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Interactions between HIV-infected monocyte-derived macrophages and human brain microvascular endothelial cells result in increased expression of CC chemokines

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The presence of perivascular monocytic infiltration is a major hallmark of HIV-1-associated dementia. Since CC chemokines are chemoattractant cytokines that are able to attract T cells and monocytes/macrophages to sites of inflammation, and since infiltrating monocytes/macrophages remain in close contact with the brain endothelium, we investigated whether interactions between HIV-1-infected macrophages and brain endothelium result in an altered chemokine production. We found an increased mRNA expression of monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein (MIP) -1α and MIP-1β, and RANTES by macrophages after HIV-1 infection. Interactions between HIV-infected macrophages and brain microvascular endothelial cells resulted in an additional upregulation of chemokine mRNA expression, during cell – cell contact as well as in a trans-well system. Since IL-1β can function as a modulator of chemokine expression we investigated if interleukin-1β could be involved in the regulation of chemokine induction. Coculturing of HIV-infected macrophages and endothelial cells resulted in immune-activation as indicated by increased mRNA expression of IL-1β. Subsequently, addition of a neutralizing antibody against IL-1β resulted in altered chemokine expression by macrophages, but not by endothelial cells. Thus, IL-1β appears to play a major role in the regulation of chemokines during cellular interactions in HIV-associated dementia, but other factors may also be involved. *Journal of NeuroVirology* (2000) **6,** 382 – 389.

Keywords: monocytes/macrophages; endothelial cells; HIV-1-associated dementia; chemokines; cytokines

Introduction

HIV-1-associated dementia (HAD) is a serious neurodegenerative disease that may affect up to one-third of children and 25% of adults with AIDS (Navia *et al*, 1986; Price *et al*, 1988). Infiltration and immune activation of monocytes are believed to play a major role in HAD (Glass *et al*, 1995; Tyor *et al*, 1992; Genis *et al*, 1992; Nuovo and Alfieri, 1996). In fact, one of the strongest predictors for HAD is the presence of massive monocytic infiltration (Glass *et al*, 1995). It is not yet fully understood how and why monocytes are leaving the blood stream and entering the brain parenchyma. An important role has been proposed for adhesion models like E-selectin and VCAM-1 (Nottet *et al*, 1996) and chemokines are currently studied intensively for their role in HIV-1-associated dementia. Chemokines are often upregulated in inflammatory processes and they play a major role in the mobilization as well as in the activation of all blood leukocytes (Furie and Randolph, 1995; Proost *et al*, 1996). Based on their function, sequence and chromosomal location two families of chemokines may be distinguished, the CC and the CXC chemokines (Baggiolini *et al*, 1997; Moser *et al*,

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1998). Whereas the CXC chemokines mainly have an effect on neutrophils, the CC chemokines mostly attract lymphocytes and monocytes/macrophages (Uguccioni *et al*, 1995; Cairns and D'Souza, 1998). Therefore, the CC chemokines are particularly interesting, considering the possible involvement of macrophages in HAD. CC chemokines have been found to enhance migration of monocytic cells across the BBB (Randolph and Furie, 1995) and into the brain parenchyma (Peterson *et al*, 1997). *In vivo*, CC chemokines are significantly elevated in brains of demented AIDS patients (Schmidtmayerova *et al*, 1996; Conant *et al*, 1998) and *in vitro*, HIV-1 was shown to induce the CC chemokine MCP-1 in macrophages and U937 promonocytic cells (Mengozzi *et al*, 1999). Thus, by increasing chemokine levels and adhesion molecules, HIV-1 infection may lead to enhanced infiltration of macrophages into the brain. Therefore, in HAD, chemokines may prove to play an important role in the attraction of large quantities of monocytes into the brain.

Although many chemokine and receptor expression studies have been performed (Lukacs *et al*, 1995; Lavi *et al*, 1998; Sanders *et al*, 1998; Persidsky *et al*, 1999), interactions between different cells in the brain and their effect on chemokine production are still poorly understood. Since infiltrating macrophages remain in close contact with endothelial cells we studied interactions between these two cell types *in vitro*. In these cocultures, levels of mRNA expression of the CC chemokines monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES (regulated upon activation, normal T-cell expressed and secreted) were investigated.

Results

MCP-1, MIP-1α, MIP-1β, and RANTES mRNA levels are upregulated in cocultures of BMVEC's and HIV-infected macrophages

Since infiltrating monocytes may interact with brain endothelial cells in HAD, the effect of interactions between these two cell types on chemokine production was studied *in vitro*. In particular, it was tested whether the combination of HIV-infected macrophages and BMVEC's resulted in increased chemokine production as compared to all other culture conditions. HIV-1-infected or uninfected macrophages were cultured alone or in the presence of BMVEC's and RNA was isolated at 48 h. To study the effect of HIV-1 infection on chemokine production by macrophages, RNA was isolated from HIV-infected macrophages. A semi-quantitative fluorescence assay was used to determine the mRNA levels of MIP-1α, MIP-1β, MCP-1, and RANTES. Because of donor–donor differences of the primary macrophages, the experiment was performed three times and the mRNA levels of MIP-1α, MIP-1β, MCP-1 and RANTES of a representative experiment are depicted in Figure 1A–D, and are expressed in relative fluorescence units (r.f.u.). Standard devia-

