTaq Master Mix 2X (Promega, Madison, WI) with the following oligonucleotide primers: forward: 5′-GGCACGGCTGTCCAAGGAGCT-3′ (Hixson and Vernier 1990), and reverse: 5′-GATGGCGCTGAGGCCGCGCT-3′. This generated a product of 262 bp. Amplified DNA products were excised at every CGCG site using *Hha*I (Promega). Restriction products were separated with an 8% polyacrylamide gel and viewed by using Sybrsafe (Invitrogen) under UV light. The number and size of the restriction products allows the identification of the different apoE genotypes as follows: E2/E2: 91 bp and 83b; E2/E3: 91 bp, 83 bp, 48 bp, and 35 bp; E3/E3: 91 bp, 48 bp, and 35 bp; E3/E4: 91 bp, 72 bp, 48 bp, and 35 bp; E4/E4: 72 bp, 48 bp, and 35 bp.

Detection of cell antigen expression

Intracellular expression of apoE, β-III-tubulin and glial fibrillary acidic protein (GFAP) were detected essentially as described earlier (McCarthy et al. 2006). Briefly, monolayers were fixed with 4% (w/v) paraformaldehyde, then permeabilized with a solution of 0.2% Triton X-100 in phosphate-buffered saline. Specific antigen expression was determined by immunofluorescent antibody (IFA) staining of fixed monolayers. ApoE was detected with mouse monoclonal anti-apolipoprotein E (Abcam, Cambridge, MA) and secondary Alexa-Fluor 546 (red; Life Technologies, Grand Island, NY). Astrocytes were detected with rabbit anti-GFAP (Dako, Carpinteria, CA) and secondary Alexa-Fluor 488 (green; Life Technologies). Neurons were detected with rabbit anti-β-III-tubulin (Covance Labs, Princeton, NJ). Immunostained monolayers were counterstained with 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA) to detect cell nuclei. Immunostained monolayers were examined using the Olympus IX81 microscope fitted with epifluorescence optics, and microscopic fields were recorded digitally using a Hamamatsu Digital Camera (C4742-95) and Slidebook 4.1 Digital Microscopy Software (Intelligent Imaging Innovations, Inc., Denver, CO).

Neurofilament-light expression by immunoblotting

The total protein content of cell cultures was determined by BCA protein assay (Pierce, Rockford, IL) of culture lysates and between 5 and 10 μg total protein were loaded onto 8% polyacrylamide gels. Quantitative immunoblotting (McCarthy et al. 2000, 2006) with chemiluminescent signal detection was used to measure NF-L expression in differentiated, NEP-derived cultures exposed to HIV-1 for 22 days. NF-L was detected with mouse monoclonal antibody to neurofilament 68 kDa (NF-L), clone DA2, isotype IgG1, kappa (Life Technologies). Immunoblots were developed with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific, Rockford, IL) and signals were viewed and quantitated using a Bio-Rad Chemidoc XRS+Molecular Imager. To control for potential loading errors in each lane, the NF-L signal density was normalized to α-tubulin. Data are presented as mean values±standard error. Statistical analyses to compare mean antigen signals were performed with PRISM4 statistical software (GraphPad, San Diego, CA).

Analysis of cytokines and growth factors present in PBMC supernatants

Cytokines and other soluble factors present in supernatants from mock- vs. HIV-infected PBMC cultures were profiled by membrane array immunoblotting, using RayBio Human Cytokine Antibody Array 6 (RayBiotech, Norcross, GA).

Results

Distribution of apolipoprotein E genotypes among human brain tissue specimens

Apolipoprotein E genotyping was performed using total cell DNA extracted from each human fetal brain specimen used to generate NEP and NEP-derived monolayers. ApoE genotypes were derived for 31 distinct human brain specimens. Twenty specimens were apoE3/E3, eight specimens were apoE3/E4, and three specimens were apoE4/E4. This distribution of human apoE genotypes parallels that previously reported by Turchan-Cholewo et al. (2006) for human fetal brain specimens also obtained from the Human Embryology Laboratory, University of Washington. No statistical difference in gestational age was found among the apoE genotype groups (one-way ANOVA, $p=0.59$). For this study, the population of human fetal CNS specimens is different from those studied previously (McCarthy et al. 2006), because no residual CNS tissue or cells from the previous study remained available for apoE genotyping.

