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

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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 γ glutamyl-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 (Teko, 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. Cultures were passaged after trypsinization and were examined by immunofluorescence using an anti-factor VIII-related Ag Ab (1:50; Dako, Carpenteria, CA) and unreactive for an isotype-matched negative control (IgG1 mouse myeloma protein; 1:50; Organon Teknika, Durham, NC) and antimedulillary mouse myeloma (IgG1; 1:50; Organon Teknika, Durham, NC) and unreactive for either HAM56 or control Abs. Thus, microglial cells were removed from the mixed culture by shaking 30 min at 4°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 × 10⁶ cells/ml) were added to the top of the insert, and inserts were washed for integrity. For wells receiving MCP-1, 400 μl MCP-1 at different concentrations were added below the culture insert. The transmigration assay was conducted for 2.5 h at 37°C. 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 and stained with a blocking Ab/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 was termed the number of cells/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 FACSscan 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 BDLS 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.
FIGURE 1. MCP-1 (100 ng/ml) induces maximal transmigration of monocytes across cocultures. MCP-1 was added to the lower chamber of cocultures at the indicated dose for 2.5 h. Monocytes that transmigrated and adhered to the plate bottom were incubated with Mac 387 Ab and quantified as the number of Mac 387-positive cells per five nonoverlapping fields (×40 magnification). A total of 20 and 100 ng/ml MCP-1 induced significant monocyte migration (p < 0.03 and p < 0.001, respectively; n = 3) as compared with untreated cultures (0 ng/ml). A dose of 200 ng/ml MCP-1 induced no more monocyte transmigration than was observed with 100 ng/ml (n = 1). As a control, 100 ng/ml MCP-1 was added to both the top and bottom chambers, so as to abolish the chemotactic gradient (+/+).

ELISA
Cocultures were washed and incubated with 50 μg/ml of one of the following Abs: anti-ICAM-1 (IgG1; Dako), anti-E-selectin (IgG2a; Becton Dickinson), anti-VCAM-1 (IgG1; Becton Dickinson), anti-IG9 (IgG3; purified in our laboratory (33)), or isotype-matched myeloma proteins as negative controls (Cappel, West Chester, PA). After 1 h, the upper and lower sides of cocultures were washed extensively with media. Monocyte transmigration was assayed as above by the addition of 100 ng/ml MCP-1 to the lower chamber supernatants.

Statistical analyses
Student’s one-tailed, paired t test was used to compare the numbers of transmigrated leukocytes and MCP-1 protein in cytokine-treated cultures with that of untreated cultures. For MCP-1-blocking experiments, significance between anti-MCP-1- and negative control-treated supernatants was similarly compared. Results were considered to be significant for p < 0.05 values.

Results
MCP-1 induces migration of monocytes across a coculture of EC and astrocytes
We first determined whether exogenously added MCP-1 induces the migration of monocytes across cocultures. A range of MCP-1 doses was added to the lower chamber of coculture inserts, and 3 × 10⁵ 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 leukocytes consisted of approximately 90% monocytes in response to MCP-1.

FIGURE 2. MCP-1 induces monocyte and lymphocyte transmigration across cocultures. MCP-1 (100 ng/ml) was added to the lower chamber of cocultures for 2.5 h. Transmigrated leukocytes were quantified by collecting adherent and nonadherent cells from the lower chamber and incubating them with Leukogate mixture Ab (see Materials and Methods). A, Significant numbers of monocytes and lymphocytes transmigrate in response to MCP-1 in the bottom chamber (+/−; p < 0.0003; n = 15). Minimal migration was observed in the absence of MCP-1 (−/−) or the presence of MCP-1 on both sides of the insert (+/+; n = 4). B, The starting population of leukocytes was gated according to forward and side scatter, and CD45-FITC and CD14-PE expression. It consisted of approximately 90% lymphocytes (CD14-negative) and 10% monocytes (CD14-positive; n = 15). C, Transmigrated leukocytes consisted of approximately 90% monocytes in response to MCP-1.
Cytokine treatment of cocultures or EC alone 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.