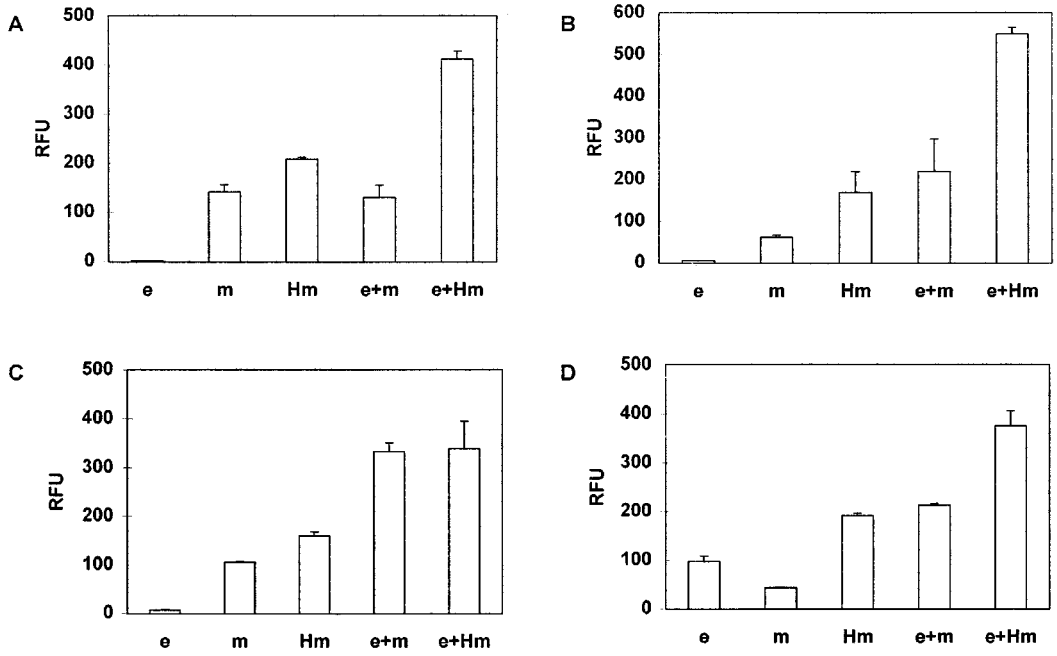

Figure 1 mRNA expression levels, expressed in relative fluorescence units (r.f.u.), of MIP-1α (**A**), MIP-1β (**B**), RANTES (**C**), and MCP-1 (**D**) in BMVEC's (e), macrophages (m), HIV-infected macrophages (Hm), cocultures of BMVEC's and macrophages (e+m), and cocultures of BMVEC's and HIV-infected macrophages (e+Hm). The depicted results are representative for at least three independent experiments and individual samples were analyzed three times by semi-quantitative PCR.

tions reflect assay reproducibility. Except for RANTES, mRNA levels of all chemokines were increased in cocultures of BMVEC's and HIV-infected macrophages as compared to all other culture conditions ($P<0.05$ according to the Mann–Whitney *U*-test). The effect of endothelial cells on chemokine expression by uninfected macrophages was studied by comparing expression levels in cocultures of uninfected macrophages and BMVEC's with uninfected macrophages alone. In addition, the effect of HIV-1 infection on chemokine expression was studied by comparing chemokine expression by HIV-1-infected macrophages with that of uninfected macrophages. Finally, the additional effect of BMVEC's was studied by comparing chemokine expression by cocultures of HIV-infected macrophages and BMVEC's with uninfected macrophages alone. These ratios were determined for three independent experiments and the mean ratios are depicted in Table 1. All chemokines were induced by HIV infection and the combination of HIV-1 infection and the presence of BMVEC's resulted in a significant increase in MIP-1α, MIP-1β and MCP-1 ($P<0.05$ according to the Mann–Whitney *U*-test) as compared to cocultures of uninfected macrophages and BMVEC's. The production of proteins was confirmed by Western blot analysis (data not shown).

Soluble factors produced by HIV-infected macrophages are responsible for induction of MCP-1 and RANTES mRNA in BMVEC's

The coculture experiments were repeated in a transwell assay to investigate whether soluble factors produced by (HIV-infected) macrophages could have an effect on chemokine production by BMVEC's. Uninfected or HIV-infected macrophages were cultured in a chamber insert with BMVEC's for 48 h and RT–PCR was performed on BMVEC's. Figure 2 shows representative results that demonstrate that when a chamber insert containing HIV-1-infected macrophages was added, BMVEC's expressed more RANTES (3.5-fold) and MCP-1 (two-fold) mRNA as compared to BMVEC's that were cultured alone ($P<0.05$ according to the Mann–Whitney *U*-test). These differences were 2.5-fold and 1.5-fold for RANTES and MCP-1, respectively, when BMVEC's were cocultured with uninfected macrophages ($P<0.05$ according to the Mann–Whitney *U*-test). No detectable mRNA levels of MIP-1α and MIP-1β were observed.

