

Granulocyte–macrophage colony-stimulating factor enhances viral load in human brain tissue: amelioration with stavudine

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Background Granulocyte-macrophage colony-stimulating factor (GM-CSF) is elevated in cerebrospinal fluid in HIV- associated dementia; in addition, therapeutic GM-CSF elevates plasma viral load.

Objective To assess the effect of GM-CSF on viral replication and the potential ameliorative effect of antiretroviral therapy.

Design A primary human brain aggregate system is used as a model of the *in vivo* situation.

Method Cultured aggregates were infected with the macrophage tropic strain HIV-1_{SF162} and then exposed to varying GM-CSF concentrations and 0.3 µmol/l stavudine. Viral replication was assessed by p24 expression in the supernatant and aggregates. Immunohistochemistry identified neurons, astrocytes, microglia and oligodendrocytes.

Results A GM-CSF concentration of 1 ng/ml resulted in a fivefold increase in microglial cells, the main HIV cellular reservoir ($P = 0.0001$). Prior GM-CSF exposure before infection of the aggregates resulted in sixfold increase in p24 levels compared with non-GM-CSF-exposed infected aggregates. Infected aggregates with or without GM-CSF had significant neuronal loss of 50% and 45%, respectively, and astrocytosis. Addition of stavudine to the infected aggregates, even in the presence of GM-CSF, reduced p24 levels to zero and prevented neuronal loss and astrocytosis.

Conclusions This study demonstrates that GM-CSF enhances viral replication while addition of stavudine prevents this potentially detrimental process.

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Introduction

Granulocyte–macrophage colony–stimulating factor (GM–CSF) is a haemopoetic growth factor necessary for proliferation, differentiation and function of mature granulocytes and monocytes [1]. It is therapeutically indicated for bone marrow support to alleviate the neutropenia, which can occur in up to 50% of individuals

with advanced HIV infection [2,3] or as a complication of antiretroviral therapy [4,5]. GM–CSF has also been shown to increase the anti–HIV activity of zidovudine and levels of therapeutically active zidovudine 5′–triphosphate [5]. However GM–CSF administration also elevates plasma viral load [6] and, while its effects in the brain tissue compartment are not known, it can cross the blood–brain barrier [7] and central nervous system side effects are

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recognized [8]. In addition, the cerebrospinal fluid level of GM-CSF is 10-fold higher in individuals with HIV-associated dementia than in cognitively unimpaired HIV-infected individuals [9].

Brain resident HIV-1, which is mainly found in microglial cells and macrophages, causes an array of damage that clinically can result in cognitive impairment including HIV-associated dementia. Neuropathological studies have found a range of inflammatory disorders [10,11], synaptic and dendritic damage and frank neuronal loss [12,13]. Our group and others have recently shown that early cognitive abnormalities are related to dendritic and synaptic damage, which worsens with rising brain viral load [12,14], while the establishment and progression of HIV-associated dementia correlates with selective neuronal loss [15]. Importantly, it has been observed that these cognitive disorders also correlate with higher levels of viral RNA in the cerebrospinal fluid, indicating that progression of HIV-related brain damage and cognitive impairment and dementia is associated with increased central nervous system viral burden [16,17]. Therefore, it is possible that the association of elevated GM-CSF with cognitive impairment is related to an elevated viral load.

Furthermore, this potential association between the presence and level of virus and cognitive disorders stresses the therapeutic importance of being able to control or suppress viral replication in the brain effectively. To date, studies in this area have been confined to animal models. The effects of different nucleoside analogues on viral replication and astrogliosis have been compared using the severe combined immunodeficiency (SCID) mouse as a model [18]. Administration of abacavir and lamivudine resulted in an 80 and 95% decrease, respectively, in the number of infected cells, while zidovudine, didanosine and stavudine resulted in variable effects. While these results demonstrated a successful reduction in infected cell HIV load with antiretroviral therapy, the utilization of the SCID model with intracerebrally injected HIV-1-infected monocyte-derived macrophages requires further validation. Similarly, the recent development of AIDS in a chimpanzee model [19] has been described, but a number of studies have shown a lack of pathogenicity [20]. Consequently, studies based on animal models, while both encouraging and informative, cannot be directly extrapolated to the human situation.

