Journal of NeuroVirology (2000) 6, 75–83

www.jneurovirol.com

Short Communication

Upregulated expression of interleukin-8, RANTES and chemokine receptors in human astrocytic cells infected with HIV-1

Manuela Cota*[,1], Andrea Kleinschmidt[4], Francesca Ceccherini-Silberstein[4], Francesca Aloisi[5], Manuela Mengozzi[1], Alberto Mantovani[2,3], Ruth Brack-Werner[4,6] and Guido Poli[1,6]

[1]AIDS Immunopathogenesis Unit, DIBIT, San Raffaele Scientific Institute, 20132 Milano, Italy; [2]Department of Immunology and Cell Biology, 'Mario Negri' Institute for Pharmacological Research, 20157 Milano, Italy; [3]Section of Pathology and Immunology, Department of Biotechnology, University of Brescia, 25123 Brescia, Italy; [4]Institute of Molecular Virology, GSF-National Research Center for Environment and Health, 85764 Neuherberg, Germany and [5]Laboratory of Organ and System Pathophysiology, Istituto Superiore di Sanità, 00161 Roma, Italy

Human immunodeficiency virus (HIV) infection of the central nervous system (CNS) affects primarily microglial cells and astrocytes. Infection of these latter cells occurs independently of CD4 and is characterised by preferential accumulation of 2 Kb mRNA, encoding mostly Nef, and by low levels of 4.5 and 9 Kb RNAs. We have investigated the potential role of chronic HIV infection of human astrocytic cells on the expression of pro-inflammatory cytokines, chemokines and their receptors by comparing the infected TH4-7-5 with its parental uninfected 85HG66 cell lines. Upregulated levels of tumour necrosis factor-α (TNF-α) and of certain chemokines, namely interleukin-8 (IL-8) and regulated upon activation normal T cell expressed and secreted (RANTES), were observed in the infected *versus* uninfected cells, whereas monocyte chemotactic protein-1 (MCP-1) was comparably expressed in both cell lines. This pattern of expression was confirmed in primary foetal astrocytes transiently transfected with HIV. In addition, CXCR1, CXCR2 and CCR2b, receptors for IL-8 and MCP-1, respectively, were also found to be upregulated in TH4-7-5 *versus* 85HG66. CXCR4, the receptor of stromal cell derived factor-1 (SDF-1) and co-receptor for syncytium inducing HIVs, was comparably expressed in infected and uninfected astrocytic cells, whereas CCR5 was not detected in either cell line. Furthermore, treatment of TH4-7-5 cells with TNF-α or IL-1β stimulated RNA and protein secretion of IL-8, MCP-1, and RANTES as well as HIV expression. Thus, our findings suggest that HIV infection of astrocytic cells can contribute to the establishment of a chronic inflammatory state in the CNS, eventually resulting in HIV encephalitis, by increasing the secretion of pro-inflammatory cytokines, such as TNF-α and several chemokines. Overexpression of chemokine receptors including CCR2b, CXCR1 and CXCR2 in infected astrocytic cells may contribute to HIV-induced damage of the CNS via autocrine/paracrine activation of astrocytes. *Journal of NeuroVirology* (2000) **6,** 75–83.

Keywords: astrocytes; HIV; cytokines; chemokines; chemokine receptors

Human immunodeficiency virus (HIV) replication in the central nervous system (CNS) is sustained primarily by infection of resident macrophages (microglia) and of astrocytes (reviewed in Bell, 1999; Brack-Werner, 1999). Unlike microglia, HIV infects astrocytes in a CD4-independent manner (Chesebro *et al*, 1990; Kunsch *et al*, 1989). Astrocyte infection is characterised by low level of production of infectious progeny virions coupled with sustained expression of the *nef* gene product (Brack-Werner *et al*, 1992; Kleinschmidt *et al*, 1994; Saito *et al*, 1994; Tornatore *et al*, 1994b). This pattern

*Correspondence: M Cota, P2-P3 Laboratories, DIBIT, Via Olgettina n. 58, 20132 Milano, Italy
[6] These two authors contributed equally to this study
Received 21 April 1999; revised 4 August 1999; accepted 17 August 1999

appears to be mostly determined by an impaired function of the viral protein Rev in these cells (Neumann *et al*, 1995; Ludwig *et al*, 1999).

Amongst other host factors, cytokines are well known modulators of HIV production: tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) induce HIV expression both in $CD4^+$ T lymphocytes and macrophages (reviewed in Vicenzi *et al*, 1997) as well as astrocytes (Tornatore *et al*, 1991; 1994a). On the other hand, HIV virions and/or gp120 Env can upregulate the secretion of several cytokines, including TNF-α, IL-1β, IL-6 and transforming growth factor-β (TGF-β) in both monocyte-derived macrophages (MDM) (Vicenzi *et al*, 1997) and astrocytic cells (da Cunha *et al*, 1995; Koka *et al*, 1995; Yeung *et al*, 1995). These cytokines have been shown capable of acting as autocrine/paracrine modulators of virus expression in infected cells (Poli and Fauci, 1995).

Chemokines, chemotactic cytokines, have recently gained attention as potent inhibitors of *in vitro* HIV infection as a consequence of their capacity to bind to and block cell surface molecules which, together with CD4, serve as receptors for HIV entry (reviewed in Littman, 1998). In particular, the CC chemokines regulated upon activation normal T cell expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α), and MIP-1β bind to CCR5 and inhibit infection of macrophage-tropic non-syncytium inducing strains of HIV in several cellular model systems (Cocchi *et al*, 1995; Littman, 1998), including primary human macrophage/microglial cells, but not astrocytes (He *et al*, 1997).

Among others investigated, the CC chemokine monocyte chemotactic protein-1 (MCP-1) was found elevated in the cerebrospinal fluid (CSF) of AIDS patients with either cytomegalovirus encephalitis (Bernasconi *et al*, 1996) or with AIDS dementia (Kelder *et al*, 1998; Conant *et al*, 1998) or HIV encephalitis (Cinque *et al*, 1998).