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 on the underside (astrocyte side) of the insert. We previously determined that the timing and dose of these cytokine treatments induce MCP-1 expression in both EC and astrocytes (35, 55). After 16 h, the top chamber was washed once with warm media, and PBMC were added for the transmigration experiment. The supernatant from the bottom chamber was untouched. A total of 3 x 10⁶ PBMC was added to the top chamber, and cultures were incubated for 2.5 h. As shown in Fig. 3, significant monocyte migration across cocultures was detected with TNF-α, IFN-γ, or IL-1β treatments (p < 0.003; n = 11 each), as compared with untreated cocultures. TNF-α, IFN-γ, or IL-1β also induced significant monocyte migration across EC cultures (p < 0.04; n = 6). When cocultures, but not EC alone, were treated with TGF-β, significant monocyte migration was also detected (p < 0.0005). This is consistent with our earlier findings that TGF-β induces MCP-1 expression in human fetal astrocytes (25). Greater monocyte migration was detected across cocultures, as compared with EC alone. These differences were significant for TGF-β and TNF-α (p < 0.04 each). We also examined leukocyte transmigration across cocultures and EC monolayers using human brain microvascular endothelial cells. Identical transmigration profiles of monocytes and lymphocytes across cytokine-treated cultures were detected with cultures established using HUVEC (data not shown).

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 in the inserts separately, 75–80% of the MCP-1 expression in cocultures was attributed to the astrocytes (Fig. 4). With TGF-β treatment, all of the MCP-1 expression was astrocyte derived. Cytokine-treated EC released significant MCP-1 protein in amounts sufficient to promote leukocyte 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 EC and astrocytes. In EC or astrocyte cultures, as well as in EC/astrocyte cocultures, we did not detect other monocyte chemotactants, such as MIP-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-positive (data not shown). Monocytes that transmigrated were CD69 positive (data not shown). MCP-1 directly mediates leukocyte migration across EC and astrocyte cocultures

Cytokine treatments induce the transmigration of activated lymphocytes

As shown in Fig. 5, significant migration of lymphocytes was observed following TGF-β, TNF-α, IFN-γ, or IL-1β treatment of cocultures (p < 0.004; n = 10), as compared with untreated conditions. The order of potency was IL-1β or TNF-α > IFN-γ >> TGF-β. With EC grown in the absence of astrocytes, the baseline trafficking of lymphocytes was greater, and only IFN-γ induced significant lymphocyte migration above that of untreated cultures (p < 0.04). We determined whether these lymphocytes were activated, because MCP-1 is chemotactic for activated, but not resting T cells (34). 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 on 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, IgG9, or isotype-matched myeloma proteins as negative controls. As shown in Fig. 8A, anti-ICAM-1 completely blocked MCP-1-induced monocyte 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.

FIGURE 5. Cytokine treatment of cocultures or EC alone induces lymphocyte 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 expression. Baseline lymphocyte migration was greater across EC than across cocultures. MCP-1 (100 ng/ml) induced lymphocyte migration across cocultures (p < 0.002; n = 15) and EC alone (p < 0.01; n = 7), as compared with untreated cultures. TGF-β, TNF-α, IFN-γ, and IL-1β induced lymphocyte migration across cocultures (p < 0.004; n = 10) as compared with untreated cultures. IFN-γ, but not TGF-β, TNF-α, or IL-1β, induced significant lymphocyte migration across EC (p < 0.04; n = 6).

FIGURE 6. The transmigrated lymphocytes express CD69, an early activation marker. Following acquisition using forward scatter, side scatter, and CD14 gates, lymphocytes were analyzed by FACS for CD69 expression. The starting population of lymphocytes was 95% negative for CD69 expression. Following TNF-α or IL-1β treatment of cocultures, the lymphocytes that transmigrated were >50% positive for CD69 expression. One experiment, representative of three such experiments, is shown. Similar findings were observed for IFN-γ treatment of cocultures and for cytokine treatment of EC alone.
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, Birdsell et al. showed that TNF-α primes leukocytes for enhanced transendothelial migration (40). Our data indicate that the expression of proinflammatory cytokines in the CNS facilitates the recruitment 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 recruitment 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 inflammation 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 important distinctions between murine and human studies, and also underscores the pleiotropic nature of TGF-β, whose effects depend 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 promote 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 cocultures by EC and/or astrocytes we detected significant MCP-1 protein. 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 across cocultures. It is possible, however, that the induction of IP-10 is more complex, involving the interactions of multiple cytokines 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 across the cocultures.

We found that the MCP-1-induced transmigration of monocytes was dependent upon ICAM-1 and partially dependent upon E-selectin. No significant role for either VCAM-1 or IG9 in this process was found. The enhanced expression of ICAM-1, VCAM-1, and E-selectin has been demonstrated in several CNS inflammatory diseases, including MS (45, 46) and HIV-1 encephalitis (47, 48). In those studies, the increased expression of adhesion molecules 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 adhesion in both EAE (49, 50) and SIV encephalitis (51). Our findings demonstrate that pathways other than VCAM-1 may be utilized 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 transendothelial 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 adhesion and transmigration could be distinguished. VLA-4 was more important than LFA-1 in mediating 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 transmigration. 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 ligands 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 additional 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 transmigrated 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 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 progression of CNS inflammatory diseases.

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