In order to bridge this gap between animal models and the disease process in humans, a human brain aggregate system [21] has recently been established. This resembles the *in vivo* situation as it involves visualizing the three-dimensional structure and cellular composition of neurons, astrocytes, oligodendrocytes and microglia

that are directly proportional to the cells found in the human cortex. Morphological characterization of the aggregate has shown all cell types to be present throughout the aggregate, with a tendency for the neurons and oligodendrocytes to be situated more centrally while astrocytes form a more peripheral band. The processes of the neurons, astrocytes and oligodendrocytes formed a dense network and the enzymes for production of the main neurotransmitters are present (G. Trillo-Pazos *et al.*, unpublished data). This model has been used to investigate the effect of GM-CSF on viral levels and cellular damage in human brain tissue infected with HIV. Furthermore, the ability of the nucleoside reverse transcriptase inhibitor stavudine to suppress viral replication and ameliorate cellular pathology was assessed.

Methods

Culturing of aggregates

In our laboratory, a human fetal brain aggregate model was established composed of all the appropriate cells found in the human cortex [21]. Human fetal brain tissue (13–18 weeks of gestation) was collected with the approval of the local ethical committee of King's College Hospital NHS Trust. Brain tissue was mechanically disaggregated and cultured onto 0.75% Noble Agar (Difco, Oxford, UK) in Hanks Balanced Salt Solution (HBSS; Sigma, Poole, UK). Cell suspensions were cultured at 1×10^6 cells/ml in DMEM/F12, 5% human serum, 5 mmol/l glutamine and 20 µg/ml gentamycin sulphate (all from Sigma). The cells formed a layer within the first 2 days and gradually the layer would fold up to a ball shape within a week. The layer of agar has a repellent effect on cells, preventing them from attaching to the surface and forming a monolayer. These aggregates were maintained in culture for 4 weeks.

Growth of viral stocks

Polymorphonuclear blood monocyte cells (PBMC) were separated from a blood pack from an HIV-negative subject by the Lymphoprep method. Briefly, blood was diluted with HBSS and poured into tubes containing Lymphoprep solution (Robbins Scientific, UK) and centrifuged. The layer consisting of lymphocytes was removed and placed into HBSS containing 10% fetal calf serum (FCS; Sigma) and centrifuged again. The pellet was resuspended in 90% FCS and 10% dimethyl sulphoxide (Sigma). The PBMC were divided into portions and frozen at -80°C until required. To grow viral stock, 5×10^6 PBMC/5 ml were maintained in culture for 2 days with phytohaemagglutinin (2 µg/ml) and interleukin 2 [20 IU/ml; National Institute for Biological Standards and Control (NIBSC), Herts, UK], followed by the addition of a

macrophage tropic HIV-1 strain SF162 (Professor J. Levy, AIDS Reagents Project, NIBSC). Samples of 22 μl were collected up to day 5. The p24 ELISA (enzyme-linked immunosorbent assay) kit (Coulter, High Wycombe, UK) was used according to manufacturer's instructions to determine the maximum level of virus produced. Supernatant from the cultures was collected and stored in portions at -80°C until required.

Viral infection of the aggregates

Two weeks after the aggregates were established, they were exposed to 1 ng/ml GM-CSF every third day with media change for up to 2 weeks. Aggregates were then exposed to 0.3 $\mu\text{mol/l}$ stavudine, either 24 h before, simultaneously with or 24 h after infection of the aggregates with HIV-1_{SF162}. Aggregates were infected with 200 μl of virus dilutions ranging up to 10^{-1} . The highest dilution that gave consistent infection, 10^{-1} , was used. The concentration of stavudine was within the concentration for 50% inhibition (IC_{50}) and within the range observed in the cerebrospinal fluid (0.009–4.1 $\mu\text{mol/l}$) [22,23]. These aggregates were compared with aggregates not exposed to GM-CSF. The following day, excess virus was washed off with HBSS and replaced with fresh media. From thereon the medium was replaced every 3 days. Experiments were terminated 12 days following infection.