Of interest, astrocytes have been shown to express several chemokines, both constitutively or upon cytokine stimulation, including MCP-1 (Weiss *et al*, 1998; Palma *et al*, 1997; Barna *et al*, 1994; Desbaillets *et al*, 1994; Hurwitz *et al*, 1995; Liu *et al*, 1996; Kasahara *et al*, 1991; Hayashi *et al*, 1995; Peterson *et al*, 1997), and IL-8 (Aloisi *et al*, 1992; Van Meir *et al*, 1992; Kasahara *et al*, 1991). Other chemokines (RANTES, MIP-1α and MIP-1β) were expressed exclusively after cytokine stimulation (Miyamoto and Kim, 1999; Hayashi *et al*, 1995; Peterson *et al*, 1997; Barnes *et al*, 1996). In addition, MCP-1 synthesis and release was recently demonstrated to be inducible by HIV replication or stimulation with exogenous Tat in both human foetal astrocytes (Conant *et al*, 1998) and MDM (Cinque *et al*, 1998; Mengozzi *et al*, 1999). In a blood brain barrier tissue culture system constituted by human endothelial cells and autologous astrocytes, exogenous MCP-1 induced the transmigration of monocytes and activated T lymphocytes in a dose-dependent manner (Weiss *et al*, 1998). In this model system TNF-α, IL-1β or TGF-β further induced MCP-1 production and consequently migration of monocytes and T cells (Weiss *et al*, 1998). Human astrocytic cells have also been shown to express several chemokine receptors, including CXCR2, CXCR4, CCR1, and CCR5 (reviewed in Hesselgesser and Horuk, 1999).

One of the obstacles in studying the influence of HIV infection on cellular functions in human astrocytic cell cultures is that only a minority ($\leqslant 5\%$) of cells are infected (Brack-Werner *et al*, 1992; He *et al*, 1997). In addition, detection of HIV-mediated changes in primary foetal astrocytes may be further hampered by constitutive expression of some cytokines or chemokines (Conant *et al*, 1998). Therefore, in the present study, we used the well-characterised TH4-7-5 chronically HIV-1 infected astrocytoma cell line consisting of $\geqslant 95\%$ HIV infected cells and its parental uninfected cell line 85HG66 (Brack-Werner *et al*, 1992; Neumann *et al*, 1995) to investigate the potential effects of persistent HIV infection on the expression of cytokines, chemokines and chemokine receptors. Our results demonstrate upregulation of several molecules in the infected *versus* uninfected astrocytic cells, which was further enhanced by cell stimulation with TNF-α or IL-1β.

Enhanced expression of cytokines, chemokines, and chemokine receptors in chronically HIV-1 infected astrocytoma cells

The chronically HIV-infected human astrocytoma cell line TH4-7-5 was established by multiple rounds of cell cloning following acute infection of parental 85HG66 astrocytoma cells with the CXCR4-dependent HIV-$1_{LAI\text{-}IIIB}$ strain (Brack-Werner *et al*, 1992). Total RNA from $2-5\times10^6$ cells was extracted by the RNA-zol method (Duotech s.r.l., Milan, Italy) from both uninfected and HIV-infected cell lines. One μg of RNA was reverse transcribed according to published procedures (Biswas *et al*, 1998). PCR amplification was performed under the following conditions: 30 s denaturation at 94°C, 45 s annealing at 60°C, and 60 s extension at 72°C. The mRNA for the cellular enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was monitored as an internal control to allow comparison of transcript levels between different samples (Biswas *et al*, 1998). The primers used are listed in Table 1. PCR products were analysed by electrophoresis in 2% agarose gel and visualised by ethidium bromide staining.

Uninfected astrocytic 85HG66 cells showed expression of TGF-β, MCP-1 and IL-8 mRNAs, whereas mRNAs for TNF-α, IL-1β, MIP-1α, MIP-1β, RANTES or SDF-1 were not detected (Figure 1). Among chemokine receptors, expression of CXCR4 but not

Table 1 Nucleotide primers in coupled RT–PCR detection

	Primer	*Sequence*	*Product size*
TNF-α	Sense	AGG CAG TCA GAT CAT CTT CTC G	302
	Antisense	TCT TGA TGG CAG AGA GGA GG	
TGF-β	Sense	CTG CGG ATC TCT GTG TCA TT	246
	Antisense	CTC AGA GTG TTG CTA TGG TG	
IL-1β	Sense	CTT CAT CTT TGA AGA AGA ACC TAT CTT CTT	331
	Antisense	AAT TTT TGG GAT CTA CAC TCT CCA GCT GTA	
IL-8	Sense	GATTTCTGCAGCTCTGTGTG	191
	Antisense	CAGAGCTCTCTTCCATCAG	
MCP-1	Sense	CAA TAG GAA GAT CTC AGT GC	188
	Antisense	GTG TTC AAG TCT TCG GAG TT	
RANTES	Sense	TGC CTC CCA TAT TCC TCG G	211
	Antisense	CTA GCT CAT CTC CAA AGA	
MIP-1α	Sense	GTC TGT GCT GAT CCC AGT GA	346
	Antisense	TTG TCA CCA GAC GCG GTG TG	
MIP-1β	Sense	GTC TGT GCT GAT CCC AGT GA	258
	Antisense	GGA CAC TTA TCC TTT GGC TA	
SDF-1	Sense	ACG AAT TCG CGC CAT GAA CGC CAA GGT CGT	451
	Antisense	CAG GAT CCT GCA AAC CTC AGG CCC GAT C	
CCR2b	Sense	AAC ATG CTG TCC ACA TCT CGT TCT	1100
	Antisense	CGT TTA TAA ACC AGC CGA GAC TTC	
CCR5	Sense	CTC GGA TCC GGT GGA ACA AGA TGG ATT AT	1100
	Antisense	CTC GTC GAC ATG TGC ACA ACT CTG ACT G	
CXCR1	Sense	GAG GTT GTG TGT GGA AGG TG	476
	Antisense	AGG TTG ATG TTT TGG CAG TG	
CXCR2	Sense	GCT CTA GAG CTG GGC AAC AAT ACA GCA AACT	493
	Antisense	CCA TCG ATG GGC ACT TAG GCA GGA GGT CTT A	
CXCR4	Sense	GCC AAC GTC AGT GAG GCA GAT G	209
	Antisense	GAG GAT GAC TGT GGT CTT GAG G	
GAPDH	Sense	CCA TGG AGA AGG CTG GGG	195
	Antisense	CAA AGT TGT CAT GGA TGA CC	

