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Cytokine Induction of MIP-1α and MIP-1β in Human Fetal Microglia

Carrie M. McManus,* Celia F. Brosnan,*† and Joan W. Berman2α†

Leukocyte infiltration into the central nervous system (CNS) is a key event in the inflammatory processes of neuroimmunologic diseases. Microglia, resident macrophages of the CNS, may contribute to this process by elaborating chemoattractants that are capable of recruiting leukocytes across the blood-brain barrier. Such factors have been detected in the CNS of animal models of multiple sclerosis and in the brains of human and nonhuman primates with AIDS encephalitis. As the expression of these chemoattractants may play an important role in the initiation and progression of neuroimmunologic diseases, we analyzed expression of the chemokines MIP-1α, MIP-1β, MCP-1, and RANTES in human fetal microglial cultures. Unstimulated microglia expressed minimal levels of MIP-1α, MIP-1β, and MCP-1, while RANTES was undetectable. In response to LPS, TNF-α, or IL-1β, both MIP-1α and MIP-1β were induced at the mRNA and protein levels in a dose- and time-dependent manner. IFN-γ did not significantly induce chemokine expression. MCP-1 was detectable in LPS- and cytokine-treated microglia. TGF-β, a cytokine with down-modulatory effects on other cell types, had little effect on chemokine expression in microglia when used concomitantly before or during treatment with LPS. These results illustrate the ability of certain inflammatory stimuli to induce expression of MIP-1α, MIP-1β, and MCP-1 by human fetal microglia. The expression of these chemoattractants may function to recruit inflammatory cells into the CNS during the course of neuroimmunologic diseases and may modulate the ability of HIV to infect the CNS. The Journal of Immunology, 1998, 160: 1449–1455.

Microglia, resident brain macrophages, serve important functions in the central nervous system (CNS). Microglia serve as APCs of the CNS, remove tissue debris during development and following trauma, regulate the proliferation of astrocytes, and produce cytokines and other soluble factors associated with an immunologic response (1–8). It has been proposed that activated microglia are involved in the pathogenesis of numerous CNS diseases such as multiple sclerosis (MS) (1) and AIDS encephalitis (9, 10); however, the precise role of microglia in these pathologies is unclear. Neuroimmunologic diseases are characterized by infiltrations of inflammatory cells across the blood-brain barrier. These cells are recruited to sites of injury where they aid in both tissue injury and repair.

Chemokines are low m.w. chemoattractant cytokines that have been shown to recruit leukocytes. MIP-1α, MIP-1β, MCP-1, and RANTES are all members of the C-C chemokine family, named for the distinct cysteine-cysteine motif found near the N termini of all members. These chemokines are characterized by their ability to attract monocytes and T cells and may be necessary for the recruitment of inflammatory cells to sites of injury (11). During the course of neuroimmunologic diseases, cells endogenous to the CNS must produce chemotactic factors that can recruit mononuclear cells across the blood-brain barrier to sites of injury. There is evidence both in vitro and in vivo for the production of chemokines in the CNS (12, 13). Hayashi et al. (12) showed that murine microglia secrete MIP-1α after stimulation with LPS, and MIP-1α has been shown by Miyagishi et al. (13) to be present in the cerebrospinal fluid of MS patients. Karpus et al. (14) demonstrated that blocking Ab to MIP-1α prevented development of both acute and relapsing experimental autoimmune encephalomyelitis (EAE) while also preventing the infiltration of mononuclear cells. Also, MCP-1 has been shown to increase in the CNS of mice with EAE, an animal model of MS (15–18). Despite these reports on chemokine expression in the CNS of rodent models of inflammation, little is known about chemokine expression in the human CNS.

Schmidtmayerova et al. (19) showed that MIP-1α and MIP-1β mRNA are up-regulated in the brain tissue of patients with AIDS dementia. In addition, MIP-1α, MIP-1β, and RANTES have recently been shown to be suppressive factors for HIV, and their receptors are cofactors for HIV entry into cells (20–22). The role of these chemokines in HIV infection of cells of the CNS provides potential new therapeutic targets for the treatment and prevention of HIV infection in the CNS and in AIDS-related dementia.