Detection of integrated viral DNA

Briefly, genomic DNA from aggregates was extracted using the Qiagen tissue kit (Qiagen, Crawley, UK) according to the manufacturer's instructions. The aggregates were left in 10% formalin solution overnight before handling. They were then washed in phosphate-buffered saline and mechanically homogenized. Tissue was lysed with proteinase K (Qiagen) overnight at 56°C . DNA was isolated, purified and stored at -20°C . A reaction mixture consisting of 3 mmol/l MgCl_2 (PE Biosystems, Warrington, UK), 5 μl 10 \times PCR (polymerase chain reaction) Gold buffer (PE Biosystems), 1 unit Ampli Taq Gold DNA polymerase (PE Biosystems), 0.2 mmol/l dNTP mix (Promega, Southampton, UK), 0.4 $\mu\text{mol/l}$ primer (MWG Biotech, Milton Keynes, UK), 10 μl DNA template made up to 50 μl with double-distilled water. Amplification was carried out on a Perkin Elmer 9700 thermal cycler (Perkin Elmer, Beaconsfield, UK): 30 cycles were performed at 94°C (30 s), 50°C (1 min) and 72°C (1 min) using primers for the protease region of *pol* (MSPF1; 5'-AGAGAGCTTCAGG TTTGG-3' and MSPR2; 5'-GGCCATCCATTC CTGGCTT-3') and reverse transcriptase region (MSRAF1; 5'-CAGTATTAGTAGGACC-3' and MSRAR2; 5'-ATCMCCCACATCYAGTACTG-3'). PCR products were analysed by gel electrophoresis in 2% agarose gels.

Analysis of viral replication by fluorescent assisted cell sorting

Following HIV-1 infection, aggregates were disaggregated into single cells and fixed in Orthopermafix (Ortho Clinical Diagnostics, Rarigan, New Jersey, USA) for 40 min and incubated with either p24 antibody conjugated to photoerythropoietin (PE, 1:100; Coulter) or PE isotype control antibody (1:280, Sigma) for 30 min. A Beckton-Dickinson (Oxford, UK) fluorescent assisted cell sorting machine was used to determine the p24 fluorescence by calculating 10 000 events. The proportion of p24 for each condition was calculated using the Cell Quest (Beckton-Dickinson) software.

Cellular quantification of aggregates

Aggregates were placed in 10% formalin and then into boiling 3% Noble agar, followed by overnight histological processing into paraffin wax. The aggregates were exhaustively sectioned throughout their entirety at 10 μm . For the identification and quantification of microglia, a systematically random sample of at least five slides were chosen. The first slide in the series was picked randomly from the first ten serial sections of the aggregate and thereafter every tenth was chosen from along the slide series. Contiguous series of sections were also sampled for the identification and quantification of neurons, astrocytes and oligodendrocytes. All slide series were processed immunohistochemically using ABC HRP kit from Dako (Cambridge, UK) according to the instructions provided. Primary antibodies to detect microglia CD68 (1:100, Dako) were used in the first series. In the other series, antibodies were applied to identify neurons with microtubule-associated protein-2 (1:100, Sigma), astrocytes with glial fibrillary acidic protein (1:1000, Dako) and oligodendrocytes with myelin oligodendrocyte (Chemicon, Harrow, UK). Methyl green (Vector, Peterborough, UK) was used as a nuclear marker. For each slide, 20 fields were chosen randomly and counted for both positively and negatively stained cells. Results were statistically analysed using one way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post hoc test.