Apolipoprotein E is expressed by differentiating astrocytes in culture

NEP were cultured as neurospheres, then inoculated onto adherent substrate and differentiated in culture conditions that produce astrocytes and neurons with very little or no microglia present (McCarthy et al. 2006). Only astrocytes synthesized apoE as detected by immunostaining, which produced intracellular co-localization of immunostain to apoE and GFAP (Fig. 1a). ApoE was detected both as cell-associated and secreted protein (Fig. 1c). No co-localization of apoE and the neuronal marker β-III-tubulin was found by immunostaining (Fig. 1b), consistent with published studies indicating that the major source of apoE in the CNS is found in astrocytes (Boyles et al. 1985; Pitas et al. 1987).

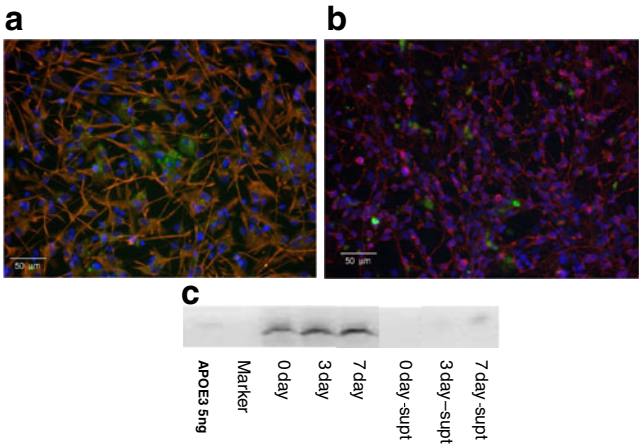

Fig. 1 Apolipoprotein E is detected by immunostain in differentiated astrocytes but not in neurons, and is secreted into the media. Untreated monolayer cultures were differentiated from NEP for 7 days, then fixed and stained for neural cell antigen and apoE as described in Methods. **a** Astrocytes were stained with anti-GFAP and secondary Alexa-Fluor 488 (*green*). **b** Neurons were detected with anti-β-III-tubulin. Immunostained monolayers were counterstained with 4,6-diamidino-2-phenylindole (*DAPI*) to detect cell nuclei. Magnification bar=50 μm. **c** Immunoblot image showing human apolipoprotein E detected in lysates of untreated, differentiating NEP at t=0, 3, 7 days of differentiation. ApoE was detected in the culture supernatant at t=7, but not earlier. Also shown is recombinant human ApoE3 (Biovision, Mountain View, CA) at 5 ng/lane

Exposure of differentiating NEP to HIV-1

To investigate the effect of HIV on neuronal cells, differentiating NEP were treated in three ways: "Untreated" cultures contained only differentiation medium. "Mock" cultures contained differentiation medium with 1:40 diluted aliquots of "mock-infected" supernatant from the mock-infected PBMCs. Mock exposure served as a model for background inflammation associated with HIV-1 infection, but not directly linked to viral proteins. "Virus-exposed" cultures contained differentiation medium with diluted aliquots of HIV-1 SF2 supernatant. At the end of the 21- to 22-day incubation period, cells were harvested for quantitative evaluation of cellular protein expression by immunoblotting. By day 22, NF-L expression was increased in mock treated cultures as compared to untreated cultures, in cells containing an ε3 allele, i.e., either apoE3/E3 (Fig. 2a), or apoE3/E4 (Fig. 2b), but not in cells homozygous for the ε4 allele (Fig. 2c). However, when HIV-1 was included in the differentiation medium, this increase in NF-L expression was negated in apoE3/E4 cultures, but was still seen in apoE3/E3 cultures (Fig. 2a and b). Thus there is a significant effect of culture treatment for the apoE3/E4 cultures (one-factor ANOVA, factor=culture treatment, $p<0.0004$). No significant change in NF-L expression was observed in the virus-exposed apoE4/E4 cultures as compared to the untreated or the mock-exposed cultures. Normalized

