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Astrocyte-Derived Monocyte-Chemoattractant Protein-1 Directs the Transmigration of Leukocytes Across a Model of the Human Blood-Brain Barrier

Jonathan M. Weiss, Sherry A. Downie, William D. Lyman, and Joan W. Berman


Abstract.

The migration of leukocytes across the blood-brain barrier (BBB) into the central nervous system is critical in the pathogenesis of central nervous system inflammatory diseases. The production of chemokines, such as monocyte-chemoattractant protein-1 (MCP-1), by endothelial cells (EC) and astrocytes may initiate and amplify this process. Using a coculture of human EC and astrocytes to model the BBB, we demonstrated that exogenous MCP-1 induces the transmigration of monocytes in a dose-dependent manner. TNF-α, IFN-γ, or IL-1β treatment of cocultures also induced significant migration of monocytes that correlates with the induction of MCP-1 protein. TGF-β, previously shown to induce MCP-1 expression in astrocytes, but not in EC, caused migration of monocytes across cocultures, but not across EC grown alone. Monocytes and lymphocytes transmigrated across cytokine-treated cocultures in greater numbers than across EC alone. Astrocytes were the main source of cytokine-induced MCP-1, supporting a role for astrocytes in facilitating leukocyte transmigration. A blocking Ab to MCP-1 inhibited MCP-1- and cytokine-induced transmigration of monocytes by 85–90%. Cytokine treatment of cocultures also resulted in the transmigration of activated, CD69-positive lymphocytes. The MCP-1-mediated transmigration of monocytes across cocultures was blocked using an Ab to ICAM-1 and inhibited by 55% using an Ab to E-selectin. These data suggest a central role for astrocyte-derived MCP-1 in directing the migration of monocytes and lymphocytes across the BBB.
system in which HUVEC were cocultured with autologous astrocytes on opposite sides of a porous insert (27). Astrocyte processes contact the EC through the insert pores and induce the expression of BBB markers, including glucose transport protein-1 and ε glutaminyl-transpeptidase (21, 27). These markers are not expressed on umbilical vein EC grown in the absence of astrocytes or in the absence of direct astrocyte-EC contact through the insert pores. The same coculture system was utilized by Hayashi et al., who also demonstrated the induction of barrier properties, as evidenced by an increased resistance to the passage of radiolabeled inulin (28). Using our coculture system, we demonstrated a role for MCP-1 in facilitating leukocyte transmigration and determined the effects of proinflammatory cytokines upon EC and astrocyte-derived chemokines that may mediate the transmigration process. We demonstrate an important role for astrocyte-derived MCP-1 in mediating the migration of monocytes and activated lymphocytes across cocultures.

Materials and Methods

Source of fetal tissue

The present study is part of an ongoing research protocol that has been approved by the Albert Einstein College of Medicine Committee on Clinical Investigation (Bronx, NY), and the City of New York Health and Hospitals Corporation. Informed consent was obtained from all participants. Fetal tissues were obtained at the time of elective termination of intrauterine pregnancy from healthy females (29).

Cell culture

Astrocyte cultures were prepared according to a modified protocol of McCarthy and de Vellis (30). Briefly, human fetal CNS tissue was separated from the meninges, minced, and digested in 0.25% trypsin (Life Technologies, Grand Island, NY), 0.1% collagenase (Sigma, St. Louis, MO). The resulting cell suspension was serially filtered through sterile 100- and 80-μm nylon mesh filters (Tetko, Elmsford, NY) and pelleted at 900 rpm. Cultures were established in RPMI media supplemented with 10% FCS and 1% penicillin/streptomycin (Life Technologies). After 12 days, microglial cells were removed from the mixed culture by shaking 30 min at 4°C. Cells were passaged after trypsinization and were examined by immunofluorescence using an anti-factor VIII-related Ag Ab (Boehringer Mannheim, Indianapolis, IN), anti-macrophage (HAMP56; IgM; 1:50; Enzo Diagnostics, Farmingdale, NY), as a marker for cells of the monocyte/macrophage lineage, and 1:100 mouse myeloma; 1:50; Organon Teknika, Durham, NC; and antimedulillary and subcapsular cortical thymic epithelial cells (IgM; 1:50; Sigma, respectively). Astrocyte cultures (3–5 passages) were >99% glial fibrillary acidic protein positive and unreactive for either HAM56 or control Abs. Thus, cells of passages 3–5 were used to ensure the absence of contaminating microglial cells.