Analysis of GM-CSF on viral replication

GM-CSF (R&D Systems, Oxon, UK) at 100, 10 and 1 ng/ml was added to aggregates for either 1 week or 2 weeks before infection with HIV-1_{SF162}. On days 0–12, 200 μl of culture media was collected and viral replication was confirmed by the p24 ELISA assay kit (Coulter). The coloured products were measured using E^{MAX} Precision Microplate reader (Molecular Devices, High Wycombe, UK) at 450 nm. Statistical analysis of p24 levels was by Wilcoxon rank sums.

Results

Four assessments were undertaken in this study: the success of the HIV infection of the aggregate protocol was verified; the effect of administration of GM-CSF on viral replication was examined; the consequence of exposure of stavudine on viral replication was assessed; and finally changes in the cell type profile in the aggregate were measured for each of the various experimental conditions.

HIV-1_{SF162}-infected aggregates were investigated by qualitative detection of the HIV-1 reverse transcriptase and protease gene products using PCR. Aggregates were obtained at 12 days after overnight exposure to HIV-1_{SF162}. HIV DNA was detected in the HIV-exposed aggregates but not in the uninfected control aggregates (Fig. 1).

Application of GM-CSF (1, 10 or 100 ng/ml) for 1 or 2 weeks to aggregates that were then infected with HIV-1_{SF162} resulted in demonstrable changes in the group exposed to GM-CSF for 2 weeks. Both the level of p24 and the number of microglial cells rose significantly in this group. On microscopic examination ($\times 100$), this increase in microglial cells was immediately apparent. The cells were uniformly distributed throughout the aggregate, and, morphologically, they were rounded in appearance, consistent with the activated amoeboid phenotype. p24 levels rose by sixfold ($P < 0.05$) (Table 1) and the proportion of microglial cells rose from 2 to 9% ($P = 0.0001$). The increases were largest in those aggregates exposed to 1 ng/ml GM-CSF (Fig. 2).

The capacity of stavudine to reduce viral replication in the HIV-1_{SF162}-infected aggregates was tested before assessing its effect in ameliorating the GM-CSF-associated changes. Stavudine was added to aggregate supernatant 24 h prior to, simultaneously with or 24 h after the exposure to HIV-1_{SF162}. Adding stavudine to

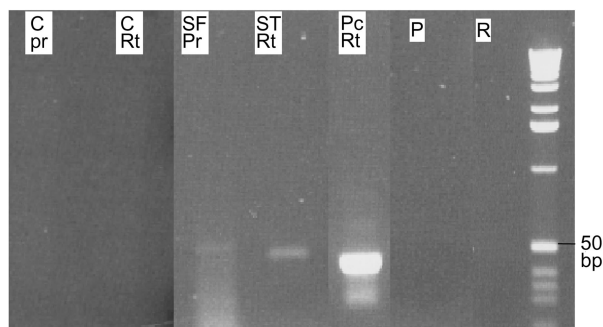


Fig. 1. Gel from polymerase chain reaction demonstrating HIV proviral DNA in HIV_{SF162}-infected brain tissue aggregates. Pr, protease; Rt, reverse transcriptase; C, control; SF, HIV-1_{SF162}; Pc, positive control; bp, base pair.

Table 1. HIV p24 in the supernatant of HIV-1_{SF162}-infected aggregates exposed to various concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF) for 2 weeks.

Week	HIV p24 ($\mu\text{g/ml}$) release with varying GM-CSF ($\mu\text{g/ml}$)			
	0	0.1	0.01	0.001
1	29	23	25	31
2	29	161	157	188