The cellular sources and regulation of chemokines have not been definitively shown in cells of the human CNS. In this study, we analyzed the kinetics of expression of the C-C chemokines MIP-1α, MIP-1β, RANTES, and MCP-1 in human fetal microglia. The proinflammatory cytokines IL-1β, TNF-α, and IFN-γ, in addition to LPS, were used to activate microglia, and analyses were performed for RNA and protein expression of the various chemokines.

TGF-β is a pleiotropic cytokine that has been shown to be protective in EAE and to regulate expression of some cytokines, as well as nitric oxide, in murine microglia (23, 24). Previous data by Maltman et al. (25) showed that TGF-β is a potent down-regulator of MIP-1α and MIP-1β mRNA and protein in murine bone marrow macrophages. Thus, we also analyzed the effects of TGF-β on the expression of MIP-1α and MIP-1β.
Here, we present the first comprehensive study of the expression of chemokines in human microglia. Our data show that human primary fetal microglia can differentially express MIP-1α, MIP-1β, and MCP-1 under inflammatory conditions, and that the LPS-induced expression of MIP-1α and MIP-1β is not significantly down-modulated by TGF-β. These findings differ from what is seen in monocytes and macrophages from other tissue sources, as well as in murine cells.

Materials and Methods

Cell culture and reagents

Human fetal CNS tissue (20–23 wk) was obtained at the time of elective termination of intrauterine pregnancy from otherwise normal healthy females. Informed consent was obtained from all participants. This tissue was used as part of an ongoing research protocol that has been approved by the Albert Einstein College of Medicine Committee on Clinical Investigation and the City of New York Health and Hospitals Corporation. The tissue was prepared similarly to that previously described by Lee et al. (26). Briefly, tissue was dissociated and incubated for 45 min at 37°C in 1 × HBSS (Life Technologies, Grand Island, NY), 1 × trypsin (Life Technologies), and DNase I (Boehringer Mannheim, Indianapolis, IN). Tissue fragments were then passed through 250- and 150-μM nylon mesh (Tetko, Inc., Briarcliff Manor, NY). Cells were washed, resuspended in complete DMEM (25 mM HEPES, 10% FCS, 1% nonessential amino acids, and 1% penicillin-streptomycin), and reseeded. Cells were seeded at 1.2 × 10^6 per 150-cm² tissue culture flask (Falcon, Becton Dickinson, Franklin Lakes, NJ) and cultured for 12 days. Microglial cells were then removed from the mixed culture by shaking 30 min at 4°C and plated in complete DMEM at a concentration of 1 × 10^6 cells per 100 × 20-mm tissue culture plate (Falcon). Cells were analyzed for the purity of the culture and shown to be ≥95% HAM56 (a microglial marker) positive. Cells were treated with between 1 ng/ml and 1 μg/ml of LPS (serotype 0111:B4) from Sigma Chemical Co. (St. Louis, MO), 10 U/ml of human rIL-1α (courtesy of Dr. C. D. Janeway, Yale University, New Haven, CT), 100 U/ml of human rIFN-γ (a gift from Dr. C. D. Janeway, Yale University, New Haven, CT), 100 U/ml of human rTNF-α (courtesy of Dr. Detlef Schlondorff, Albert Einstein College of Medicine, Bronx, NY) (29), and 18S (30). The MIP-1α and MIP-1β expression was measured with a 10-ng/ml treatment of LPS. A representative pooled protein data from ELISA analyses of cell culture supernatants was analyzed for chemokine expression. Concentrations of both MIP-1α and MIP-1β were increased at all doses of LPS tested. There is a similar induction after 8 h of treatment with LPS, a potent activator of cells of the monocyte/macrophage cell lineage, was used to activate microglia, and these cultures were then analyzed for mRNA and protein expression of MIP-1α or MIP-1β. Northern blot analysis of these data showed that MIP-1α and MIP-1β mRNA (Fig. 2A). There is a similar induction of these chemokines at all doses after 8 h of treatment. Cell supernatants were analyzed by ELISA for protein expression of both MIP-1α and MIP-1β (Fig. 2B). Both chemokines were strongly induced at all doses of LPS tested.