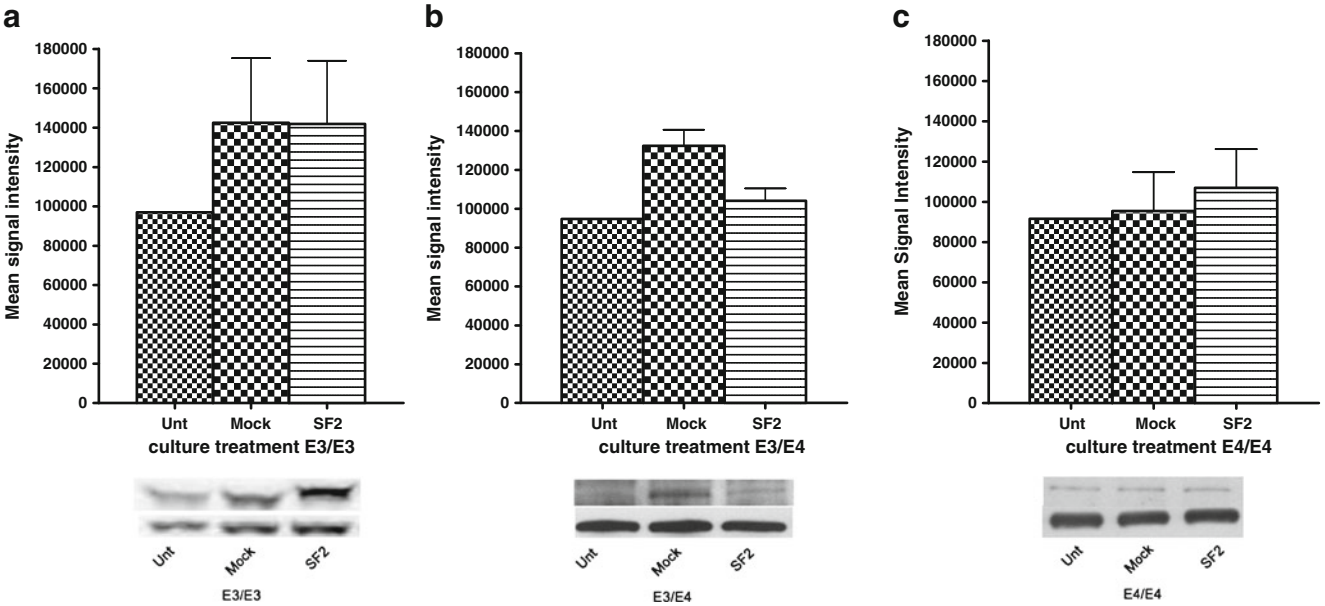

Fig. 2 Mean NF-L expression is increased in mock-exposed cultured cells harboring the apolipoprotein ε3 allele, but this increase is abrogated by exposure to HIV-1 in cells with the E3/E4 genotype. Human NEP were differentiated in monolayer cultures into a mixed population of astrocytes and neurons while treated as described, then lysed at t=21 or 22, and assayed for NF-L by immunoblotting. **a** Mean signal intensity values from 20 independent assays of 20 E3/E3 specimens, including control (untreated, mock) and viral-exposed. **b** Mean signal intensity values from ten independent assays of eight independent E3/E4 specimens. **c** Mean signal intensity values from four independent assays of three independent E4/E4 specimens. Immunoblot images are from representative assays of apoE3/E3 (*left*), apoE3/E4 (*middle*), apoE4/E4 (*right*) cells. One-factor ANOVA (factor=culture treatment) found significant culture treatment effect ($p<0.0004$) for the E3/E4 cohort, but not the E3/E3 or E4/E4 cohort

neuronal NF-L expression (virus-exposed NF-L signal divided by corresponding mock-exposed NF-L signal) was decreased by 20–25% in the apoE3/E4 cells exposed to HIV-1 strain SF2; normalized NF-L levels were not significantly decreased in the virus-exposed apoE3/E3, or apoE4/E4 cells (Fig. 3). The difference between normalized NF-L expression in virus-exposed apoE3/E4 versus apoE3/E3 cells was significant ($p=0.046$, unpaired t-test).