Table 1 Effect of HIV infection and BMVEC's on chemokine mRNA expression by macrophages.

	mϕ+BMVEC/ mϕ[a]	*HIV mϕ/ mϕ*[b]	*HIV mϕ+ BMVEC/mϕ*[c]
MIP-1α	3.2	3.8	8.1
MIP-1β	4.8	4.3	9.2
RANTES	3.5	2.7	3.7
MCP-1	3.9	3.2	6.3

Data show results that are representative of three independent experiments. [a]Ratio between chemokine mRNA levels of mixed cultures of uninfected macrophages and BMVEC's (mϕ+BMVEC's) and uninfected macrophages (mϕ). [b]Ratio between chemokine mRNA expression levels of HIV-infected macrophages (HIV mϕ) and chemokine mRNA expression levels of uninfected macrophages (mϕ). [c]Ratio between chemokine mRNA expression levels in mixed cultures of HIV-infected macrophages and BMVEC's (HIV mϕ+BMVEC's) and chemokine mRNA expression levels of HIV-infected macrophages (HIV mϕ).

Soluble factors produced by BMVEC's are responsible for the induction of MIP-1α, MIP-1β, and RANTES in macrophages

The coculture experiments were repeated in a transwell assay to investigate whether soluble factors produced by BMVEC's could have an effect on chemokine production by macrophages. Uninfected or HIV-infected macrophages were cultured in a chamber insert with BMVEC's in the lower compartment and RT–PCR was performed on the macrophages. Figure 3 shows chemokine expression by uninfected and HIV-infected macrophages alone or when cultured in the (trans-well) presence of BMVEC's. Soluble factors produced by BMVEC's caused an increase in MIP-1α, MIP-1β, RANTES and MCP-1 mRNA expression by uninfected macrophages, and an increase in MIP-1α, MIP-1β and RANTES expression by HIV-infected macrophages. Except for MCP-1, chemokine production was higher in HIV-infected macrophages that were cocultured in the trans-well presence of BMVEC's than in all other culture conditions ($P<0.05$ according to the Mann–Whitney *U*-test).

HIV-1 expression increases in the presence of BMVEC's

HIV-1 replication was monitored in cocultures of BMVEC's and HIV-infected macrophages and compared to virus replication in infected macrophages alone. The results are depicted in Figure 4. HIV-1

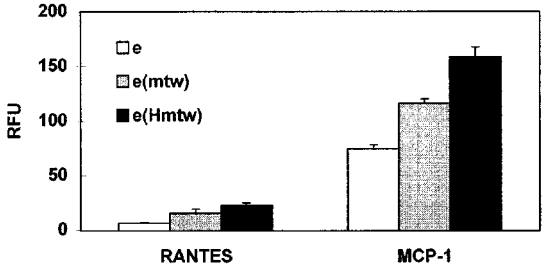

Figure 2 mRNA expression levels of RANTES and MCP-1 in BMVEC's (e), and BMVEC's after being cocultured in the presence of macrophages or HIV-infected macrophages in a chamber insert (e (mtw) and e (Hmtw), respectively). The depicted results are representative for at least three independent experiments and individual samples were analyzed three times by semi-quantitative PCR.

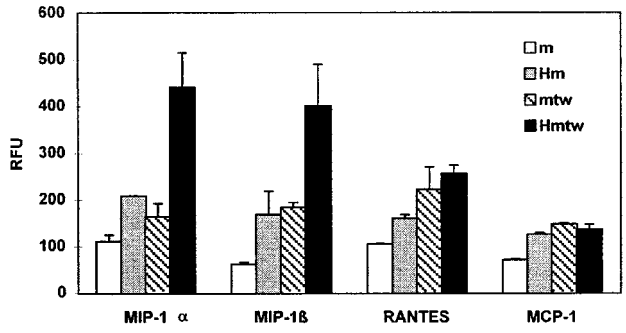

Figure 3 Chemokine expression by macrophages (m), HIV-infected macrophages (Hm), and uninfected and HIV-infected macrophages after being cultured in a chamber insert with BMVEC's in the lower compartment (m(tw), and Hm(tw), respectively). The depicted results are representative for at least three independent experiments and individual samples were analyzed three times by semi-quantitative PCR.

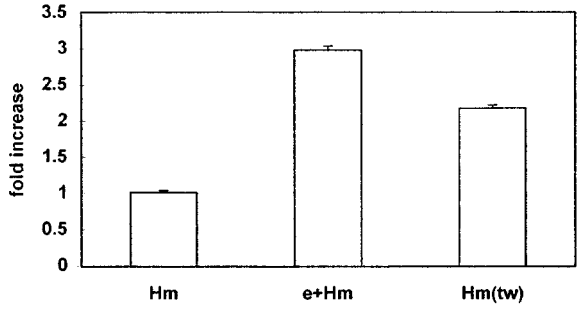

Figure 4 HIV-1 (tat-rev) expression by HIV-infected macrophages alone (Hm), cocultures of BMVEC's and HIV-infected macrophages (e+Hm) and by HIV-infected macrophages that were cultured in a chamber insert with BMVEC's in the lower compartment (Hm(tw)). The depicted results are representative for at least three independent experiments and individual samples were analyzed three times by semi-quantitative PCR.

expression increased threefold when infected macrophages were cocultured with BMVEC's and increased 2.2-fold when the macrophages were cocultured with the BMVEC's in the trans-well system. Thus, HIV-1 expression by macrophage was significantly higher in both coculture conditions as compared to HIV-infected macrophages alone ($P<0.05$, according to the Mann–Whitney *U*-test). In conclusion, soluble factors appear to be involved in the induction of HIV-1 expression.