The kinetics of LPS-induced expression of these chemokines was measured with a 10-ng/ml treatment of LPS. A representative Northern blot analysis is shown in Figure 3A illustrating a time-dependent response for MIP-1α and MIP-1β expression. The densitometric analysis of these data showed that MIP-1α and MIP-1β mRNA were induced after 8 h of treatment with LPS, increased after 12 h, declined over the next 24 h, and returned to almost untreated levels by 48 h after treatment (Fig. 3B). Figure 3C shows pooled protein data from ELISA analyses of cell culture supernatants. The protein expression induced by LPS continued to increase over all time points examined, with similar induction of MIP-1α and MIP-1β.

Immunofluorescence

Adherent cells were washed twice with PBS, fixed with ice-cold methanol, and blocked in 1% BSA for 1 h. The primary Ab, either HAM56 (specific for fixed macrophages and used at a dilution of 1:50, Enzo Diagnostics, Farmingdale, NY), glial fibrillary acidic protein (GFAP) (an astrocyte-specific marker used at a dilution of 1:50, Enzo Diagnostics, Farmingdale, NY), or mouse myeloma IgG1 or IgM (used at a dilution of 1:100, Cappel Research Products, Durham, NC) was incubated overnight at 4°C. Cells were then washed and incubated with fluorescein isothiocyanate–labeled secondary Abs for 1 h. The slides were photographed at ×10 magnification using an inverted Olympus IMT-2 microscope with an Olympus 35-mm camera (Olympus Corporation, Lake Success, NY). The pictures were taken with Kodak Elite II 35-mm color film for slides and then developed. After developing, the slides were captured and processed with Adobe Photoshop (Adobe Systems Inc., Mountain View, CA).

RNA extraction and Northern blot analysis

Total RNA was extracted from microglial cultures using Tri-Reagent (Molecular Research Center, Cincinnati, OH). RNA was electrophoresed through a 1% agarose gel and transferred to Hybond nylon membrane (Amer sham, Cleveland, OH). Membranes were then prehybridized for 1 h at 65°C and hybridized overnight at 65°C with [α-32P]-labeled cDNA inserts as probes (Random Primer Labeling Kit, Amersham). Prehybridization and hybridization were performed in a buffer consisting of 25% SSPE, 10% SDS, 50% Denhardt’s solution, 2.5% and 20% SDS, and salmon sperm DNA. Hybridization was conducted using the following cDNA inserts as probes: MIP-1α, MIP-1β (courtesy of Dr. Barbara Sherry, Picower Institute for Medical Research, Manhasset, NY) (27, 28), MCP-1 (courtesy of Dr. Detlef Schlondorff, Albert Einstein College of Medicine, Bronx, NY) (29), and 18S (30). The MIP-1α and MIP-1β probes have been shown to be specific for the individual chemokine. After hybridization, blots were washed twice at room temperature with wash buffer (2 × SSC, 0.1% SDS, 0.005% NaP inorganic phosphate), once at 55°C in buffer at a 1:2 dilution, and then at 65°C in wash buffer at a 1:2 dilution. Blots were then exposed to x-ray film (Fisher, Springfield, NJ) at −70°C. Densitometry was performed on multiple film exposures using a computing densitometer (Molecular Dynamics, Sunnyvale, CA).

Chemokine ELISA

 Supernatants from microglial cell cultures were analyzed for chemokine proteins. ELISA kits for MIP-1α, MIP-1β, MCP-1, and RANTES were purchased from R&D Systems and used according to the manufacturer’s instructions.

Results

Characterization of human fetal cell cultures

Human CNS tissue was cultured and microglia were obtained from a mixed culture of CNS cells. Figure 1 shows the morphology and purity of the microglial cell cultures that were used throughout this study. More than 95% of the cells were positive for HAM56, a microglial marker, as determined by immunofluorescence. Occasionally, some astrocytes were detected by GFAP reactivity, but those detected represented <5% of the cells.