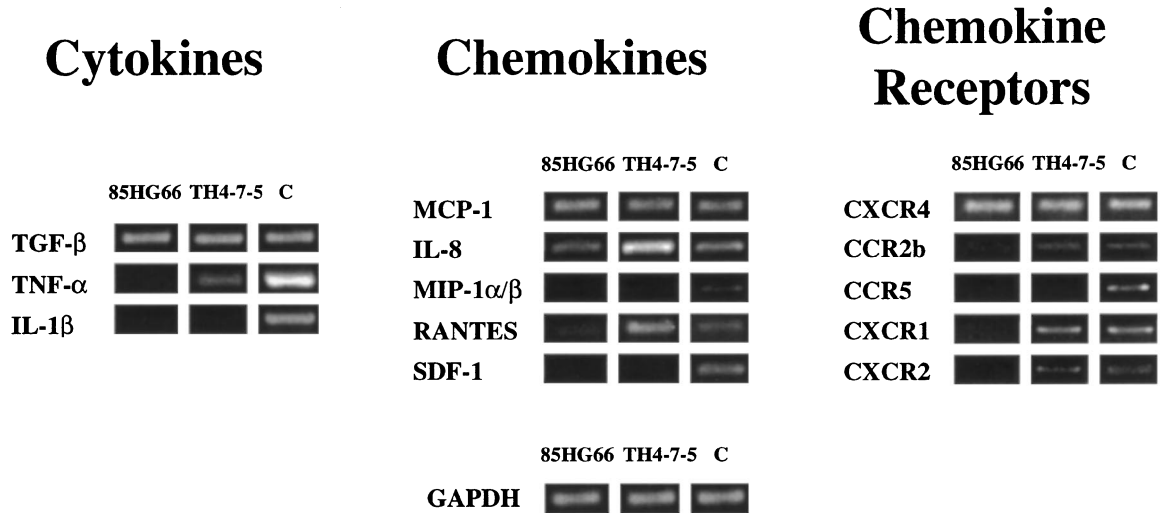


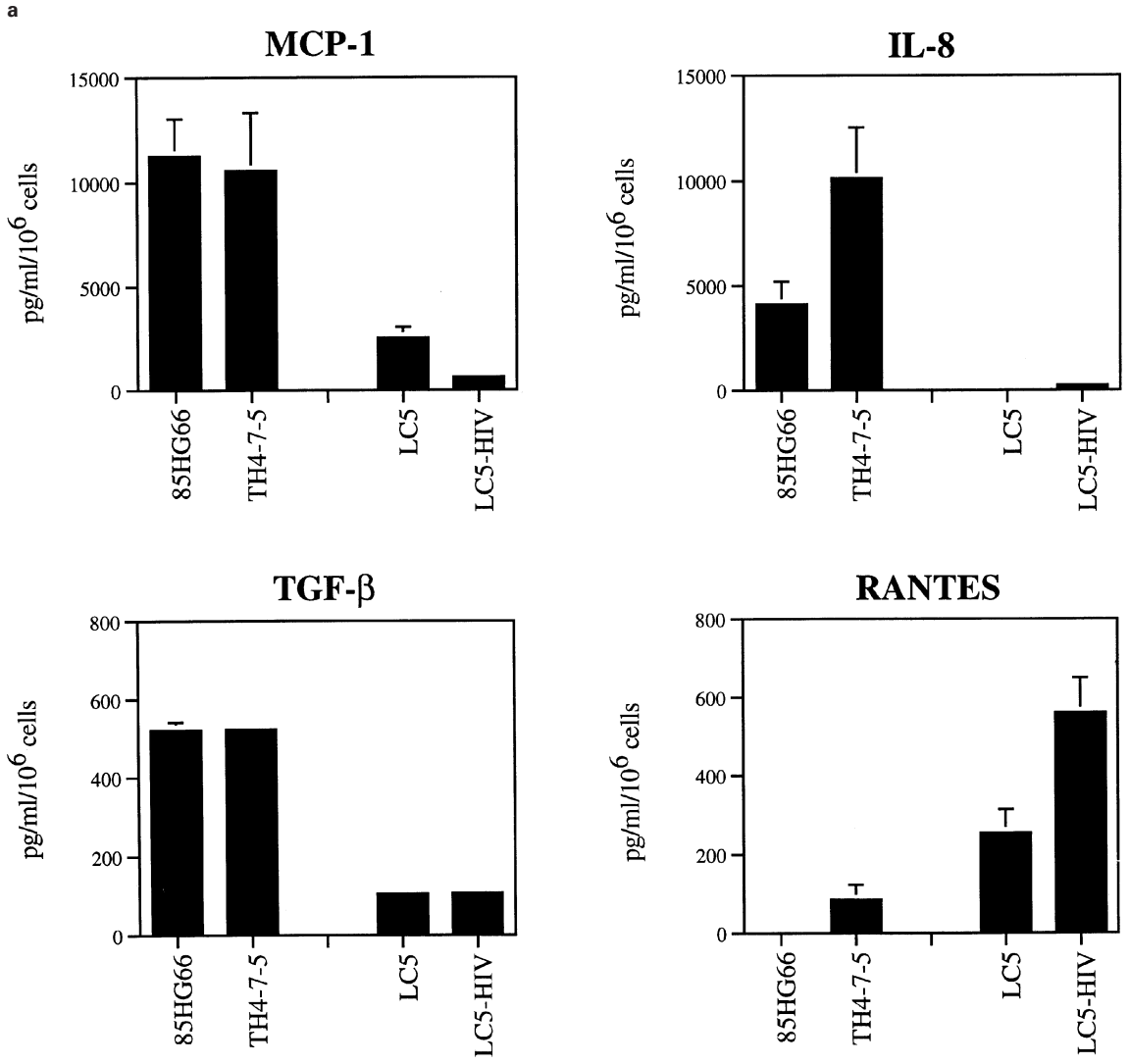
Figure 1 Expression of cytokines, chemokines and chemokine receptors in astrocytoma cell lines. Total RNA was extracted from parental uninfected 85HG66 and HIV-infected TH4-7-5 cell lines. The indicated cytokines, chemokines and chemokine receptors were analysed by RT–PCR (lane C indicates a positive control for each PCR reaction).