IL-1β mRNA expression is increased in cocultures of macrophages and BMVEC's

Since IL-1β is a potent regulator of chemokine expression, we investigated whether IL-1β could be involved in the induction of chemokines in the cocultures. Figure 5 shows representative results of IL-1β expression by BMVEC's and macrophages under different culture conditions. HIV infection of macrophages induced IL-1β mRNA expression 2.8-fold and overall expression of IL-1β was increased

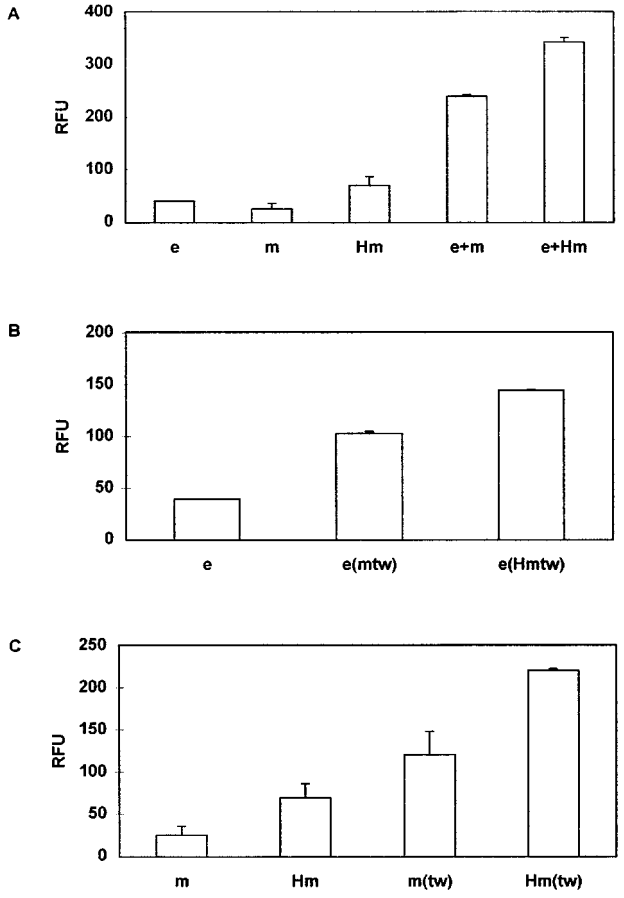

Figure 5 (**A**) IL-1β mRNA expression by BMVEC's (e), macrophages (m), HIV-infected macrophages (Hm) and cocultures of BMVEC's and uninfected (e+m) and infected macrophages (e+Hm). (**B**) IL-1β mRNA expression by BMVEC's (e), and by BMVEC's after being cocultured with a chamber insert containing uninfected or HIV-infected macrophages (e(mtw) and e(Hmtw), respectively. (**C**) IL-1β mRNA expression by uninfected and infected macrophages (m and Hm) and uninfected and HIV-infected macrophages after being cultured in a chamber insert with BMVEC's in the lower compartment (m(tw) and Hm(tw), respectively). The depicted results are representative for at least three independent experiments and individual samples were analyzed three times by semi-quantitative PCR.

in cocultures of macrophages and BMVEC's (Figure 5A). Coculturing the cells using the chamber insert also resulted in an increase of IL-1β expression by BMVEC's (Figure 5B) as well as by uninfected and HIV-infected macrophages (Figure 5C, $P<0.05$ according to the Mann–Whitney *U*-test). Thus, HIV infection as well as coculturing the two cell types both induce high levels of IL-1β mRNA in both BMVEC's and macrophages.

Chemokine expression by HIV-infected macrophages, but not by BMVEC's, is decreased in a trans-well assay after addition of a neutralizing antibody against IL-1β

To investigate whether the observed increase in chemokine production by cocultures of HIV-in-

fected macrophages and BMVEC's was IL-1β dependent, trans-well coculture experiments were repeated in the presence or absence of a neutralizing antibody against IL-1β. Subsequently, chemokine and HIV-1 mRNA expression by HIV-infected macrophages and BMVEC's was measured. Representative results are depicted in Figure 6. BMVEC's again enhanced chemokine expression by HIV-infected macrophages and this enhancement was at least partly abolished when anti-IL-1β was added to the coculture (Figure 6A). Thus, except for MIP-1β, in the presence of anti-IL-1β cocultures of BMVEC's and HIV-infected macrophages no longer demonstrated an increase of chemokine production as compared to other culture conditions (P=0.8, 0.73, 0.61 for MCP-1, MIP-1α, and RANTES, respectively, and P=0.05 for MIP-1β according to the Mann–Whitney U-test). In addition, HIV-1 expression decreased by 33% (data not shown). In the presence of an isotype-matched control antibody there was still a significant increase in chemokine mRNA expression ($P<0.05$, according to the Mann–Whitney U-test, data not shown).