LPS induction of MIP-1α and MIP-1β in human fetal microglia

Highly purified human fetal microglial cultures were analyzed for their ability to express chemokines under different treatment conditions. LPS, a potent activator of cells of the monocyte/macrophage cell lineage, was used to activate microglia, and these cultures were then analyzed for mRNA and protein expression of MIP-1α or MIP-1β. Northern blot analysis of cultures stimulated with increasing doses of LPS showed potent induction of both MIP-1α and MIP-1β mRNA (Fig. 2A). There is a similar induction of these chemokines at all doses after 8 h of treatment. Cell supernatants were analyzed by ELISA for protein expression of both MIP-1α and MIP-1β (Fig. 2B). Both chemokines were strongly induced at all doses of LPS tested.

The kinetics of LPS-induced expression of these chemokines was measured with a 10-ng/ml treatment of LPS. A representative Northern blot analysis is shown in Figure 3A illustrating a time-dependent response for MIP-1α and MIP-1β expression. The densitometric analysis of these data showed that MIP-1α and MIP-1β mRNA were induced after 8 h of treatment with LPS, increased after 12 h, declined over the next 24 h, and returned to almost untreated levels by 48 h after treatment (Fig. 3B). Figure 3C shows pooled protein data from ELISA analyses of cell culture supernatants. The protein expression induced by LPS continued to increase over all time points examined, with similar induction of MIP-1α and MIP-1β.
TGF-β does not modulate MIP-1α or MIP-1β induction by LPS

TGF-β, a cytokine with anti-inflammatory properties, can significantly down-modulate MIP-1α and MIP-1β expression in murine bone marrow macrophages (25). In addition, Suzumura et al. have shown that TGF-β suppresses cytokine-induced activation of murine microglia (31). We have previously shown that TGF-β down-modulates LPS-induced IL-1β expression in human fetal microglia (data not shown). However, we have also found that in human fetal microglia, TGF-β was unable to modulate chemokine expression. Figure 4 shows a representative experiment of TGF-β pretreatment before treatment with LPS. As can be seen in Figure 4A, mRNA expression was increased after LPS treatment alone; pretreatment with TGF-β did not alter this expression. The protein data follow what is seen with mRNA expression in that there was no significant modulation by TGF-β. Cotreatment of microglia with TGF-β and LPS for 20 h had no significant effect on mRNA or protein expression of MIP-1α or MIP-1β as compared with treatment with LPS alone (data not shown). Pretreatment of microglia with TGF-β for 16 h before 4 h of treatment with LPS (data not shown) also had no significant effect on mRNA or protein expression of MIP-1α or MIP-1β.

Proinflammatory cytokines induce MIP-1α and MIP-1β expression in human fetal microglia

The proinflammatory cytokines TNF-α, IL-1β, and IFN-γ were tested for their ability to induce MIP-1α or MIP-1β at the mRNA and protein levels. These cytokines are all made by cells endogenous to the CNS (5, 6, 32) and have been shown to play a role in CNS inflammatory diseases (9, 33). TNF-α induced MIP-1α mRNA as shown in Figure 5A. TNF-α induction was similar to that of LPS in that it followed the same kinetics. Induction was observed at 8 h, increased at 12 h, and then decreased to basal level by 48 h. Protein expression shows that TNF-α potentiated both MIP-1α and MIP-1β to similar levels. The amount of protein expressed continued to increase up to the last time point analyzed, which was 48 h.

IL-1β also induced MIP-1α and MIP-1β expression as shown by the graph of protein in Figure 6A. Over time, MIP-α was not induced as potently by IL-1β as compared with MIP-1β. MIP-1β protein increased over time up to 48 h, and MIP-1α increased only until 24 h and then remained at a similar level up to 48 h. Figure 6B shows that IFN-γ could not induce either MIP-1α or MIP-1β by guest on November 9, 2022 http://www.jimmunol.org/ Downloaded from
protein at any time points analyzed. It is important to note that astrocytes, a possible contaminating cell type in our cultures, do not produce either MIP-1α or MIP-1β in response to these factors (data not shown).

We also determined whether cytokines associated with a Th2 response could induce MIP-1α or MIP-1β. We found that IL-4 and IL-10, as well as MCP-1, could not significantly induce expression of either chemokine at the mRNA or protein level (data not shown).