of CCR5, CCR2b, CXCR1 or CXCR2 was observed in 85HG66 cells (Figure 1). Chronically infected TH4-7-5 cells revealed upregulated expression of TNF-α, IL-8 and RANTES, whereas TGF-β and MCP-1 RNA levels were comparable in both cell lines. Of interest, mRNA expression of both CXCR1 and CXCR2, receptors for IL-8, and of CCR2b, the MCP-1 receptor, was consistently observed in the infected, but not in the uninfected cell line, whereas CXCR4 was detected at similar levels in both cell

lines (Figure 1). No evidence of expression was obtained for IL-1β, MIP-1α, MIP-1β, SDF-1 and CCR5 mRNAs in either cell line (Figure 1).

Cytokine and chemokine secretion in infected *versus* uninfected astrocytic and fibroblastoid cell lines

The synthesis and release of some cytokines and chemokines in infected and uninfected cell lines was further investigated. Culture supernatants and cell lysates, prepared by three consecutive freeze-thaw cycles were tested by ELISA (Laboserve/BioSource, Giessen, Germany; and R&D Systems, Wiesbaden, Germany) (Bernasconi *et al*, 1996; Cinque *et al*, 1998). In agreement with the RT–PCR results, IL-8 and RANTES were found upregulated in infected *versus* uninfected cells, whereas MCP-1 and TGF-β were comparably secreted by both cell lines (Figure 2A). TNF-α, IL-1β, and MIP-1β were undetectable in all tested culture supernatants (not shown). For comparison, cytokine and chemokine secretion was also analysed in a chronically HIV-1 infected fibroblastoid cell line and its uninfected parental cells (LC5-HIV and LC5, respectively) (Mellert *et al*, 1990). The pattern of secretion for RANTES and TGF-β was the same observed for astrocytic cells, whereas MCP-1 was found decreased in infected *versus* uninfected fibroblastoid cell lines, and IL-8 was barely detectable in both infected and uninfected cells (Figure 2A). Thus, upregulated expression of RANTES seems to be a common feature of HIV infected cells belonging to different lineages. No evidence of a cytoplasmic compartmentalisation was observed by measuring the cell-associated concentrations of TGF-β, IL-8 and MCP-1 after repeated freeze-thaw cycles (not shown).



Cytokine and chemokine secretion by primary foetal astrocytes transfected with or without HIV-1 proviral DNA

Primary foetal astrocytes were prepared from two different human embryonic brains according to published procedures (Aloisi *et al*, 1992). Third and fourth passage cultures were used for transfection experiments. Eighty to ninety per cent of the cells were positive for GFAP (glial fibrillary acidic protein) by immunohistochemical staining with a rabbit anti-cow GFAP antibody (DAKO, Germany) and Cy3-labelled goat anti-rabbit IgG as detection antibody (DIANOVA, Germany). Cells were resuspended in minimal essential medium containing D-valine (Life Technologies, Germany) and seeded onto poly-L-lysine coated culture dishes at a density of 0.8×10^5 cells/well (24-well plate) or 4×10^5 cells/well (6-well plate). Twenty-four hours later cells were transfected with the molecular HIV-1 clone HXB2 (pHXB2, Hahn *et al*, 1984, 0.625 μg/10^5 cells) and with a plasmid directing expression of the green fluorescent protein (GFP) (pFred143, Stauber *et al*, 1998, 0.125 μg/10^5 cells), or with pFred143 alone as control, by calcium phosphate coprecipitation using a commercially available kit (CellPhect, Pharmacia, Germany). Expression of GFP was analysed microscopically and by FACS at different