BMVEC's expressed very low levels of MIP-1α and MIP-1β, and anti-IL-1β did not alter this (data not shown). MCP-1 and RANTES mRNA expression by BMVEC's was upregulated when HIV-infected or uninfected macrophages were added, and this upregulation was not abolished when anti-IL-1β was added (Figure 6B). We also noted that the presence of an isotype-matched control antibody did not affect the upregulation of chemokine mRNA expression ($P<0.05$, according to the Mann–Whitney U-test, data not shown).

Addition of an isotype-matched negative control to the cultures did not affect chemokine mRNA expression.

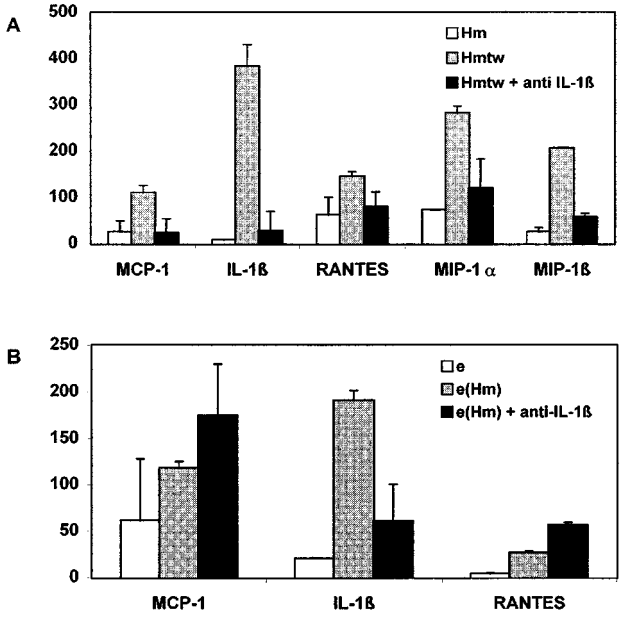

Figure 6 (**A**) Chemokine and IL-1β mRNA expression by HIV-infected macrophages (Hm), HIV-infected macrophages from a chamber insert with BMVEC's in the lower compartment without anti-IL-1β (Hm(tw)) and in the presence of anti-IL-1β (Hm(tw)+anti-IL-1β). (**B**) MCP-1, RANTES, and IL-1β expression by BMVEC's (e), and BMVEC's cultured in the presence of HIV-infected macrophages in a trans-well without and with anti-IL-1β (e(Hm) and e(Hm)+anti-IL-1β, respectively). The depicted results are representative for at least three independent experiments and individual samples were analyzed three times by semi-quantitative PCR.

Discussion

This study demonstrates that endothelial cells may participate in a highly active manner in the neuropathogenesis of AIDS. Initially, HIV-1-infected macrophages are shown to have a selective advantage to enter the brain by upregulating adhesion molecules and secreting matrix metalloproteinases to permeabilize the BBB (Nottet *et al*, 1997a). Since human monocytes will be attracted by high levels of CC chemokines, increased levels of CC chemokines in perivascular areas are likely to stimulate transendothelial migration. Subsequently, enhanced filtration of monocytes may result in increased production of inflammatory and neurotoxic molecules and may thereby augment progression to HAD. *In vivo*, MIP-1α and MIP-1β mRNA levels are elevated in brain tissue of demented AIDS patients (Schmidtmayerova *et al*, 1996) and MCP-1 was shown to be increased in the cerebrospinal fluid of patients with HAD (Conant *et al*, 1998). We show that, *in vitro*, infected as well as uninfected macrophages may engage into intensive interactions with brain endothelium, and this could have profound consequences on disease progression. Expression of CC chemokines increased significantly when HIV-1-infected macrophages were cocultured with BMVEC's. The increase was due to higher expression by HIV-1-infected macrophages for MIP-1α, MIP-1β and RANTES, whereas upregulation of MCP-1 and RANTES was observed mostly in the BMVEC's, as can be concluded from the trans-well experiments. This implies that soluble factors produced by BMVEC's stimulate MIP-1α, MIP-1β and RANTES expression by HIV-1-infected macrophages, whereas soluble factors produced by HIV-1-infected macrophages induce MCP-1 and RANTES production by BMVEC's. However, one should realize that there probably is extensive cross-talk between the cell-types and that the induction of chemokines by BMVEC's or macrophage products is probably an end result of induction and production of a variety of other factors by the two cell-types. In addition, in this study mRNA levels were measured that are likely to parallel protein levels, but deviations may exist.