To identify candidate factors in the PBMC-derived supernatants that may explain the apoE-related increase in NF-L expression in mock-treated NEP cultures, we analyzed the PBMC-derived supernatants by membrane array immunoblotting. The arrays were chosen to profile cytokines, chemokines, and growth factors, and to estimate their relative concentrations (Table 1). Supernatants from both mock-infected and HIV-1(SF2)-infected PBMCs were enriched for cytokines IL-5 and IL-6, and for growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF) and epidermal growth factor (EGF). Most of these factors were further enriched in the SF2-infected supernatant compared to the mock-infected supernatant. HIV-1 (SF2)-infected PBMC supernatant was enriched in cytokines IL-1α/β, the monocyte chemoattractant protein (MCP) chemokines, the CCR5-using chemokine RANTES, and several neurotrophins (NT-3, BDNF, PDGF). TNF-α, IFN-γ, and the CXCR4-using chemokine stromal cell-derived factor type 1 (SDF-1) were not abundant in the PMBC supernatants.

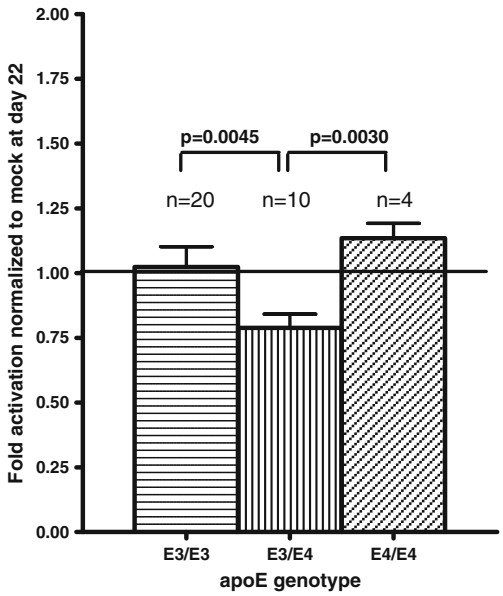

Fig. 3 Normalized NF-L expression is decreased in virus-exposed apoE3/E4 cultures, and normalized NF-L expression in virus-exposed apoE3/E4 cultures differed significantly from that of the apoE3/E3 or apoE4/E4 cultures ($p=0.0045$ and 0.0030, respectively, unpaired t-tests). NF-L expression from virus-exposed cells was normalized to that of the corresponding mock-exposed cells, i.e., virus-exposed NF-L signal divided by corresponding mock-exposed NF-L signal. The data were stratified by apoE genotype, as indicated. Bar graphs are mean± standard error of the normalized NF-L signal. A normalized value of 1 equals the control value (*horizontal line*). The number of independent assays is indicated

Discussion

In this study, cultures of human NEP differentiated into neurons and astrocytes in the presence of factors derived from mock-infected or HIV-1-infected PBMC supernatants. In this microenvironment, neuronal NF-L expression showed variations related to the apolipoprotein E genotype of the cells. When mock-infected supernatant was added to differentiation medium during the 21-day incubation, maturing neurons with one or two ε3 alleles showed an increase in NF-L expression by day 21, as compared to "untreated" maturing neurons incubated in differentiation medium only (Fig. 2a,b). However, when HIV-1(SF2)-infected supernatant was added, maturing neurons containing both the ε3 and ε4 alleles (genotype apoE3/E4) showed no such increase in NF-L expression by day 21. Maturing neurons with two ε4 alleles (genotype apoE4/E4) showed no changes in NF-L expression from untreated controls when either mock-infected supernatant or HIV-1(SF2)-infected supernatant was added to differentiation medium.

