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Matrix metalloprotease-9 release from monocytes increases as a function of differentiation: implications for neuroinflammation and neurodegeneration

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Abstract

Naïve monocytes extravasate in response to monocyte chemoattractant-1 (MCP-1) and subsequently, following differentiation within tissue, carry out effector functions. Consistent with this concept, expression of the MCP-1 receptor CCR2 decreases with monocyte differentiation, as production of cytokines increases (Fantuzzi et al., 1999). Because matrix metalloproteases (MMPs) may also play an important role in the ability of monocytes to migrate into tissues and/or to promote pathogen clearance/tissue injury, we have examined production of matrix metalloprotease-9 as a function of both monocyte differentiation in vitro and expression of CCR2. Increased time in culture, which is linked to monocyte differentiation, resulted in enhanced production of MMP-9, assessed by gelatin substrate zymography. Further, CCR2-negative monocytes produced greater quantities of MMP-9 than did naïve CCR2-positive cells. Our results indicate that MMP-9 release increases during monocyte differentiation, consistent with a prominent role in effector functions. Because extracellular matrix proteins are important to cell structure and survival (Wee Yong et al., 1998), increased expression of MMP-9 could contribute to tissue damage following monocyte differentiation.

Keywords: MMP-9; MCP-1; Monocytes; CCR-2

1. Introduction

Several central nervous system (CNS) diseases, including HIV dementia, feature parenchymal invasion by hematogenous monocytes that locally differentiate into macrophages. In many such conditions, macrophage-derived products make a substantial contribution to tissue damage (Epstein and Gendelman, 1993; Glass et al., 1995; Kuroiwa et al., 1999).

Matrix metalloproteases (MMPs) are a family of zinc-containing endopeptidases, that selectively degrade components of the extracellular matrix [ECM] (Wee Yong et al., 1998). These proteins can also activate membrane-bound cytokines and receptors. MMPs are grouped according to their major substrates, although there is substantial overlap. Despite promiscuous substrate specificities, MMPs exhibit functional differentiation, as they vary with respect to cellular source, inducibility and efficiency of substrate utilization. Further, MMP activity is regulated at the levels of proenzyme activation and interaction with specific tissue inhibitors of metalloproteinases (TIMPs).

MMPs are implicated in diverse physiological processes, including leukocyte migration across capillary basement membranes (Leppert et al., 1995; Leppert et al., 1996). Several lines of evidence raise the possibility that MMP-9 may be important for extravasation by naïve monocytes, as MMP-9 degrades collagen type IV and entactin, both of which are major components of capillary basement membranes.

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Additionally, expression of MMP-9 could be envisioned as a component of the differentiated monocyte/macrophage effector program. MMP-9 is expressed by monocytes after exposure to varied inflammatory stimuli (Zhang et al., 1998). In this view, MMPs produced within tissue sites could be toxic to pathogens or parenchymal cells. ECM proteins facilitate cell survival, supporting the notion that MMPs could mediate cytotoxic effects (Nichol et al., 1995; Bozzo et al., 1997; Libby et al., 1999). This concern is heightened by the observation that tissue levels of MMPs are elevated in pathological conditions characterized by an excess of activated macrophages (Wee Yong et al., 1998). MMPs produced within the brain parenchyma may also amplify inflammatory reactions by promoting CNS entry of peripheral blood leukocytes. Consistent with this concept, injection of MMP-9 into the brain parenchyma induced leukocyte infiltration of the CNS (Rosenberg et al., 1992; Anthony et al., 1998).

The recruitment of leukocytes into tissue is contingent on the expression of adhesion molecules, chemokines and chemokine receptors (Sasseville et al., 1992; Conant et al., 1998; Cinque et al., 1998; Kelder et al., 1998). With respect to monocytes, MCP-1 and its receptor, CCR2, are critically important to transendothelial migration. (Weiss et al., 1998). MCP-1 is a relatively potent and selective monocyte chemoattractant (Uguccioni et al., 1995) and moreover, recent studies in gene-targeted mice demonstrate that MCP-1 is uniquely essential to monocyte recruitment in vivo (Lu et al., 1998).

CCR2 is a critical receptor for MCP-1 mediated transendothelial migration of monocytes (Kurihara et al., 1997, Boring et al., 1997; Kuziel et al., 1997; Gu et al., 1998; Dawson et al., 1999). Although there are salient differences between CCR2−/− and MCP-1−/− mice, both genetic models display a near-complete defect in monocyte recruitment to the peritoneum following thioglycollate installation. Moreover, CCR2 antagonists block the ability of human monocytes to migrate in response to MCP-1 (Weber et al., 1999a).