HUVEC were obtained by digesting umbilical cords with type II collagenase (2 mg/ml; Worthington Biochemical, Freehold, NJ) and were grown on 0.2% gelatin (Fisher Scientific, Pittsburgh, PA)-coated tissue culture plates in M199 media (Life Technologies) supplemented with 20% newborn calf serum (Life Technologies), 5% heat-inactivated human serum (Biocell Laboratories, Rancho Dominguez, CA), 1% penicillin/streptomycin, and 12 ng/ml endothelial cell growth factor (Sigma). Human brain microvascular endothelial cells were purchased from Cell Systems (Kirkland, WA). EC cultures were >99% factor VIII positive, as demonstrated by immunofluorescence using an anti-factor VIII-related Ag Ab (IgG1; 1:100; Dako, Carpentrya, CA) and unreactive for an isotype-matched negative control (IgG1 mouse myeloma protein; 1:50; Organon Teknika).

Establishment of EC and astrocyte cocultures

Cocultures of umbilical vein EC and astrocytes were established on opposite sides of a gelatin-coated, 3-μm pore-size tissue culture insert (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ). Astrocytes (1 × 10⁵) were first seeded onto the underside of the insert. Cells were allowed to attach for 4–5 h, at which time the insert was placed into a 24-well tissue culture plate containing complete M199 media. EC (1.6 × 10⁵) were applied to the gelatin-coated opposite side of the insert. Cocultures were maintained until 16 h before the transmigration assay, at which time inserts were transferred to new wells containing cytokines using M199 media supplemented with only 1% newborn calf serum and no human serum, so as to minimize the effect of serum on MCP-1 expression (31). These media were utilized throughout the transmigration assay. The transmigration assay was always conducted 3 days following establishment of cocultures.

Reagents

EC and astrocytes on the insert were treated with either 1 ng/ml TGF-β1 (endotoxin <0.1 ng/μg; R&D Systems, Minneapolis, MN), 100 U/ml TNF-α (endotoxin <0.1 ng/μg; R&D), 200 U/ml IFN-γ (endotoxin <0.2 ng/mg; Genzyme, Cambridge, MA), or 2 IU/ml IL-1β (endotoxin < 2.5 EU/mg; National Cancer Institute, Frederick, MD) for 16 h. Human rMCP-1 was purchased from Genzyme. For cytokine treatments, 400 μl media containing cytokine were placed in a 24-well plate, and the insert tops were gently washed with warmed media. For MCP-1-blocking experiments, cultures were washed and preincubated with 10 μg/ml mAb 1077 to MCP-1 (a generous gift of Dr. Charles Mackay, formerly of Leukosite, Cambridge, MA) or IgG1 mouse myeloma protein (Organon Teknika) as a negative control for 1 h.

Isolation of PBMC and PBMC transmigration

Human PBMC were isolated from freshly drawn human blood by Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) centrifugation, as previously described (32). Briefly, whole blood was diluted 1/3 with PBS, layered over Ficoll (1:2.5), and centrifuged at 1400 rpm for 40 min at 18°C. The layer containing PBMC was removed and washed with cold PBS containing 1% FCS. Cells were spun at 1400 rpm for 20 min at 4°C and resuspended with cold media. Cells were brought to a final concentration of 1.5 × 10⁶ PBMC/ml with room temperature media. The tops of the inserts were washed again with warmed media. PBMC (200 μl or 3 × 10⁵ cells) were added to the top of the insert, and inserts were washed with cold media to minimize variability, one blood donor was used for the majority of experiments.