Comparison of the kinetics of MIP-1α and MIP-1β protein expression induced by LPS and cytokine treatment of human fetal microglia

MIP-1α and MIP-1β were significantly induced by LPS over a time course of 48 h. Comparison of their expression from pooled data of several experiments (n ≥ 4) shows that MIP-1α and MIP-1β had similar kinetics as well as levels of induction following treatment with LPS (Fig. 3C). Figure 7 shows a comparison of cytokine treatments for pooled protein data from several experiments (n ≥ 4). TNF-α was an effective inducer of MIP-1α, and this induction was similar to that of LPS. IL-1β was a weaker inducer of MIP-1α, and IFN-γ did not induce this chemokine at any time. MIP-1β expression differed from MIP-1α in that TNF-α and IL-1β were similar inducers of MIP-1β over time, whereas IL-1β was only a weak inducer for MIP-1α. Similar to MIP-1α, IFN-γ did not induce MIP-1β. Although IL-1β and TNF-α are inducers of MIP-1α and MIP-1β, their potencies of induction were not as strong as LPS. These results reflect the differential expression of these chemokines in response to various inflammatory factors.

LPS induces expression of MCP-1 in human fetal microglia

MCP-1 is another member of the C-C chemokine family that is known to correlate with the course of disease in EAE (15). Human fetal microglia, as shown in Figure 8, expressed significant levels
of MCP-1 mRNA after treatment with LPS. This expression continued to increase over time to up 48 h, the last time point assayed. These kinetics differ from what was seen with MIP-1α and MIP-1β, both of which had an earlier peak of mRNA expression at 12 to 24 h. MCP-1 protein was also detected in the cell supernatants of these cultures after LPS and cytokine treatment (data not shown).

RANTES is undetectable in human fetal microglia

Untreated cells, as well as LPS-, TNF-α-, IL-1β-, or IFN-γ-treated cells, were analyzed for RANTES mRNA or protein expression. RANTES was undetectable under all conditions by both Northern blot analysis and ELISA analysis (data not shown).

Discussion

The results presented detail the release of chemokines in response to endotoxin and proinflammatory cytokines. Human fetal microglia expressed MIP-1α and MIP-1β in response to LPS, TNF-α, and IL-1β and did not respond to IFN-γ. LPS treatment also elicited MCP-1 expression. These responses were dose and time dependent. TGF-β did not significantly modulate LPS-induced expression of MIP-1α or MIP-1β. We also showed that human fetal microglia were not induced to express these chemokines after treatment with IL-4, IL-10, or MCP-1. The expression of these chemokines from cells endogenous to the CNS, particularly the human CNS, has not been extensively studied. This is the first report of MIP-1α and MIP-1β mRNA and protein expression as well as MCP-1 expression from human primary microglial cells.

Chemokines, or chemotactic cytokines, are characterized by their ability to induce migration of leukocytes. The C-C chemokine family, MIP-1α, MIP-1β, MCP-1, RANTES, etc., is characterized by its ability to recruit monocytes and T cells (11). A hallmark of CNS inflammation is the infiltration of monocytes/macrophages and T cells across the blood-brain barrier to sites of injury. This is seen in MS and EAE as well as in AIDS encephalitis (9, 34). Thus, the release of chemokines by cells endogenous to the CNS could play a role in recruiting inflammatory cells into the CNS and therefore could be crucial to the pathogenesis of CNS inflammatory disease. Several groups have shown that chemokines are expressed in the CNS of animal models of inflammation (14–18). We have shown previously that MCP-1 may be an important mediator of CNS inflammation, using a rat model of EAE. MCP-1 was detected at the onset of inflammation and its levels increased and decreased according to the course of disease activity (15). Godiska et al. and others demonstrated that many chemokines including MIP-1α, MIP-1β, JE (MCP-1), and RANTES were induced during the course of EAE in mice (15–18). Karpus et al. (14) illustrated a distinct role for one chemokine, MIP-1α, in murine EAE. This group showed that Abs to MIP-1α blocked the development of EAE as well as leukocyte infiltration into the CNS. All of these findings point to an important role for chemokines in CNS inflammation.
We found that the Th1-associated cytokines TNF-α and IL-1β induced expression of MIP-1α and MIP-1β from human fetal microglia. In contrast, we found that Th2-associated cytokines did not induce this expression. These data are consistent with recent findings by Schrum et al. (35) implicating MIP-1α and MIP-1β in type 1 cytokine-mediated inflammation. EAE, a Th1-mediated disease, can be abrogated by blocking Abs to MIP-1α (14), a Th1-associated chemokine, but not MCP-1 (14), a Th2-associated factor (36).