Numerous studies have linked CCR2 expression to the activation status of monocytes. For example, CCR2 expression is drastically and rapidly reduced following treatment of monocytes with pro-inflammatory stimuli including IFN-γ, LPS or TNF-α (Penton-Rol et al., 1998; Weber et al., 1999b). In addition, CCR2 expression decreases with cell culture associated monocyte differentiation (Fantuzzi et al., 1999).

The current study was performed to establish the relationship between between MMP-9 release and monocyte differentiation in vitro. Our results indicate that differentiated, CCR2-negative cells produce greater amounts of MMP-9 than naïve monocytes, suggesting that this enzyme may play a salient role in tissue injury, in CNS conditions in which abundant monocyte infiltrates are present.

2. Materials and methods

2.1. Isolation of monocytes

Peripheral blood derived monocytes were obtained from normal donors by elutriation or Ficoll gradient followed by adherence purification, as indicated in the text. Monocytes obtained by counterflow centrifugal elutriation were prepared as previously described (Zhang et al., 1998). Adherence-purified monocytes were obtained from peripheral blood mononuclear cells isolated through the use of cell preparation tubes (Becton-Dickinson) by a previously described technique (Gartner and Popovic, 1990).

2.2. Cell separation

CCR2-positive monocytes were obtained through positive selection using an anti-human CCR2-phycoerythrin (PE) conjugated antibody (R & D Systems, Minneapolis, MN) and anti-PE coated magnetic beads (Miltenyi Biotec, Auburn CA) according to the manufacturer's instructions. Briefly, elutriated cells were incubated with 0.75 μg anti-CCR2 per 10^6 cells for 30 min at room temperature. The total volume for this incubation was adjusted to 100 μl/10^6 cells with MACS buffer (degassed, calcium-free PBS with 0.2 mM EDTA and 0.5% BSA). In subsequent steps, cells were kept on ice or at 4°C.

Following incubation with the primary antibody, cells were washed with 14 ml MACS buffer and pelleted (300 RCF for 15 min). Cells were then incubated with the secondary, anti-PE antibody (20 μl/10^6 cells) for 15 min at 4°C in a total volume of 100 μl/10^6 cells. Monocytes were washed again and pelleted prior to their separation on a magnetic column (LS+ separation column, Miltenyi Biotec) as per the manufacturer's instructions.

2.3. Flow cytometry/CCR2 surface expression

To assess purity of the cell fractions and/or cell surface expression of CCR2, flow cytometric analysis was performed using a FACSTAR^{PLUS} machine and CellQuest software. Three-colour staining with the following antibodies was performed: fluorescein (FITC) labeled CD14(Becton Dickinson)/phycoerythrin (PE) labeled CCR2/Allophycocyanin (APC) labeled CD4 (Becton Dickinson) and IgG FITC/IgG PE/IgG APC (isotype matched control with antibodies from Becton Dickinson). Two-color staining was also done using APC labeled CD14 (Becton Dickinson) and PE labeled CCR2.

For experiments that analyzed CCR2 surface expression as a function of time in culture, PBMCs were isolated by ficoll gradient and plated in minimal essential media (MEM, GIBCO) with 10% FBS at a density of 0.5×10^6/6.4 mm well. Non-adherent cells were removed 2 h after

plating. Twenty-four and forty-eight hours later, cells were placed on ice for 30 min and vigorously shaken. This removed the majority of adherent cells from both time points (>90%) which were then stained and analyzed by flow cytometry.

2.4. Cytokines

Recombinant human cytokines were obtained from R & D Systems, reconstituted in phosphate buffered saline and stored in aliquots at −70°C.

2.5. Stimulation of monocytes and gelatin substrate zymography

Monocytes were plated at approximately 2.5×10^5 cells per 200 μl/6.4 mm well in MEM with 10% FBS. Following adherence, cells were washed and treated for 16 h with serum-free MEM, or with serum-free MEM containing the indicated cytokine (5 ng/ml IL-1β, 10 ng/ml TNF-α, or 10 ng/ml MCP-1). Supernatants were then sampled and frozen for zymography and the media was replaced with MEM containing 10% FBS until day 5, at which time cells were similarly washed and stimulated in serum free media. Supernatants from monocytes which were cultured and/or stimulated for 1 versus 5 days were run on the same gel, and in additional experiments (not shown) we determined that freezing supernatants for 5 days did not have a noticeable effect on MMP-9 activity as detected by zymography. Of note, those wells which were used for 5 day supernatants were also washed at day 1 so as to remove cells in equal number from the wells used for both conditions. Colony stimulating factors were not added at any time. In addition, based on microscopic evaluation, cell confluence did not differ between wells used for the day 1 and day 5 supernatants.