Monocyte immunocytochemistry and quantification

Monocytes that had transmigrated and adhered to the bottom of the well were fixed briefly in 5% Formalin. Monocytes adherent to the bottom of the insert were blocked with 1% BSA/PBS, cells were incubated with a monocyte-specific mAb (Mac 387; Dako) overnight at 4°C. Cells were washed and incubated with peroxidase-conjugated secondary Abs for 1 h. For visualization, cells were washed and stained with diaminobenzidine (Sigma). The number of transmigrated cells was calculated by counting the number of stained cells under ×40 magnification. Five nonoverlapping fields were counted, and the average of five fields determined the number per field. The transmigration of cells transmigrated is equivalent to the number of cells/field × 20.

Flow-cytometric analysis

For the majority of experiments, the cells that had transmigrated were collected from the culture supernatant below the insert. Cells adherent to the well bottoms were detached with 0.5 mM EDTA/PBS and pooled with the previously collected cells. Cells were centrifuged 5 min at 300 × g and resuspended in a final volume of 400 μl media. A total of 10 μl Leukocyte Ab mixture (Becton Dickinson, San Jose, CA) was added for a 30-min incubation at 4°C. This mixture contains FITC-conjugated anti-CD45, a pan-leukocyte marker, and PE-conjugated anti-CD14, a monocyte marker. For some experiments, a FITC-conjugated CD14 Ab was used in combination with a PE-conjugated CD69 Ab (Becton Dickinson). Cells were centrifuged, fixed in 2% paraformaldehyde, and analyzed within 24 h by FACS.

Leukocyte transmigration was analyzed with a FACScan flow cytometer (Becton Dickinson). The numbers of monocytes and lymphocytes per sample were determined by acquisition and analysis of List Mode Data files using Lysis II software and the Consort 32 computer system. The instrument was calibrated with CALIBRITE beads and autocontrol software BDIS using a 4-parameter, 4-decade logarithmic fluorescence amplifier. Gated acquisition was determined by a combination of gates based on forward scatter, side scatter, and CD45-FITC reactivity. The number of cells in each sample and the starting leukocyte sample was determined by analyzing the number of events recorded in a 3-min interval.

Adhesion molecule-blocking studies

Cocultures were stimulated with TNF-α (100 U/ml) on the EC side to induce the expression of adhesion molecules. For ICAM-1, the contribution of basal expression (untreated) was also examined. The length of TNF-α treatment was 4 h for E-selectin and IG9, and 7 h for VCAM-1.
We first determined whether exogenously added MCP-1 induces the migration of monocyes across cocultures. A range of MCP-1 doses was added to the lower chamber of coculture inserts, and $3 \times 10^5$ PBMC were added to the upper chamber. After 2.5 h, the number of transmigrated monocytes was quantified by staining cells that had passed through the filter and adhered to the bottom of the plate with an Ab for monocytes, Mac 387. As shown in Fig. 1, MCP-1 induced the dose-dependent transmigration of monocytes, with 100 ng/ml MCP-1 resulting in maximal migration ($p < 0.001; n = 3$). Significant transmigration was observed using a dose of 20 ng/ml MCP-1 as well ($p < 0.03$). Transmigration could be detected by 1 h and was maximal after 2.5 h (data not shown). In the absence of exogenously added MCP-1, there was a low level of monocyte transmigration due to minimal baseline expression of MCP-1 by astrocytes (Fig. 1). The addition of 100 ng/ml MCP-1 to both the upper and lower chambers, so as to abolish the chemokine gradient, inhibited monocyte transmigration to levels below this basal level of migration (Fig. 1).