Recent findings indicating that MIP-1α, MIP-1β, and RANTES act as suppressive factors for HIV and that their receptors play a role as cofactors for HIV entry into host cells also propose new roles for these chemokines and raise interesting questions, especially in the context of HIV infection of the CNS (20–22). Schmidtmayorova et al. (19) showed that HIV-1-infected monocytes produced MIP-1α and MIP-1β chemokine messages and that mRNA levels of these chemokines were elevated in the brains of patients with HIV encephalitis. They also indicated that cells with cytoplogic features of microglia/macrophages and astrocytes were expressing this message. Sasseville et al. (37) showed that there was increased expression of MIP-1α and MIP-1β protein in the brains of macaque monkeys with SIV-induced AIDS encephalitis and that cells with the morphology of monocytes/microglia appeared to be expressing these chemokines. Recent data by He et al. show that human microglia express CD4 as well as CCR3 and CCR5, chemokine receptors that function as cofactors for HIV-1 infection (38). It is of interest that MIP-1α and MIP-1β are ligands for CCR5 and may be important as suppressive factors that compete with HIV for this receptor to block infection of microglial cells. Future studies on the regulation of receptor expression on microglia in response to HIV infection as well as proinflammatory stimuli will help clarify the role of chemokines in CNS disease. It will be important to determine chemokine and chemokine receptor expression by cells in the CNS to understand the mechanism by which HIV infects these cells and how it then mediates damage resulting in encephalitis and dementia. We are currently pursuing these studies.

The data presented here illustrate both some distinct differences and some similarities between human microglial chemokine expression and chemokine expression by similar cell types, monocytes, and macrophages in both humans and rodents. Our data are consistent with Hayashi et al. (12), who showed that LPS induces MIP-1α from murine microglial cells as well as from other groups showing LPS induction of MIP-1α and MIP-1β from human PBMCs and alveolar macrophages (39, 40). However, Hayashi et al. (12) also demonstrated that murine microglia do not express MCP-1 in response to LPS, whereas we found that human microglia are potently induced by LPS to express MCP-1. Similarly, previous studies have shown that IL-1β induces MIP-1α in human PBMCs and alveolar macrophages, and although we found that IL-1β was a potent inducer of MIP-1α, it only weakly induced MIP-1α in our studies (39). TNF-α and IFN-γ do not induce MIP-1α in human PBMCs or alveolar macrophages (39). Human fetal microglia are also not induced by IFN-γ to produce MIP-1α or MIP-1β. In our studies, TNF-α potently induced MIP-1α and MIP-1β expression. This differs from what was reported by Berkman et al. (39) and Martin et al., whose studies demonstrated no induction of these chemokines by TNF-α in human or murine macrophages (41). Another major difference between our studies and those of others is the inability of TGF-β to modulate MIP-1α or MIP-1β expression in human fetal microglia, whereas it strongly down-regulates their expression in murine bone marrow macrophages (25). These differences illustrate the complexity of cytokine/chemokine interaction and emphasize how this interplay varies between cells of similar lineage as well as between the same cells of different species. This also highlights the differences between the CNS and other organs and tissues.

Our results show for the first time that human primary fetal microglia can be induced to express MIP-1α, MIP-1β, and MCP-1 mRNA and protein in response to various proinflammatory stimuli including LPS. An understanding of the different profiles of chemokine expression from cells endogenous to the CNS and from cells that can infiltrate into the CNS during inflammation will be important in the development of novel therapeutic strategies to treat neuroimmunologic diseases.

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References