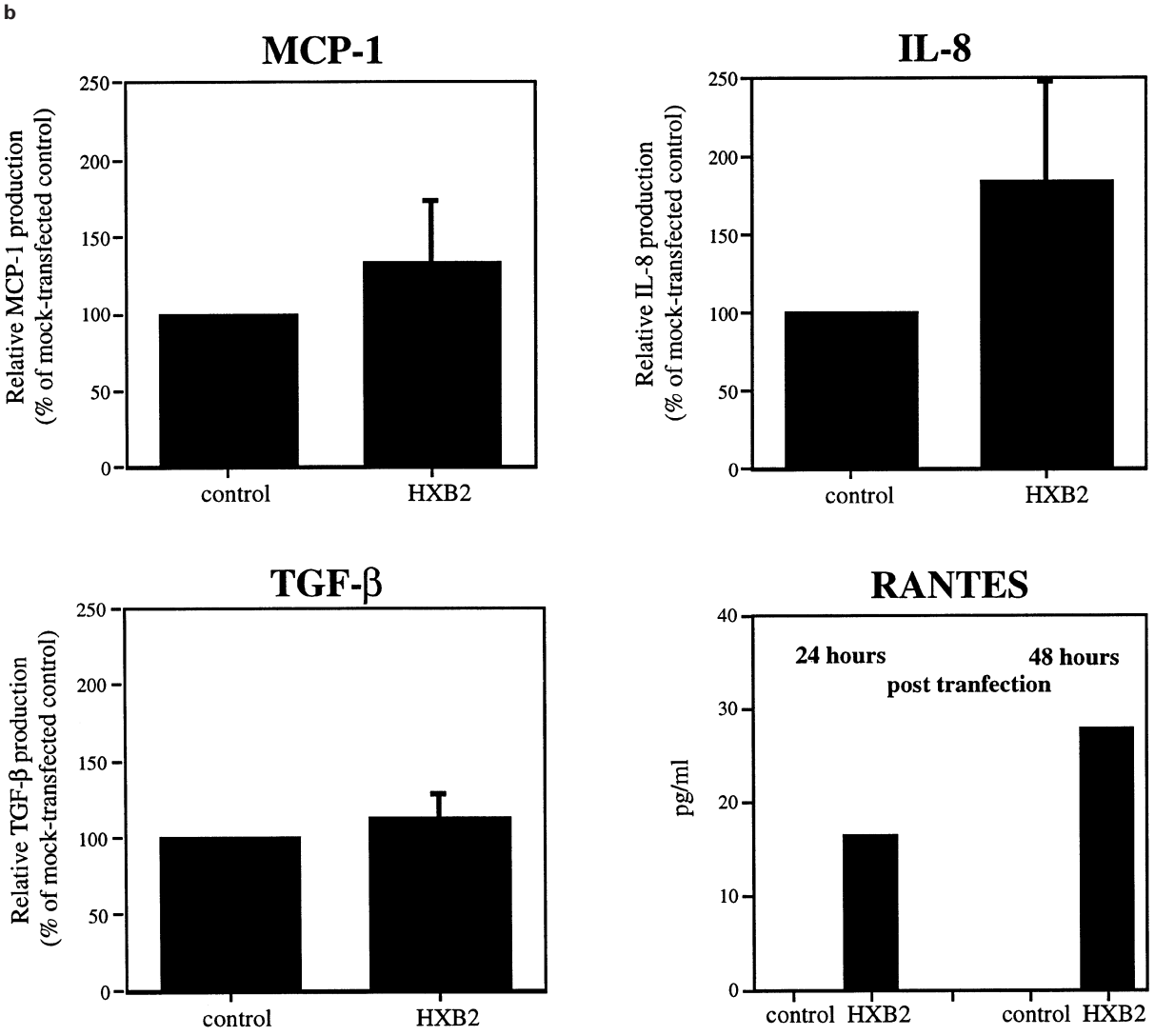


Figure 2 Effect of HIV infection on synthesis and release of MCP-1, IL-8, RANTES and TGF-β by astrocytic cells. (**a**) Chronically HIV-1 infected astrocytoma cells. Proteins were measured by ELISA in culture supernatants of uninfected (85HG66) and HIV-infected (TH4-7-5) astrocytoma and fibroblastoid cell lines (LC5/LC5-HIV) 5 days after seeding. Bars represents mean values of three parallel cultures; concentration values were normalised to 10^6 cells. (**b**) HIV-1 transfected human primary foetal astrocytes. Proteins were measured by ELISA in culture supernatants of mock-transfected (pFred143) and HIV-transfected (pHXB2/pFred143) primary astrocytes. Bars represent mean values of three independent transfection experiments (MCP-1, IL-8) or different time points of one experiment (TGF-β, RANTES). Each experiment was performed with at least duplicate cultures.

time points (24, 48, 72, 144 h) after transfection. Typically, 10–20% of transfected cells were positive for GFP fluorescence. Culture supernatants were tested in parallel for cytokine and HIV-p24 content by ELISA. HIV-transfected primary foetal astrocytes showed increased levels of IL-8 and RANTES, whereas secretion of MCP-1 and TGF-β was similar with and without HIV (Figure 2B). This pattern agrees with the results obtained with chronically HIV-1 infected astrocytoma cell line TH4-7-5 and its uninfected parental cell line 85HG66 (Figure 2A).

Cytokine-mediated modulation of chemokines and HIV expression in TH4-7-5 *versus* 85HG66 astrocytoma cells

85HG66 and TH4-7-5 cells were cultivated in 24-well plastic plates (Falcon, Becton-Dickinson Labware, Lincoln Park, NJ, USA) (3×10^5 cells/well in 2 ml) either unstimulated or stimulated with phorbol-12, myristate-13, acetate (PMA, 10^{-7} M; Sigma Chemical Corp., St. Louis, MS, USA) or individual cytokines including TNF-α (1 ng/ml), TGF-β (2 ng/ml), and IL1-β (2 ng/ml)(R&D Abingdon, UK). Cells were harvested 4 h after stimulation, pelleted and stored at -80°C for assessment of mRNA expression by RT–PCR. In parallel, culture supernatants were collected 24 h after stimulation and stored at -20°C for analysis of secreted proteins. PCR conditions were optimised to achieve linearity between the input cDNA and either the chemokine or GAPDH amplification products, respectively, as described (Graziosi *et al*, 1996). Cell culture supernatants from a parallel culture of TH4-7-5 were harvested 6 days after cytokine stimulation in order to measure HIV production by Mg^{2+}-dependent reverse transcriptase (RT) activity, as previously described (Biswas *et al*, 1998). Increased IL-8 mRNA and protein expression was observed after stimulation of both infected and uninfected astrocytic cells with TNF-α, IL1-β, or PMA (Figure 3). TNF-α and IL1-β strongly upregulated MCP-1 RNA and protein in infected TH4-7-5 cells, but to much lower levels in their uninfected