To determine what other cells were transmigrating with monocytes and in what proportion, we pooled the adherent and nonadherent populations of transmigrated cells and analyzed them using an Ab mixture that detects lymphocytes as well as monocytes (Leukogate mixture consisting of CD45-FITC and CD14-PE; Becton Dickinson). As shown in Fig. 2A, the addition of 100 ng/ml MCP-1 to the lower chamber resulted in the significant transmigration of monocytes across a coculture of EC and astrocytes ($p < 0.0003; n = 15$). With this method, we also demonstrated monocyte migration using as little as 5 or 10 ng/ml MCP-1 (data not shown). Lymphocytes migrated to a lesser, yet significant, degree ($p < 0.002$ as compared with untreated cocultures). The migrated sample reflected a dramatic shift in the ratio of monocytes to lymphocytes. Whereas the starting CD45-positive leukocyte population consisted of approximately 90% lymphocytes and 10% monocytes (CD14-positive; $n = 15$), Transmigrated leucocytes consisted of approximately 90% monocytes in response to MCP-1.
monocytes (Fig. 2C). The few lymphocytes that migrated are likely to be activated, given that MCP-1 is chemotactic for activated T cells (34). In the absence of MCP-1, there was minimal migration of either monocytes or lymphocytes (Fig. 2A). As before, the addition of MCP-1 to the upper and bottom chambers abolished the chemotactic gradient and completely inhibited migration (Fig. 2A).

Cytokine treatment of EC and astrocytes induces MCP-1 and monocyte migration

To determine whether proinflammatory cytokines activate EC and astrocytes to express functional MCP-1, cocultures or EC cultured alone were treated for 16 h with TNF-α, IFN-γ, or IL-1β. The upper chambers were washed once and PBMC were added. After 2.5 h, transmigrated cells were collected from the lower chamber and analyzed by FACS for CD45 and CD14 expression and compared with untreated (UT) cultures. TGF-β induced monocyte migration across cocultures (p < 0.0005; n = 11), but not EC alone. TNF-α, IFN-γ, or IL-1β each induced significant monocyte migration across cocultures (p < 0.003; n = 11) and EC alone (p < 0.04; n = 6). Differences between monocyte migration across cocultures and EC alone were significant for TGF-β and TNF-α (p < 0.04), but not for IFN-γ or IL-1β.

FIGURE 3. Cytokine treatment of cocultures or EC alone induces monocyte transmigration. Cocultures or EC grown in the absence of astrocytes were treated for 16 h with TGF-β, TNF-α, IFN-γ, or IL-1β. The upper chambers were washed once and PBMC were added. After 2.5 h, transmigrated cells were collected from the lower chamber and analyzed by FACS for CD45 and CD14 expression and compared with untreated (UT) cultures. TGF-β induced monocyte migration across cocultures (p < 0.0005; n = 11), but not EC alone. TNF-α, IFN-γ, or IL-1β each induced significant monocyte migration across cocultures (p < 0.003; n = 11) and EC alone (p < 0.04; n = 6). Differences between monocyte migration across cocultures and EC alone were significant for TGF-β and TNF-α (p < 0.04), but not for IFN-γ or IL-1β.

Astrocytes are a main source of MCP-1 in coculture supernatants. Aliquots of the supernatant from the lower chamber were collected and analyzed by ELISA for MCP-1 protein expression. Cytokine treatment of cocultures induced MCP-1 protein (⁎⁎, p < 0.00001; n = 11). Cytokine-treated astrocytes, grown in the absence of EC, expressed significant MCP-1 (⁎, p < 0.004; n = 5) to levels nearly equivalent of cocultures. TNF-α, IFN-γ, or IL-1β-treated EC produced significant MCP-1 into the lower chamber (⁎, p < 0.004; n = 8), but to a lesser extent than cocultures or astrocytes alone.

The fact that astrocyte-derived chemokines mediate a large proportion of this migration across cocultures was confirmed by analyzing by ELISA the supernatants from the lower chamber for MCP-1 expression (Fig. 4). Cytokine-treated cocultures released significant levels of MCP-1 protein into the lower chamber, as compared with untreated cocultures (p < 0.00001). By comparing MCP-1 expression in cocultures with that of EC or astrocytes grown on the inserts separately, 75–80% of the MCP-1 expression was astrocyte derived. Cytokine-treated EC released significant MCP-1 protein in amounts sufficient to promote monocyte migration (p < 0.004; n = 8), albeit to a lesser extent than cocultures or astrocytes alone.