aggregates significantly reduced p24 levels in the supernatant [statistical analysis of variance (F) between group differences, within group differences (4,10) = 10.19; $P = 0.001$, log-transformed data]. p24 levels in aggregates of the control group were of a background intensity 2.7 $\mu\text{g/ml}$ (SD, 0.8). In aggregates exposed to stavudine 24 h prior to HIV infection, p24 levels in the supernatant rose to 30.7 $\mu\text{g/ml}$ (SD, 24.5), but this was not statistically different from uninfected control levels ($P = 0.053$, Tukey's HSD post-hoc test). In contrast, when stavudine was added at the same time or after the infection of the aggregates the p24 levels were as high as in the infected aggregates with no stavudine: 55.5 $\mu\text{g/ml}$ (SD, 14.2) 53.5 $\mu\text{g/ml}$ (SD, 8.2), and 60.1 $\mu\text{g/ml}$ (SD, 3.2), respectively. Similarly, FACS analysis of p24 immunofluorescent cells revealed no p24-immunopositive cells in the stavudine pre-exposed group. The simultaneous addition of stavudine and HIV-1_{SF162} produced only 9% p24 immunofluorescence. Adding stavudine, even 24 h after viral infection, still resulted in lower p24 levels than in infected aggregates without stavudine (Fig. 3).

Finally, the alteration in cellular composition of the aggregates associated with HIV-1 infection, and its modification by 1 ng/ml GM-CSF and stavudine, was assessed, (Table 2). Statistically significant alterations were observed in the proportions of neurons in the various conditions [$F(5,20) = 16.96$; $P = 0.0001$]. Neurons constituted 34% of the cell population in control aggregates, both in the presence and absence of

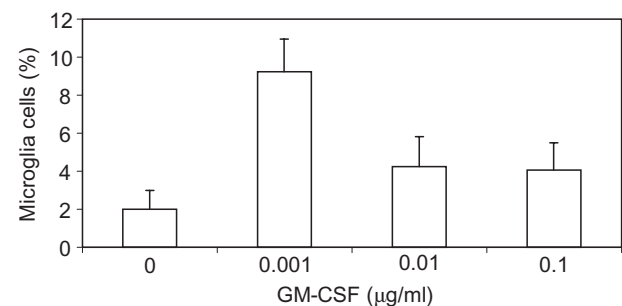


Fig. 2. The percentage of microglial cells in aggregates when exposed to various concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF).

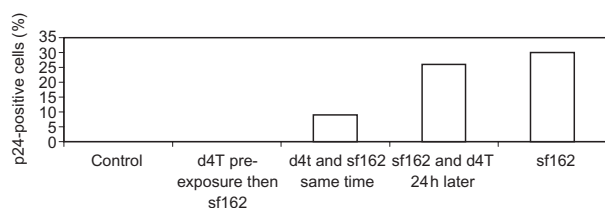


Fig. 3. The proportion of HIV p24-positive cells as estimated by fluorescent assisted cell sorting for control aggregates, those exposed to HIV_{SF162} and 0.3 $\mu\text{mol/l}$ stavudine simultaneously, and those aggregates only infected with HIV_{SF162}.

GM-CSF. When infected with HIV-1_{SF162}, neurons were significantly reduced to 17% ($P = 0.001$) and 15% ($P = 0.0001$), respectively, in the absence and presence of GM-CSF. Addition of stavudine restored the neuronal number to control levels. Similarly, for astrocytes, there were significant differences across the groups [$F(5,20) = 18.41$; $P = 0.0001$]. However, HIV infection of the aggregates was associated with large rises in astrocyte numbers both in the GM-CSF-naive ($P = 0.004$) and GM-CSF-exposed ($P = 0.0001$) aggregates. Oligodendrocyte numbers were not obviously affected by HIV infection. However, there was an apparent increase in oligodendrocyte numbers [$F(5,20) = 0.0001$; $P = 0.003$, Tukey's HSD post-hoc test] in the GM-CSF-naive group infected with HIV and exposed to stavudine. Finally, addition of GM-CSF had a significant impact on microglial cell numbers. Microglial cells constituted approximately 2–4% in the GM-CSF-naive aggregates but rose to 15% in the GM-CSF-exposed control aggregates [$F(5,20) = 16.28$; $P = 0.003$, Tukey's HSD post-hoc test] and 16% when infected with HIV. Even following the addition of stavudine, the microglial cell proportion remained at 10%, although this was significantly lower than in the infected aggregates ($P = 0.011$).