Increases in NF-L expression may be a result of exposure to soluble factors present in both mock-infected and viral-infected PBMC supernatants. Indeed, neurite outgrowth has been shown to be stimulated by factors derived from mitogen-activated immune cells or bone marrow-derived stromal stem cells (Kim et al. 2010). In particular, both GM-CSF and IL-6 have been identified as neurotrophic factors (Kannan et al. 2000; Oh et al. 2010; Shuto et al. 2001). In this study, both GM-CSF and IL-6 were detected in mock-infected and viral-infected PBMC supernatants (Table 1), providing a basis for the neurotrophic response seen in mock-exposed differentiating NEP containing an ε3 allele. In addition, it is possible that supernatants carry some apoE proteins produced by the PBMC themselves; and therefore, these results might be influenced by their apoE genotypes. However, this is not likely to be a significant influence, since these results were reproduced using mock supernatant or viral supernatant preparations from different PBMC donors. Moreover, apoE in blood-derived cells is mainly produced by differentiated macrophages (Mahley 1988), which are not as plentiful in these PBMC cultures that were not treated to enhance macrophage production.

Neurons with the heterozygous apoE3/E4 genotype produced about 20–25% less NF-L during a 21-day incubation in differentiation medium with added HIV-1 supernatant, as compared to parallel cultures in differentiation medium with

Table 1 Profile of factors secreted by PBMCs in mock-infected and HIV(SF2)-infected cultures

	Mock	SF2		Mock	SF2		Mock	SF2
Cytokine			Pro-inflammatory factors			Chemokines		
Eotaxin-2	**0.221**	**0.539**	IL-1α	0.020	**0.48972**	MCP-1	*1.929*	***4.284224***
Leptin	0.003	0.080	IL-1β	0.014	**0.669856**	MIP-1 d	0.065	0.056014
Eotaxin-3	0.003	0.028	IL-2	0.005	0.106887	RANTES	**0.373**	**0.849686**
LIGHT	0.081	0.190	IL-3	0.017	0.048024	SDF-1	0.033	0.054877
Flt-3 ligand	0.040	0.035	IL-5	**0.585**	***2.439389***	MCP-2	*1.639*	***5.359593***
Angiogenin	0.007	0.160	IL-6	*1.813*	***6.313113***	MCP-3	0.028	**0.287176**
Fractalkine	0.016	0.047	IL-7	0.012	**0.249873**	TARC	0.146	**0.21257**
GCP-2	0.025	0.037	IL-15	0.055	**0.269618**	MCP-4	0.057	0.143138
M-CSF	0.145	0.087	IL-16	**0.261**	**0.369075**			
BLC	**0.345**	**0.207**	TNF-α	0.000	0.005649	Growth factors		
MDC	**0.338**	*1.339*	IFN-γ	0.021	0.006022	NT-3	0.139	**0.348075**
BMP-4	0.083	0.125	GM-CSF	**0.800**	**0.696556**	BDNF	0.096	**0.492757**
MIG	**0.274**	0.086	Anti-inflammatory factors			GDNF	0.050	0.061052
BMP-6	0.025	0.039	IL-4	0.068	0.090738	EGF	**0.221**	*1.217048*
I-309	0.027	0.034	IL-10	**0.544**	*1.482612*	IGF-1	0.004	0.155844
CK B 8-1	0.048	0.011	IL-13	*1.449*	***4.610908***	PDGF-BB	0.059	**0.404461**
MIP-3α	0.082	**0.472**	TNF-β	0.051	0.186633	FGF-6	0.007	0.032417
IGFBP-1	0.003	0.056	IL-1ra	0.174	0.053639	FGF-7	0.006	0.012078
NAP-2	**0.429**	*1.938*				SCF	0.050	0.061612
IGFBP-2	0.037	**0.217**				TGF-b1	0.010	0.007837
Eotaxin	**0.410**	**0.398**				TGF-b3	0.038	0.015352
IGFBP-4	0.008	0.065				CNTF	0.015	0.027396
PARC	0.047	0.114						

PBMC culture supernatants were analyzed by membrane array immunoblotting using RayBio Human Cytokine Antibody Array 6 (RayBiotech, Norcross, GA), which detects cytokines, chemokines, interleukins, and growth factors. Values are the mean of signals from duplicate wells for each factor, expressed as a fraction of the signal values from positive controls on the same membrane