We also analyzed by ELISA the upper chamber supernatants of TNF-α- or IL-1β-treated EC and detected approximately 150 and 110 ng/ml MCP-1, respectively, as compared with 30 ng/ml MCP-1 in the lower chamber supernatants for each cytokine. Thus, EC production of MCP-1 was predominantly into the upper chamber. The upper chamber supernatants of cytokine-treated astrocytes contained equivalent MCP-1 protein as the lower chamber (70–100 ng/ml). In our protocol, we removed the upper chamber supernatant before the addition of PBMC, so as to mimic what might be occurring in vivo as a result of the blood flow removing any unbound chemokine.

Exogenous addition of 100 ng/ml MCP-1 to the lower chamber of EC or astrocyte cultures resulted in an approximately 50% increase (150 ± 12 and 160 ± 37 ng/ml, respectively) in MCP-1 expression by both cell types. We are currently investigating this apparent autocrine regulation of chemokine expression in both EC and astrocytes. In EC or astrocyte cultures, as well as in EC/astrocyte cocultures, we did not detect other monocyte chemoattractants, such as MIF-1α, MIP-1β, or RANTES (data not shown). By ELISA, IFN-γ treatment of cocultures inconsistently induced 10–20 ng/ml IP-10 in the lower chamber (data not shown).
lymphocytes, the transmigrated lymphocytes were CD69-negative. The percentage of lymphocytes that transmigrated were CD69 positive (data not shown). Monocytes did not transmigrate were CD69 positive (data not shown). Monocytes that transmigrated were CD69 negative, except for a minimal increase for CD69 expression, and thus were in an activated state (36) (Fig. 6). Expression of CD69 was used to assess the state of activation of the lymphocytes. As compared with the starting population of PBMC, which consisted of 95% CD69-negative lymphocytes, the transmigrated lymphocytes were >50% positive for CD69 expression, and thus were in an activated state (36) (Fig. 6). Even in the case of untreated cultures, the few lymphocytes that did transmigrate were CD69 positive (data not shown). Monocytes were negative for CD69 expression, except for a minimal increase with IFN-γ treatment (data not shown), consistent with previous findings that IFN-γ induces CD69 expression in monocytes (36).

To determine whether the increase in lymphocyte CD69 expression could be due to their exposure to cytokines, we treated PBMC with TNF-α or IL-1β for 2.5 h, since this is the maximum length of time that these cells would encounter the cytokines in our transmigration assay. No difference in CD69 expression was detected for either TNF-α- or IL-1β-treated lymphocytes, as compared with untreated lymphocytes (data not shown).

MCP-1 directly mediates leukocyte migration across EC and astrocyte cocultures

To determine whether the transmigration of monocytes in our system was specifically due to MCP-1, the supernatant from the lower chamber was incubated with a neutralizing Ab to MCP-1 for 1 h before addition of PBMC. As shown in Fig. 7, preincubation with mAb 10F7 (10 μg/ml) resulted in the marked inhibition of monocyte transmigration across cocultures. The percentage of inhibition was 90% for treatments with MCP-1 or IFN-γ, and 85% for treatments with TGF-β, TNF-α, or IL-1β (p < 0.05; n = 4). An isotype-matched, mouse myeloma protein was used at 10 μg/ml as a negative control, and this did not have any effect on leukocyte migration for any treatment condition. Anti-MCP-1 pretreatment inhibited the TNF-α- or IL-1β-induced lymphocyte migration across cocultures by 55 and 45%, respectively (p < 0.04; data not shown). Although IP-10 was detected inconsistently by ELISA in IFN-γ-treated cocultures, experiments using an IP-10-blocking Ab (R&D; 10 μg/ml) had no effect on monocyte migration (data not shown). No IP-10 was detected in TNF-α- or IL-1β-treated cocultures, suggesting the presence of an additional chemokine(s) that is not MIP-1α, MIP-1β, or RANTES, responsible in part for mediating T cell migration.