Discussion

This study clearly demonstrates that GM-CSF has both a potent effect on microglial proliferation, transforming resting ramified microglia into proliferating rod-shaped cells with activated thymidine kinase, as reported previously [24–26], and causes a marked increase in viral replication in the HIV-infected brain aggregate. In the clinical situation, HIV infection of the brain is associated with encephalitis, which is characterized by a significant increase in microglial cells [11]. These cells are also the cellular sites of productive infection [27]. Furthermore, stavudine both reduced viral replication and prevented the neuronal loss and astrocyte proliferation induced by HIV infection. These cellular changes are consistent with that observed in the brains of individuals who have died of HIV disease [11,23]. This is the first study to demonstrate the ameliorative effects of an antiretroviral drug on a three-dimensional human aggregate model that closely resembles the *in vivo* situation.

Microglia are the cellular reservoirs for HIV and so a process which enhances their numbers in the setting of HIV infection will potentially exacerbate HIV-related brain damage. GM-CSF elevates production of HIV in cultured primary mononuclear phagocytes [26] and systemic GM-CSF increases plasma viral load by $1 \log_{10}$ [6]. GM-CSF is also known to cross the blood–brain barrier [7]. However, the situation is not entirely clear as it has been reported that GM-CSF suppressed HIV replication in monocyte-derived macrophages [28], while it has also been observed that viral replication was enhanced by GM-CSF in plasma-derived monocyte/macrophage cells [5]. GM-CSF is known to be active even in picomolar range [29]. It has been detected at concentrations as low as 40 pg/ml in serum and 20 pg/ml in cerebrospinal fluid in healthy subjects [29]. In patients with HIV-associated

Table 2. Cell constituents of aggregates infected with HIV-1_{SF162} and exposed to granulocyte-macrophage colony-stimulating factor (GM-CSF) and stavudine. The morphometric quantification was performed 12 days following infection.

Condition	Percentage of cells (%) ^a			
	Neurons	Astrocytes	Oligodendrocytes	Microglia
No GM-CSF				
Control	34 (3)	35 (6)	28 (3)	4 (0)
SF162	17 (3)	48 (3)	33 (3)	2 (1)
SF162 and stavudine ^b	26 (4)	35 (5)	37 (1)	2 (0)
GM-CSF ^c				
Control	34 (3)	25 (3)	26 (2)	15 (3)
SF162	15 (3)	43 (4)	25 (3)	17 (6)
SF162 and stavudine ^b	27 (6)	35 (4)	28 (2)	10 (2)

^aNumber of estimations in parentheses.

^bStavudine 0.3 $\mu\text{mol/l}$.

^cGM-CSF, 1 ng/ml.

dementia, approximately 45 pg/ml GM-CSF had been detected in cerebrospinal fluid [9]. *In vitro* studies have demonstrated an increase in microglial numbers with 2 ng/ml GM-CSF. We have demonstrated that exposure of microglia in the human brain aggregate model to 1 ng/ml GM-CSF enhanced viral replication significantly, with a sixfold increase in p24 antigen levels. This indicates that the therapeutic effects of administering GM-CSF *in vivo* for bone marrow support because of either HIV-related or antiretroviral therapy-mediated leucopenia [30,31] may be counterbalanced by potentially detrimental effects in the central nervous system.