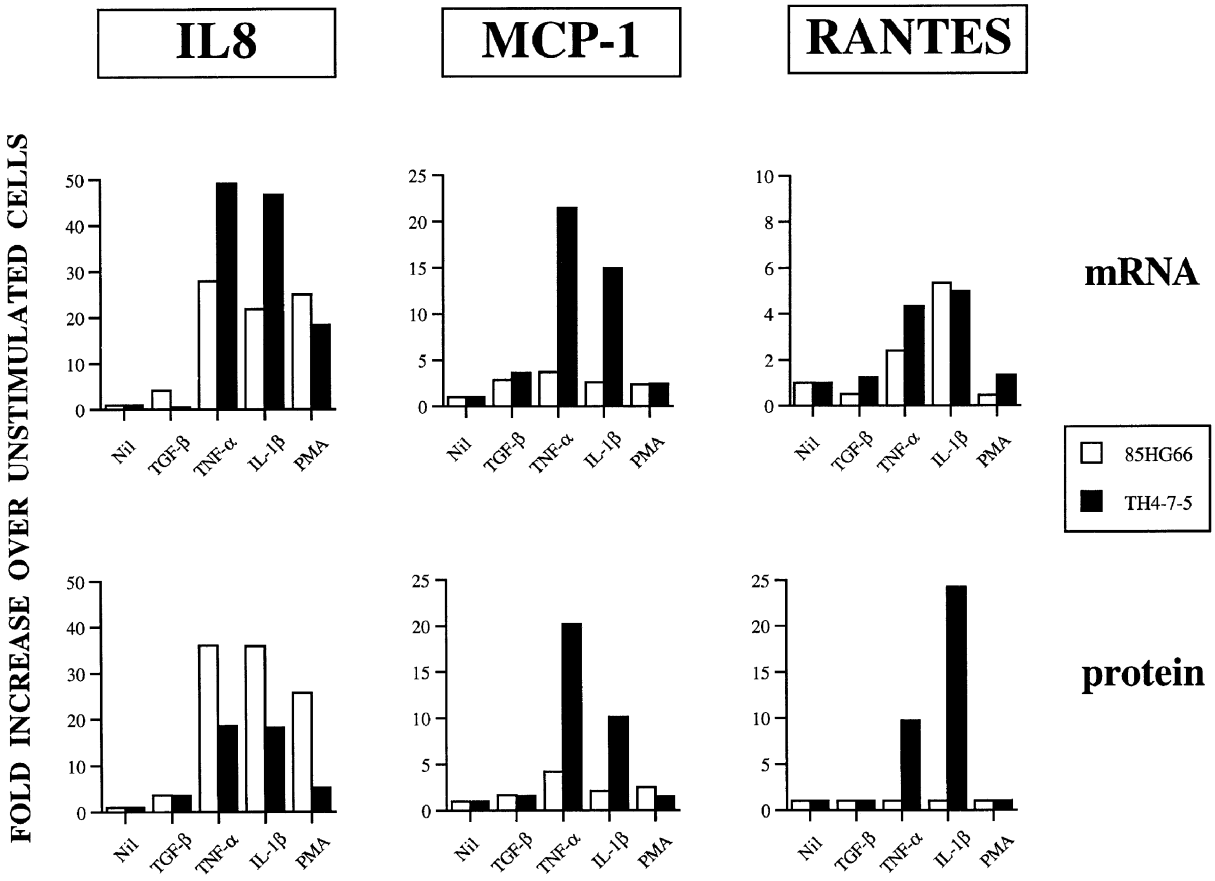


Figure 3 MCP-1, IL-8 and RANTES mRNA expression and protein secretion in cytokine stimulated astrocytoma cells. Uninfected (85HG66) and HIV infected (TH4-7-5) astrocytoma cells were stimulated with the compounds indicated at the bottom. Chemokine mRNA expression was analysed by RT–PCR 4 h after stimulation; analogous patterns were observed in cells stimulated for 24 h (not shown). Chemokines were measured by ELISA in culture supernatants 24 h after stimulation.

counterpart. A modest increase in the RNA levels of RANTES was also observed in both the infected and uninfected cells stimulated with TNF-α and IL1-β, whereas RANTES protein was upregulated only in infected, but not in the uninfected cells (Figure 3). The reasons for this discrepancy between RNA and protein levels of RANTES are unclear, but they may be explained in part with the higher sensitivity of PCR *versus* ELISA assays. However, we cannot rule out that either a translational block or storage of RANTES in cytoplasmic granules may occur (Wagner *et al*, 1998). A very similar pattern of expression of MCP-1, IL-8 and RANTES has been reported by Oh *et al* (1999) in two different uninfected astrocytic cell lines and in primary human adult astrocytes stimulated with lipopolysaccharide or cytokines. Finally, TNF-α, IL-1β and PMA also induced low levels of HIV expression in TH4-7-5 cells (Figure 4) indicating convergence, but also selectivity of these cytokines in their effects on virus *versus* cytokine and chemokine expression.

In summary, we have observed upregulated expression of TNF-α, of both CC (RANTES) and CXC (IL-8) chemokines and of chemokine receptors (CXCR1, CXCR2 and CCR2b) in chronically infected TH4-7-5 astrocytoma cells in comparison with the uninfected parental cell line. Upregulated secretion of RANTES and IL-8 was confirmed in primary foetal astrocytes transfected with HIV-1 proviral DNA. Upregulation of these chemokines may contribute to recruiting immune cells, such as monocytes and T lymphocytes from the blood stream to the brain, and may also locally attract microglial cells. Activated T lymphocytes and monocytes secrete pro-inflammatory cytokines like TNF-α and IL-1β, which may increase chemokine and virus production by astrocytes and thus contribute to viral spreading within the brain. In support of this hypothesis, we have previously demonstrated that chronically HIV-1-infected astrocytic cells may initiate productive infection of monocytic cells during experiments of *in vitro* cocultivation (Brack-Werner *et al*, 1992; Neumann *et al*, 1995).

Finally, we have provided evidence that HIV infection of astrocytoma cells induces the expression of receptors for IL-8 (CXCR1 and CXCR2) and for MCP-1 (CCR2b), chemokines which are both expressed by the same cells. Thus, autocrine/paracrine pathways of chemokine-mediated activation of astrocytes may play a role in the pathogenesis of HIV encephalitis and, at a clinical level, of the AIDS dementia complex. In this regard, we and

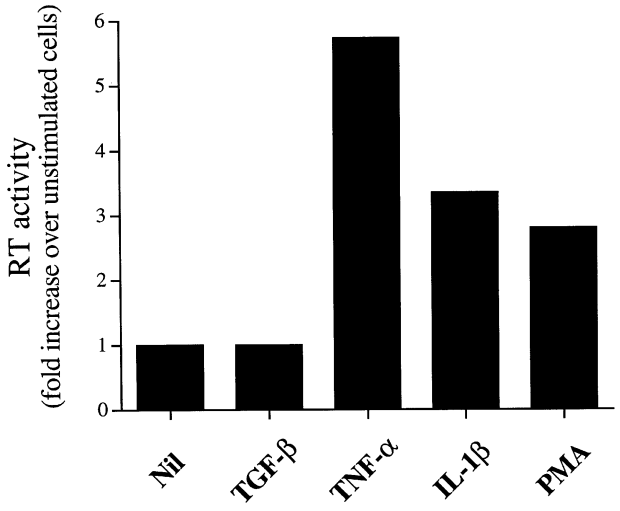


Figure 4 HIV expression in cytokine stimulated TH4-7-5 astrocytoma cells. Cytokine-stimulated cell culture supernatants were harvested 6 days after stimulation and tested for HIV RT activity. These results are representative of three independent experiments.

others have reported increased levels of MCP-1 in the CSF of AIDS patients either with HIV-encephalitis (Cinque *et al*, 1998), or AIDS dementia (Conant *et al*, 1998).