ICAM-1 and E-selectin mediate MCP-1-mediated monocyte transmigration across cocultures

We determined the roles of adhesion molecules in the MCP-1-induced transmigration of monocytes across cocultures. Cocultures were treated with TNF-α on the EC side (as described in Materials and Methods). Cocultures were washed and preincubated with mAbs to either ICAM-1, E-selectin, VCAM-1, IG9, or isotype-matched myeloma proteins as negative controls. As shown in Fig. 8A, anti-ICAM-1 completely blocked MCP-1-induced monocyte
transmigration across cocultures ($p < 0.03$; $n = 3$). Results were similar, irrespective of whether cocultures were left untreated or stimulated with TNF-$\alpha$. As shown in Fig. 8B, anti-E-selectin treatment reduced monocyte transmigration across TNF-$\alpha$-stimulated cocultures by 55% ($p < 0.05$; $n = 4$). Pretreatment with either anti-VCAM-1 or anti-IG9 had no significant effect upon MCP-1-induced monocyte transmigration ($n = 2$ each; data not shown). In all cases, the expression of the respective adhesion molecules by EC in the cocultures was confirmed by immunocytochemistry (data not shown).

Discussion

These findings indicate an important role for astrocyte-derived MCP-1 in directing the migration of monocytes and activated lymphocytes across a coculture of EC and astrocytes that models the BBB. We demonstrate that cytokines induce MCP-1 expression by astrocytes and subsequent migration of monocytes and lymphocytes. We therefore suggest that astrocyte-derived MCP-1 plays a key role in leukocyte trafficking into the CNS. The production of proinflammatory cytokines by the astrocytes (6, 7) and infiltrating leukocytes further enhances MCP-1 expression by astrocytes and subsequent leukocyte transmigration. The transmigrating leukocytes and other cells, such as microglia, may then serve as sources of additional chemoattractants, such as MIP-1-$\alpha$, MIP-1-$\beta$, and RANTES (37, 38), that contribute to this ongoing process.

Numerous in vivo studies have suggested a role for the expression of MCP-1 in the pathogenesis of CNS inflammatory disease. MCP-1 is expressed in the brain and cerebrospinal fluid of patients with AIDS dementia (14). This was the only chemokine detected in the CSF of these individuals. During the course of EAE, the marked infiltration of monocytes and reactive T lymphocytes is preceded by MCP-1 expression by astrocytes, EC, and additional leukocytes (17–19). In addition, MCP-1 was found to play a role in relapsing disease (16). In a mouse transgenic model in which MCP-1 expression was targeted to the brain using a myelin basic protein promoter, the overexpression of MCP-1 in the brain resulted in a significant mononuclear infiltrate (20). In humans, reactive astrocytes within and surrounding the MS lesion also expressed MCP-1, implicating this chemokine in the development of MS lesions (15).

Our findings are in agreement with others who have demonstrated an important role for proinflammatory cytokines in promoting the transmigration of activated leukocytes. In a study on the migration of uninfected and HIV-infected monocytes, Persidsky et al. found that the activation state, not infection, of macrophages...
correlated with their migratory potential (39). Furthermore, Bird- 
sall et al. showed that TNF-α primes leukocytes for enhanced 
transendothelial migration (40). Our data indicate that the expres-
sion of proinflammatory cytokines in the CNS facilitates the re-
cruitment of activated leukocytes via astrocyte-derived MCP-1.

We found that TGF-β potently induced MCP-1 expression by 
astrocytes and facilitated the subsequent transmigration of 
monocytes across cocultures. A role for TGF-β in the recruit-
ment of mononuclear cells into the CNS was also found by 
Wyss-Coray et al., who showed that transgenic mice whose 
astrocytes produce high levels of TGF-β had increased inflam-
mation and disease severity in EAE (41). We believe that this 
may be attributed, in part, to TGF-β acting on the astrocytes 
themselves to induce MCP-1 expression. It would be important 
to examine chemokine expression in these transgenic mice to 
characterize this possible mechanism. This proinflammatory 
role for TGF-β contrasts with previous findings that TGF-β 
treatment ameliorates EAE (42, 43). This may indicate impor-
tant distinctions between murine and human studies, and also 
underscores the pleiotropic nature of TGF-β, whose effects de-
pend upon the cytokine and cellular environment (44).