HIV is associated with a triad of neuropathological damage: astrocytosis, neuronal damage to dendrites and synapses, and neuronal death [12,32]. Macrophage-tropic strains of HIV are normally found in the brain [33,34], and viral proteins, such as gp120, gp41 and Tat, and other cellular products, such as cytokines triggered by the HIV-1 infection, either directly or indirectly contribute to neuronal injury [11,34–36]. The viral load in the brain is related both to the degree of neuronal damage and to the clinical establishment of HIV-related cognitive impairment [12,13]. Therefore, any strategy that reduces brain viral burden will also prevent or ameliorate neuronal damage and cognitive deficits. In this regard, we assessed the ability of the nucleoside reverse transcriptase inhibitor stavudine to reduce microglial cell numbers. In the GM-CSF-naïve aggregates, microglial cell density was low and not significantly different even in the HIV-infected situations. However, in those aggregates exposed to GM-CSF, the proportion of microglia was significantly higher, especially in the HIV-infected group, while addition of stavudine appeared to reverse this increase. Similarly, stavudine, especially when added prior to infection with HIV, caused a significant reduction in the supernatant p24 antigen level and in p24-immunopositive cells during FACS analysis. Furthermore, stavudine reduced the high astrocyte counts observed in the infected aggregates, a phenomenon similar to the astrocytosis observed in the clinical situation, to control values. Therefore, the effect of stavudine on HIV replication ameliorated the potential neuronal loss, astrocytosis and reduction in microglial cell numbers in the GM-CSF-exposed aggregates and prevented the rise in viral load observed with GM-CSF.

These observations have important therapeutic implications. While the exact timing of brain infection in relation to systemic infection is not entirely clear, it has been demonstrated that HIV is usually only detected as proviral DNA in asymptomatic HIV infection [37,38]. Only during advanced HIV disease can appreciable levels of the virus be detected in the brain. Stavudine should be able to suppress viral replication in the central nervous system effectively if it can penetrate the

blood–brain barrier at therapeutic levels. Although penetration of this barrier was not assessed in this investigation, stavudine was used at a concentration of 0.3 $\mu\text{mol/l}$, which is within the IC_{50} range and similar to concentrations observed in the cerebrospinal fluid [22,23]. Tissue distribution studies have shown detectable brain levels of stavudine [22,39], which may be achieved by diffusion [39]. Cytotoxicity assays indicate that stavudine is 20- to 100-fold less toxic than zidovudine [40]. Neurotoxicity is only observed with high concentrations of stavudine [39,41], significantly in excess of those used in this investigation. Clinically, it has been reported that stavudine decreases the HIV-1 RNA content of cerebrospinal fluid [41,42].

Accumulating evidence indicates that the suppression of HIV replication in the brain, and, therefore, prevention of neuronal damage, improves the clinical outcome for many patients. In addition, brain resident virus is a reservoir that can reseed into the systemic circulation and tissues via the lymphatic system [43]. Clinical studies have indicated the value of antiretroviral treatment in both improving cognitive functioning and reversing brain imaging deficits [44–46]. This is important, as even in cases of mild cognitive impairment there is a significant impact on quality of life. However, HIV-1 has a rapid turnover rate that can result in the emergence of drug-resistant mutants. It is possible that administration of drug regimens that do not suppress viral replication in the brain may encourage the development of viral resistance in the brain that can reseed into the systemic circulation and result in treatment failure. While information on treatment of resistant virus in the brain is limited, the ability of stavudine to suppress viral replication in the brain should be considered when clinicians prescribe a regimen of highly active antiretroviral therapy.

Our findings indicate that GM-CSF is potentially detrimental to the brain, and that antiretroviral drugs that penetrate the blood–brain barrier, such as stavudine, may provide neuroprotective cover against the effects of HIV. The theoretical risk of enhanced brain viral replication with GM-CSF administered alone was raised in 1992 [9]. The potentially toxic effects of GM-CSF are highlighted by the reports that GM-CSF levels in the cerebrospinal fluid are significantly higher in HIV-infected individuals with HIV-associated dementia [9], and in patients in the active phase of multiple sclerosis, with Alzheimer's disease and with vascular dementia [47]. In conclusion, the results of the present investigation indicate that therapeutic use of GM-CSF alone in HIV-infected patients may result in an increase in brain HIV burden, which, in turn, can cause a rise in systemic viral load and increase the risk of progression of HIV disease. We suggest that addition of antiretroviral therapy that penetrates the blood–brain barrier will minimize the GM-CSF-associated rise in

brain resident virus, diminish the opportunity for systemic reseeding and provide neuroprotective cover.

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