A soluble factor that could be responsible for at least some part of the communication between

BMVEC's and macrophages is IL-1β. Immune activation and subsequent production of pro-inflammatory cytokines, like IL-1β, appears to be highly associated with HAD (Boven *et al*, 1999; Tyor *et al*, 1992; Vitkovic *et al*, 1994). In addition, in the U1 monocytic celline model, it was demonstrated that IL-1β can induce HIV-1 expression (Fan *et al*, 1994) and HIV-1 infection of macrophages is able to upregulate IL-1β production (Esser *et al*, 1996, 1998). This is in agreement with our findings that show that in primary human macrophages HIV-1 expression is increased when infected macrophages were cocultured with BMVEC's. Furthermore, it has been found that IL-1β can induce MCP-1 in endothelial cells (Martin *et al*, 1997; Goebeler *et al*, 1997) and that it can upregulate MIP-1α and MIP-1β in microglia (McManus *et al*, 1998). Thus, it seems that IL-1β can indeed play a highly regulatory role in the interactions between BMVEC's and macrophages. We demonstrate that BMVEC's express higher levels of IL-1β when cocultured in the trans-well presence of macrophages, significantly more when cultured with HIV-1 infected macrophages and vice versa. Thus, it appears that both cell types can immune activate each other without the need for cell–cell contact, and that subsequent IL-1β production could very well be responsible for the observed effects on chemokine and HIV-1 expression. Indeed, when we add neutralizing antibodies against IL-1β to the cocultures, chemokine expression by HIV-infected macrophages decreases. However, the antibody did not result in inhibition of MCP-1 mRNA expression by BMVEC's and even a small increase was observed. Thus, this suggests that, in contrast to BMVEC's, chemokine expression by macrophages is IL-1β dependent. In addition, HIV-1 expression by trans-well cultured macrophages almost returns to control levels in the presence of anti-IL-1β, which also suggests a role for IL-1β.

Thus, in HAD, immune activation appears to result in many events that may eventually lead to destruction of neuronal networks and functions. The actions of cytokines on brain cells have been found to result in increased oxidative stress (Lee *et al*, 1993; Liu *et al*, 1996; Boven *et al*, 1999), increased levels of chemokines (Rollins *et al*, 1990; Brown *et al*, 1994; Takahashi *et al*, 1995; McManus *et al*, 1998) and can even be directly neurotoxic (Rothwell and Strijbos, 1995; Talley *et al*, 1995). Therefore, therapeutic strategies that are aimed to decrease inflammatory processes may prove to be highly successful in preventing HAD.

Materials and methods

Isolation and culture of primary human monocytes, macrophages and BMVEC

Peripheral blood mononuclear cells were isolated from heparinized blood from HIV-1-, HIV-2-, and hepatitis B-seronegative donors and obtained on Ficoll-Hypaque density gradients. Cells were washed twice and monocytes were purified by countercurrent centrifugal elutriation. Cells were $>98\%$ monocytes by criteria of cell morphology on May-Grünwald-Giemsa-stained cytosmears and by nonspecific esterase staining using α-naphtylacetate (Sigma Chemical Co., St. Louis, MO, USA) as substrate. Monocytes were cultured in suspension at a concentration of 2×10^6 cells/ml in Teflon flasks (Nalgene, Rochester, NY, USA) in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated human AB serum negative for anti-HIV antibodies, 10 μg/ml gentamicin, and 10 μg/ml ciproflaxin (Sigma). As previously described, HIV-1 infection of non-adherent macrophages, especially when using a low multiplicity of infection, appears much more reproducible than infection of macrophages that were first allowed to adhere (Nottet *et al*, 1997b).

Human brain microvascular endothelial cells (BMVEC) were obtained from Cell Systems Corp. (Kirkland, WA, USA) and propagated as adherent monolayers on highly purified type 1 collagen-coated T-75 tissue culture flasks (Costar, Cambridge, MA, USA).

HIV-1 infection and cocultivation of macrophages and BMVEC

After 7 days monocyte-derived macrophages were recovered from the Teflon flasks and infected with HIV-$1_{Ba\text{-}L}$ at a multiplicity of infection of 0.01 for 2 h. HIV-infected and mock-infected macrophages were washed twice to remove unbound virus and cultured in Teflon flasks for an additional 5 days to establish a chronic infection. Then, the 5-day-old macrophages were washed and added directly or in a chamber insert to 24-well plates containing a monolayer of BMVEC in a 1 : 1 ratio for 48 h. For the IL-1β blocking experiments, 5 μg/ml anti-human IL-1β (R&D systems, Minneapolis, MN, USA) was added. Coculture experiments were performed three times and each experiment was analyzed three times using semi-quantitative PCR. Since substantial donor–donor differences exist for primary human monocyte-derived macrophages, representative results are shown and statistics were performed based on rank using the Mann–Whitney *U*-test to demonstrate consistent phenoma.

RT–PCR detection of chemokines

Cocultures of macrophages and BMVEC's were homogenized and lysed in 1 ml TRIzol (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's guidelines. Total RNA was isolated and dissolved in diethylpyrocarbonate (DEPC)-treated water and 1 μg of RNA was used for the synthesis of complementary DNA and PCR reactions were performed as described previously. For semi-quantification every primer pair was tested at different cycle numbers to determine the

linear range. GAPDH mRNA levels were measured at 23 cycles, whereas cDNA had to be subjected to 25 cycles to be in the linear range to detect MIP-1α, MIP-1β, and MCP-1 and 35 cycles for RANTES.