EC are a major source of MCP-1 (23, 24), and a role for EC-
derived MCP-1 in facilitating leukocyte transmigration across 
cocultures cannot be excluded. We show in this study that cytokine 
treatment induced MCP-1 expression to levels sufficient to pro-
mote a small, but significant leukocyte transmigration across EC 
monolayers. MCP-1 bound to extracellular matrix components 
may also mediate the initial attraction of the leukocytes for the 
endothelium, leading to firm adherence. Additionally, when we 
performed ELISA analysis on the upper chamber supernatants 
of cytokine-treated EC cultures, we detected significant MCP-1-pro-
tein. We removed the upper chamber supernatants and washed 
the insert tops before adding PBMC for the assay, since this is 
what may occur in vivo as a result of blood flow. Thus, EC-derived 
MCP-1 that is not matrix bound would not play a significant role 
in our system.

In our studies, Abs to MCP-1 blocked 85–90% of the monocyte 
and 45–55% of the lymphocyte migration across cocultures. 
Although astrocyte expression of IP-10 has been noted in EAE (18), 
we detected little or no IP-10 and found no significant role for this 
chemokine in mediating either monocyte or lymphocyte migration 
among cocultures. It is possible, however, that the induction of 
IP-10 is more complex, involving the interactions of multiple cy-
tokines with cell types. We also did not detect MIP-1α, MIP-1β, 
or RANTES in the cytokine-treated cocultures, suggesting that, for 
lymphocytes, other chemokines contribute to their transmigration 
among the cocultures.

We found that the MCP-1-induced transmigration of monocytes 
was dependent upon ICAM-1 and partially dependent upon E-se-
lectin. No significant role for either VCAM-1 or ICAM-1 in this 
process was found. The enhanced expression of ICAM-1, VCAM-1, 
and E-selectin has been demonstrated in several CNS inflamma-
tory diseases, including MS (45, 46) and HIV-1 encephalitis (47, 
48). In those studies, the increased expression of adhesion mole-
cules by EC and/or astrocytes was generally correlated with the 
extent of leukocyte infiltration. VLA-4/VCAM-1 interactions have 
been shown to play an important role in mediating leukocyte ad-
hesion in both EAE (49, 50) and SIV encephalitis (51). Our find-
ings demonstrate that pathways other than VCAM-1 may be util-
ized in the transmigration of leukocytes across the BBB in 
response to MCP-1. Furthermore, our data are consistent with 
those of several groups who showed that, using EC only, the trans-
endothelial transmigration of monocytes is dependent upon LFA-1 
and Mac1, which each bind ICAM-1 (52, 53). In these studies, the

two processes of leukocyte adherence and transmigration could be 
distinguished. VLA-4 was more important than LFA-1 in mediat-
ing the initial binding of monocytes to cytokine-activated EC. Once 
the monocytes had adhered to the EC, the addition of anti-
VLA-4 Ab had no significant effect on their subsequent transmi-
igration. In contrast, the migration of monocytes could be blocked 
using an Ab to CD18, the integrin chain common to both LFA-1 
and Mac1. Thus, in our coculture system: 1) LFA-1 or Mac-1 
binding to ICAM-1 may be sufficient for monocyte binding, as 
well as transmigration, or 2) VLA-4 on monocytes may bind li-
gands other than VCAM-1, such as fibronectin (54). Our mAb to 
VCAM-1 binds to domains 6 and 7. Thus, it is also possible that 
other domains of VCAM-1 may mediate monocyte adhesion that 
would not have been detected with our reagent. Future studies 
using activated and/or HIV-infected leukocytes may suggest addi-
tional mechanisms whereby these cells cross the BBB and enter 
the CNS.

MCP-1 appears to play a critical role in both the initiation and/or 
amplification stages of CNS inflammatory diseases. The transmi-
grated leukocytes and other CNS elements, such as microglia, 
may be sources of additional MCP-1, as well as other chemokines 
and cytokines (38), that contribute further to this process.

This is the first report of cytokine-induced MCP-1 causing the 
migration of leukocytes across a coculture of human EC and as-
astrocytes and the role that astrocyte-derived factors play in this 
process. Strategies aimed at regulating astrocyte-derived MCP-1 
expression may therefore yield useful approaches to limit the pro-
gression of CNS inflammatory diseases.

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