In conclusion, our findings support a general model, whereby HIV-induced activation of astrocytes leads to secretion of cytokines, chemokines as well as expression of chemokine receptors, ultimately contributing to the establishment and maintenance of inflammation in the infected brain.

Acknowledgements

We thank Markus Neumann and Silvano Sozzani for critical discussions. We are grateful to Pietro Transidico and Stefan Schlicht for assistance in performing ELISAs, and to Cecilia Gobbi for help with statistical evaluation. We thank George Pavlakis for providing pHXB2 and pFred143 plasmids. Work at GSF was partially funded by the Deutsche Forschungsgemeinschaft (DFG, SFB 464), whereas research at the San Raffaele and at the Istituto Superiore di Sanità (ISS) was funded entirely by grants of the National Program for Research Against AIDS, Rome, Italy. M Mengozzi is recipient of a fellowship from the ISS.

References

Aloisi F, Carè A, Borsellino G, Gallo P, Rosa S, Bassani A, Cabibbo A, Testa U, Levi G, Peschle C (1992). Production of hemolymphopoietic cytokines (IL-6, IL-8, colony-stimulating factors) by normal human astrocytes in response to IL-1b and tumor necrosis factor-α. *J Immunol* **149:** 2358–2366.

Barna BP, Pettay J, Barnett GH, Zhou P, Iwasaki K, Estes ML (1994). Regulation of monocyte chemoattractant protein-1 expression in adult human non-neoplastic astrocytes is sensitive to tumor necrosis factor (TNF) or antibody to the 55-kDa TNF receptor. *J Neuroimmunol* **50:** 101–107.

Barnes DA, Huston M, Holmes R, Benveniste EN, Yong VW, Scholz P, Perez HD (1996). Induction of RANTES expression by astrocytes and astrocytoma cell lines. *J Neuroimmunol* **71:** 207–214.

Bell JE (1999). The neuropathology of adult HIV infection. *Res Neurol* **154:** 816–829.

Bernasconi S, Cinque P, Peri G, Sozzani S, Crociati A, Torri W, Vicenzi E, Vago L, Lazzarin A, Poli G, Mantovani A (1996). Selective elevation of monocyte chemotactic protein-1 in the cerebrospinal fluid of AIDS patients with cytomegalovirus encephalitis. *J Infect Dis* **174:** 1098–1101.

Biswas P, Delfanti F, Bernasconi S, Mengozzi M, Cota M, Polentarutti N, Mantovani A, Lazzarin A, Sozzani S, Poli G (1998). Interleukin-6 induces monocyte chemotactic protein-1 in peripheral blood mononuclear cells and in the U937 cell line. *Blood* **91:** 258–265.

Brack-Werner R, Kleinschmidt A, Ludvigsen A, Mellert W, Neumann M, Herrmann R, Khim MCL, Burny A, Muller-Lantzsch N, Stavrou D, Erfle V (1992). Infection of human brain cells by HIV-1: restricted virus production in chronically infected human glial cell lines. *AIDS* **6:** 273–285.

Brack-Werner R (1999). Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS* **13:** 1–22.

Chesebro B, Buller R, Portis J, Wehrly K (1990). Failure of human immunodeficiency virus entry and infection in CD4-positive human brain and skin cells. *J Virol* **64:** 215–221.

Cinque P, Vago L, Mengozzi M, Torri W, Ceresa D, Vicenzi E, Transidico P, Vagani A, Sozzani S, Mantovani A, Lazzarin A, Poli G (1998). Elevated cerebrospinal fluid levels of monocyte chemotactic protein-1 correlate with HIV-1 encephalitis and local viral replication. *AIDS* **12:** 1327–1332.

Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P (1995). Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* **270:** 1811–1815.

Conant K, Garzino-Demo A, Nath A, McArthur JC, Halliday W, Power C, Gallo R, Major EO (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. *Proc Natl Acad Sci USA* **95:** 3117–3121.

da Cunha A, Jackson RW, Vitkovic L (1995). HIV-1 non-specifically stimulates production of transforming growth factor-β1 transfer in primary astrocytes. *J Neuroimmunol* **60:** 125–133.

Desbaillets I, Tada M, De Tribolet N, Deserens AC, Hamou MF, Van Meir EG (1994). Human astrocytomas and gliomas express monocyte chemoattractant protein-1 (MCP-1) in vivo and in vitro. *Int J Cancer* **58:** 249–247.

Graziosi C, Gantt KR, Vaccarezza M, Demarest JF, Daucher M, Saag MS, Shaw GM, Quinn TC, Cohen OJ, Welbon CC, Pantaleo G, Fauci AS (1996). Kinetics of cytokine expression during primary human immunodeficiency virus type 1 infection. *Proc Natl Acad Sci USA* **93:** 4386–4391.

Hahn BH, Shaw GM, Arya SK, Popovic M, Gallo RC, Wong-Staal F (1984). Molecular cloning and characterization of the HTLV-III virus associated with AIDS. *Nature* **312:** 166–169.

Hayashi M, Lou Y, Laning J, Strieter RM, Dorf ME (1995). Production and function of monocyte chemoattractant protein-1 and other β-chemokines in murine glial cells. *J Neuroimmunol* **60:** 143–150.

He HL, Chen YZ, Farzan M, Choe HY, Ohagen A, Gartner S, Busciglio J, Yang XY, Hofmann W, Newman W, Mackay CR, Sodroski J, Gabuzda D (1997). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385:** 645–649.

Hesselgesser J, Horuk R (1999). Chemokine and chemokine receptor expression in the central nervous system. *J Neurovirol* **5:** 13–26.

Hurwitz AA, Lyman WD, Berman JW (1995). Tumor necrosis factor α and transforming growth factor β upregulate astrocyte expression of monocyte chemoattractant protein-1. *J Neuroimmunol* **57:** 193–198.

Kasahara T, Mukaida N, Yamashita K, Yagisawa H, Akahoshi T, Matsushima K (1991). IL-1 and TNF-alpha induction of IL-8 and monocyte chemotactic and activating factor (MCAF) mRNA expression in a human astrocytoma cell line. *Immunology* **74:** 60–67.