Aliquots of 5 μl of the biotinylated PCR product were semi-quantitatively analyzed using a fluorescent digoxigenin detection ELISA kit (Boehringer Mannheim) according to manufacturer's protocol as described previously (Boven *et al*, 1999). All data were normalized against GAPDH mRNA levels. The sequences of the primers and probes are depicted in Table 2.

Table 2 Sequences of the olignucleotide primers and probes in reverse transcriptase polymerase chain reaction

Target (product size)		*Sequence 5′–3′*
GAPDH	Sense	CCATGGAGAAGGCTGGGG
(195 bp)	Antisense	CAAAGTTGTCATGGATGACC
	Probe	CTGCACCACCAACTGCTTAGC
MIP-1α	Sense	TGCATCACTTGCTGCTGACACG
(333 bp)	Antisense	CAACCAGTCCATAGAAGAGG
	Probe	CTGACTACTTTGAGACGAGC
MIP-1β	Sense	CCAAACCAAAAGAAGCAAGC
(310 bp)	Antisense	AGAAACAGTGACAGTGGACC
	Probe	ACATCTCCTCCATACTCAGG
MCP-1	Sense	GCGAGCTATAGAAGAATCACC
(230 bp)	Antisense	ATAAAACAGGGTGTCTGGGG
	Probe	GACAAGCAAACCCAAACTCC
RANTES	Sense	CTTTGTCACCCGAAAGAACC
(352 bp)	Antisense	GTTTCATCATGTTGGCCAGG
	Probe	TTGCTCTTGTCCTAGCTTGG
HIV-1 tat/rev	Sense	GGCTTAGGCATCTCCTATGGC
(123 bp)	Antisense	TGTCGGGTCCCCTCGTTGCTGG
	Probe	CTTTGATAGAGAAACTTGATGAGTCTG
IL-1β	Sense	GCATCCAGCTACGAATCTCCGACC
(328 bp)	Antisense	CACTTGTTGCTCCATATCCTGTCCC
	Probe	GGACCAGACATCACCAAGCTTTTGCTG

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References

Baggiolini M, Dewald B, Moser B (1997). Human chemokines; an update. *Annu Rev Immunol* **15:** 675–705.

Boven LA, Gomes L, Hery C, Gray F, Verhoef J, Portegies P, Tardieu M, Nottet HSLM (1999). Increased peroxynitrite activity in AIDS Dementia Complex: Implications for the neuropathogenesis of HIV-1 infection. *J Immunol* **162:** 4319–4327.

Brown Z, Gerritsen ME, Carley WW, Strieter RM, Kunkel SL, Westwick J (1994). Chemokine gene expression and secretion by cytokine-activated human microvascular endothelial cells. Differential regulation of monocyte chemoattractant protein-1 and interleukin-8 in response to interferon-gamma. *Am J Pathol* **145:** 913–921.

Cairns JS, D'Souza MP (1998). Chemokines and HIV-1 second receptors: the therapeutic connection. *Nat Med* **4:** 563–568.

Conant K, Garzino Demo A, Nath A, McArthur JC, Halliday W, Power C, Gallo RC, 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.

Esser R, Glienke W, von Briesen H, Rubsamen Waigmann H, Andreesen R (1996). Differential regulation of proinflammatory and hematopoietic cytokines in human macrophages after infection with human immunodeficiency virus. *Blood* **88:** 3474–3481.

Esser R, Glienke W, Andreesen R, Unger RE, Kreutz M, Rubsamen Waigmann H, von Briesen H (1998). Individual cell analysis of the cytokine repertoire in human immunodeficiency virus-1-infected monocytes/macrophages by a combination of immunocytochemistry and in situ hybridization. *Blood* **91:** 4752–4760.

Fan ST, Hsia K, Edgington TS (1994). Upregulation of human immunodeficiency virus-1 in chronically infected monocytic cell line by both contact with endothelial cells and cytokines. *Blood* **84:** 1567–1572.

Furie MB, Randolph GJ (1995). Chemokines and tissue injury. *Am J Pathol* **146:** 1287–1301.

Genis P, Jett M, Bernton EW, Boyle T, Gelbard HA, Dzenko K, Keane RW, Resnick L, Mizrachi Y, Volsky DJ, Epstein LG, Gendelman HE (1992). Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease. *J Exp Med* **176:** 1703–1718.

Glass JD, Fedor H, Wesselingh SL, McArthur JC (1995). Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. *Ann Neurol* **38:** 755–762.

Goebeler M, Yoshimura T, Toksoy A, Ritter U, Brocker EB, Gillitzer R (1997). The chemokine repertoire of human dermal microvascular endothelial cells and its regulation by inflammatory cytokines. *J Invest Dermatol* **108:** 445–451.

Lavi E, Kolson DL, Ulrich AM, Fu L, Gonzalez Scarano F (1998). Chemokine receptors in the human brain and their relationship to HIV infection. *J Neurovirol* **4:** 301–311.