This table summarizes the profile of factors with signal value >0.05. Mean signal values above 0.2 are highlighted in *bold*, above 1 are highlighted in *italics*, and above 2 are highlighted as *bold-italics*

mock-infected supernatant. This difference amounts to an abrogation of the increase in NF-L observed in apoE3/E3 or apoE3/E4 cells when mock supernatant was added to differentiation medium (Fig. 2a,b). Hence, there is a relative decrease in NF-L expression with virus exposure in apoE3/E4 neurons (Fig. 2b). Contrary to cultures containing the ε3 allele, cultures homozygous for the ε4 allele showed no increase in NF-L expression when exposed to mock supernatant, suggesting that the E3 isoform is "beneficial" and the E4 isoform is neutral. Thus, exposure to HIV does not negate the effect of mock exposure in apoE4/E4 neurons because there was none. Even though the relative virus-associated decrease in NF-L expression in apoE3/E4 neurons is modest (20–25%), it is consistent with published data measuring neurite extension in adult mouse cortical neurons, where addition of recombinant apoE3 increased neurite growth by 25%, and addition of apoE4 decreased it by a similar percentage (Nathan et al. 2002). At the level of brain function, it is likely that a 25% percent change in neurite elaboration may result in clinically measurable cognitive dysfunction.

Why would NF-L expression be relatively decreased in HIV-1(SF2)-exposed apoE3/E4 neurons but not in HIV-1 (SF2)-exposed apoE3/E3 neurons? Apolipoprotein E binding to neurons results in isoform specific effects: binding of apoE3 increases neurite length, binding of apoE4 decreases it (Bellosta et al. 1995; Nathan et al. 2002). Viral gp120 may compete with apolipoprotein E binding at the cell surface, as suggested by studies indicating that addition of apoE4 increases HIV entry and apoE3 decreases it (Burt et al. 2008). The amphipathic domain of apolipoprotein E may interact with gp41 during the fusion process, similarly to T-20 (efurvitide) (Burt et al. 2008). To make matters even more complex, cell-surface proteoglycans such as heparan sulfate proteoglycan (HSPG) have been shown to bind both apolipoprotein E (Chen et al. 2011) and stabilize the binding of HIV (Mondor et al. 1998; Roderiquez et al. 1995). This

allows for competitive inhibition of each other's binding to the cell surface. Differential neurite outgrowth has been associated with the extent of internalization of the different isoforms, and internalization of the apoE3 isoform is 2-fold that of apoE4. This isoform specific accumulation was independent of the apoE specific receptors, but dependent on the HSPG on the cell surface (Ji et al. 1998). Thus, HIV-1 could act at the cell surface to interfere differentially with internalization of apoE3, leading to a subsequent relative decrease in neurite elaboration.

Apolipoprotein E isoforms may also interact with chemokine receptors on neuronal cells, thus potentiating viral gp120 effects on the neurotrophic action of apoE3. Recent studies showed that addition of apoE3 to dorsal root ganglion cell cultures also increased the expression of CXCR4, and that blocking this receptor with AMD3100 decreased the apoE3-related neurotrophic effects (Kosacka et al. 2009). HIV-1 gp120 from strain SF2 can bind to the CXCR4 receptor, thus blocking apoE3-mediated neurotrophic effects on neurite elaboration through CXCR4 signaling. Recombinant SF2 gp120 was previously shown to mimic the effects on NF-L expression observed with whole virus (McCarthy et al. 2006). Thus, it is highly plausible that the relative decrease in NF-L expression seen in HIV-1(SF2)-exposed apoE3/E4 neurons (Fig. 3) is due to gp120 interfering with the neurotrophic action of apoE3, at the CXCR4 site and possibly other cell surface sites. In homozygous apoE3/E3 neurons, this negative action of SF2 gp120 may be overcome by the abundance of the apoE3 isoform.

Clinical studies have implicated the apoε4 allele as a host risk factor for neurocognitive disease in HIV-1 infection, though the clinical evidence remains variable and inconclusive. Our observations with virus-exposed human NEP differentiated in vitro imply that apoliprotein E isoforms are a factor affecting neuronal susceptibility to HIV-1-associated synaptodendritic injury and neurite decline. Viral gp120 can interfere with the neurotrophic effects of apoE3, and thus the neurotrophic actions of the apoE3 isoform may be an important host-determined mechanism affecting susceptibility to HAND.

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Conflict of interest All authors declare that there are no conflicts of interest.

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