Kelder W, McArthur JC, Nance-Sproson T, McClernon D, Griffin DE (1998). Beta-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia. *Ann Neurol* **44:** 831–835.

Kleinschmidt A, Neumann M, Moller C, Erfle V, Brack-Werner R (1994). Restricted expression of HIV-1 in human astrocytes: molecular basis for viral persistence in the CNS. *Res Virol* **145:** 147–153.

Koka P, He K, Zack JA, Kitchen S, Peacock W, Fried I, Tran T, Yashar SS, Merrill JE (1995). Human immunodeficiency virus 1 envelope proteins induce interleukin 1, tumor necrosis factor α, and nitric oxide in glial cultures derived from fetal, neonatal, and adult human brain. *J Exp Med* **182:** 941–952.

Kunsch C, Hartle HT, Wigdahl B (1989). Infection of human fetal dorsal root ganglion glial cells with human immunodeficiency virus type 1 involves an entry mechanism independent of the CD4 T4A Epitope. *J Virol* **63:** 5054–5061.

Littman DR (1998). Chemokine receptors keys to AIDS pathogenesis. *Cell* **93:** 677–680.

Liu J, Zhao ML, Brosnan CF, Lee SC (1996). Expression of type II nitric oxide synthetase in primary human astrocytes and microglia: role of IL-1beta and IL-1 receptor antagonist. *J Immunol* **157:** 3569–3576.

Ludwig E, Ceccherini-Siberstein F, van Empel J, Erfle V, Neumann M, Brack-Werner R (1999). Diminished Rev-mediated stimulation of HIV-1 protein synthesis is a hallmark of human astrocytes. *J Virol* **73:** 8279–8289.

Mellert W, Kleinschmidt A, Schmidt J, Festl H, Emler S, Roth WK, Erfle V(1990). Infection of human fibroblasts and osteoblast-like cells with HIV-1. *AIDS* **4:** 527–535.

Mengozzi M, De Filippi C, Transidico P, Biswas P, Cota M, Ghezzi S, Vicenzi E, Mantovani A, Sozzani S, Poli G (1999). Human immunodeficiency virus replication induces monocyte chemotactic protein-1 in human macrophages and U937 promonocytic cells. *Blood* **93:** 1851–1857.

Miyamoto Y, Kim SU (1999). Cytokine-induced production of macrophage inflammatory protein-1 alpha (MIP-1alpha) in cultured astrocytes. *J Neurosci Res* **55:** 245–251.

Neumann M, Felber BK, Kleinschmidt A, Froese B, Erfle V, Pavlakis GN, Brack-Werner R (1995). Restriction of human immunodeficiency virus type 1 production in a human astrocytoma cell line is associated with a cellular block in *rev* function. *J Virol* **69:** 2159–2167.

Oh JW, Schwiebert LM, Benveniste EN (1999). Cytokine regulation of CC and CXC chemokine expression by human astrocytes. *J Neurovirol* **5:** 82–94.

Palma C, Minghetti L, Astolfi M, Ambrosini E, Ceccherini Silberstein F, Manzini S, Levi G, Aloisi F (1997). Functional characterization of substance P receptors on cultured human spinal cord astrocytes: Synergism of substance P with cytokines in inducing interleukin-6 and prostaglandin E2 production. *Glia* **21:** 183–193.

Peterson PK, Hu S, Salak-Johnson J, Molitor TW, Chao CC (1997). Differential production of and migratory response to β chemokines by human microglia and astrocytes. *J Infect Dis* **175:** 478–481.

Poli G, Fauci AS (1995). The role of cytokines in the pathogenesis of HIV disease. In: *Human Cytokines: Their Role in Health and Human Disease* Aggarwal BB and Puri RK (eds), Blackwell Scientific Publications, Inc. Cambridge, MA, USA pp 421–449.

Saito Y, Sharer LR, Epstein LG, Michaels J, Mintz M, Louder M, Golding K, Cvetkovich TA, Blumberg BM (1994). Overexpression of nef as a marker for restricted HIV-1 infection of astrocytes in postmortem pediatric central nervous system. *Neurology* **44:** 481–487.

Stauber RH, Horie K, Carney P, Hudson EA, Tarasova NI, Gaitanaris GA, Pavlakis GN (1998). Development and applications of enhanced green fluorescent protein mutants. *Biotechniques* **24:** 462–466, 468–471.

Tornatore C, Nath A, Amemiya K, Major EO (1991). Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin 1beta. *J Virol* **65:** 6094–6100.

Tornatore C, Meyers K, Atwood W, Conant K, Major E (1994a). Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. *J Virol* **68:** 93–102.

Tornatore C, Chandra R, Berger JR, Major EO (1994b). HIV-1 infection of subcortical astrocytes in the pediatric central nervous system. *Neurology* **44:** 481–487.

Van Meir E, Ceska M, Effenberger F, Walz A, Grouzmann E, Desbaillets I, Frei K, Fontana A, de Tribolet N (1992). Interleukin-8 is produced in neoplastic and infectious diseases of the human central nervous system. *Cancer Research* **52:** 4297–4305.

Vicenzi E, Biswas P, Mengozzi M, Poli G (1997). Role of pro-inflammatory cytokines and β-chemokines in controlling HIV replication. *J Leuk Biol* **62:** 34–40.

Wagner L, Yang OO, Garcia-Zepeda EA, Ge Y, Kalams SA, Walker BD, Pasternack MS, Luster AD (1998). Beta-chemokines are released from HIV-1-specific cytolitit T-cell granules complexed to proteoglycans. *Nature* **391:** 908–911.

Weiss JM, Downie SA, Lyman WD, Berman JW (1998). Astrocyte-derived monocyte-chemoattractant protein-1 directs the transmigration of leukocytes across a model of the human blood brain barrier. *J Immunol* **161:** 6898–6903.

Yeung M, Pulliam L, Lau AS (1995). The HIV envelope protein gp120 is toxic to human brain-cell cultures through the induction of interleukin-6 and tumor necrosis factor-α. *AIDS* **9:** 137–143.