Lee SC, Dickson DW, Liu W, Brosnan CF (1993). Induction of nitric oxide synthase activity in human astrocytes by interleukin-1 beta and interferon-gamma. *J Neuroimmunol* **46:** 19–24.

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

Lukacs NW, Strieter RM, Elner V, Evanoff HL, Burdick MD, Kunkel SL (1995). Production of chemokines, interleukin-8 and monocyte chemoattractant protein-1, during monocyte:endothelial cell interactions. *Blood* **86:** 2767–2773.

Martin T, Cardarelli PM, Parry GC, Felts KA, Cobb RR (1997). Cytokine induction of monocyte chemoattractant protein-1 gene expression in human endothelial cells depends on the cooperative action of NF-kappa B and AP-1. *Eur J Immunol* **27:** 1091–1097.

McManus CM, Brosnan CF, Berman JW (1998). Cytokine induction of MIP-1 alpha and MIP-1 beta in human fetal microglia. *J Immunol* **160:** 1449–1455.

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.

Moser B, Loetscher M, Piali L, Loetscher P (1998). Lymphocyte responses to chemokines. *Int Rev Immunol* **16:** 323–344.

Navia BA, Jordan BD, Price RW (1986). The AIDS dementia complex: I. Clinical features. *Ann Neurol* **19:** 517–524.

Nottet HS, Persidsky Y, Sasseville VG, Nukuna AN, Bock P, Zhai QH, Sharer LR, McComb RD, Swindells S, Soderland C, Gendelman HE (1996). Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *J Immunol* **156:** 1284–1295.

Nottet HS, Bar DR, van Hassel H, Verhoef J, Boven LA (1997a). Cellular aspects of HIV-1 infection of macrophages leading to neuronal dysfunction in in vitro models for HIV-1 encephalitis. *J Leukoc Biol* **62:** 107–116.

Nottet HS, Moelans II, de Vos NM, de Graaf L, Visser MR, Verhoef J (1997b). N-acetyl-L-cysteine-induced up-regulation of HIV-1 gene expression in monocyte-derived macrophages correlates with increased NF-kappaB DNA binding activity. *J Leukoc Biol* **61:** 33–39.

Nuovo GJ, Alfieri ML (1996). AIDS dementia is associated with massive, activated HIV-1 infection and concomitant expression of several cytokines. *Mol Med* **2:** 358–366.

Persidsky Y, Ghorpade A, Rasmussen J, Limoges J, Liu XJ, Stins M, Fiala M, Way D, Kim KS, Witte MH, Weinand M, Carhart L, Gendelman HE (1999). Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis. *Am J Pathol* **155:** 1599–1611.

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

Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988). The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* **239:** 586–592.

Proost P, Wuyts A, van Damme J (1996). The role of chemokines in inflammation. *Int J Clin Lab Res* **26:** 211–223.

Randolph GJ, Furie MB (1995). A soluble gradient of endogenous monocyte chemoattractant protein-1 promotes the transendothelial migration of monocytes in vitro. *J Immunol* **155:** 3610–3618.

Rollins BJ, Yoshimura T, Leonard EJ, Pober JS (1990). Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. *Am J Pathol* **136:** 1229–1233.

Rothwell NJ, Strijbos PJ (1995). Cytokines in neurodegeneration and repair. *Int J Dev Neurosci* **13:** 179–185.

Sanders VJ, Pittman CA, White MG, Wang G, Wiley CA, Achim CL (1998). Chemokines and receptors in HIV encephalitis. *AIDS* **12:** 1021–1026.

Schmidtmayerova H, Nottet HS, Nuovo G, Raabe T, Flanagan CR, Dubrovsky L, Gendelman HE, Cerami A, Bukrinsky M, Sherry B (1996). Human immunodeficiency virus type 1 infection alters chemokine beta peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. *Proc Natl Acad Sci USA* **93:** 700–704.

Takahashi M, Masuyama J, Ikeda U, Kasahara T, Kitagawa S, Takahashi Y, Shimada K, Kano S (1995). Induction of monocyte chemoattractant protein-1 synthesis in human monocytes during transendothelial migration in vitro. *Circ Res* **76:** 750–757.

Talley AK, Dewhurst S, Perry SW, Dollard SC, Gummuluru S, Fine SM, New D, Epstein LG, Gendelman HE, Gelbard HA (1995). Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crmA. *Mol Cell Biol* **15:** 2359–2366.

Tyor WR, Glass JD, Griffin JW, Becker PS, McArthur JC, Bezman L, Griffin DE (1992). Cytokine expression in the brain during the acquired immunodeficiency syndrome. *Ann Neurol* **31:** 349–360.

Uguccioni M, D'Apuzzo M, Loetscher M, Dewald B, Baggiolini M (1995). Actions of the chemotactic cytokines MCP-1, MCP-2, MCP-3, RANTES, MIP-1 alpha and MIP-1 beta on human monocytes. *Eur J Immunol* **25:** 64–68.

Vitkovic L, da Cunha A, Tyor WR (1994). Cytokine expression and pathogenesis in AIDS brain. In: *HIV, AIDS and the Brain*. Price RW, Perry SW, (eds). Raven Press: New York, pp 203–222.