Zymography, using 20 μl of cell culture supernatant was performed with precast gels (BioRad) according to the manufacturer's instructions. Molecular weights were determined by comparison with protein molecular weight standards (BioRad), and purified active or pro- human MMP-9 (Calbiochem, La Jolla, CA) were run as positive controls.

3. Results

3.1. Following 1 day in culture, cytokine-stimulated monocytes release less MMP-9 than do cells which have been cultured for 5 days

Fig. 1 shows the results of zymographic analysis of supernatants from unstimulated and cytokine stimulated adherence purified monocyte/macrophages following 1 or 5 days in culture as indicated. The predominant band represents pro-MMP-9, which runs with an apparent molecular weight of 92 kDa. The cleaved form of MMP-9 runs at a slightly lower molecular weight but due to its tight association with extracellular matrix proteins, may be difficult to detect in cell culture supernatants. Based on molecular weight, the uppermost band, which can be observed in the day 5 culture supernatants, may represent

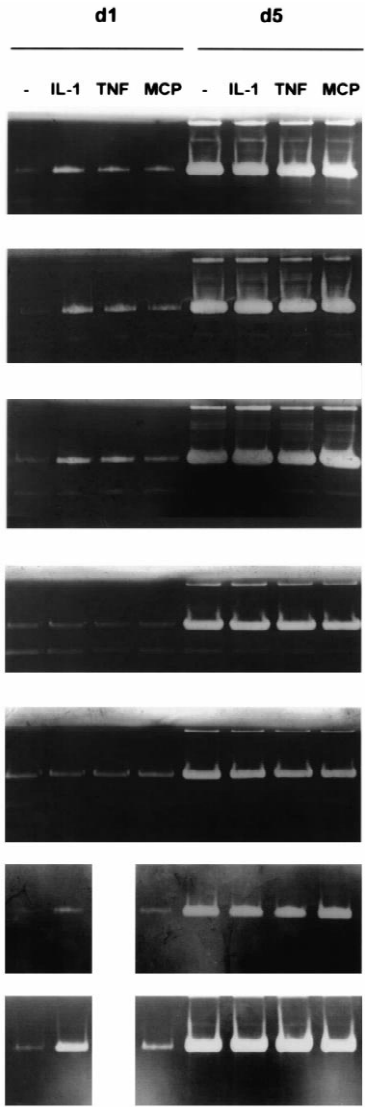

Fig. 1. (a) Zymographic analysis of supernatants from unstimulated and cytokine stimulated adherence purified monocyte/macrophages following 1 or 5 days in culture as indicated. Each row represents results obtained using monocytes from a different donor. The predominant band, which runs with an apparent molecular weight of 92 kDa, represents pro-MMP-9 activity. That band shown in the four lowermost images and indicated by the arrow is the purified active form of human MMP-9, which was run as a positive control. In some images, a faint band which runs with an apparent molecular weight less than that of active MMP-9 is likely to represent MMP-2. (b) Zymographic analysis showing the migration of purified pro-MMP-9 along with that of supernatants from cultured monocytes (5 days). The uppermost bands in lane 1 may represent concatamers of MMP-9.

MMP-9 dimer activity. In addition, the very faint band which runs with a lower molecular weight than does pro-MMP-9 and can be seen in some but not all rows may represent pro-MMP-2 activity.

Monocytes from seven normal donors were studied; the release of pro-MMP-9 was greater at 5 days than at 1 (Fig. 1). As expected, cells from different donors showed variable cytokine-responsiveness. After 5 days in culture, MMP-9 production in response to cytokines was diminished, likely because constitutive MMP-9 release was near-maximal and/or cell surface cytokine receptor expression had diminished (Fantuzzi et al., 1999).

3.2. Monocytes obtained by elutriation also show increased MMP-9 release at 5 days, as opposed to 1 day, in culture

Fig. 2 shows the results of an experiment using monocytes obtained by elutriation rather than adherence purification. The predominant band represents pro-MMP-9 activity, which is significantly greater in supernatants from cells that were maintained for 5 days in culture, as compared to those maintained for 1 day in culture.

3.3. CCR2 expression on monocytes decreases with increased time in culture

CCR2 expression is reduced during monocyte activation and/or differentiation. To determine whether CCR2 receptor expression may decrease with time in culture following isolation and culture techniques similar to those used for those experiments from which the data was shown in Fig. 1, we stained monocyte/macrophages from a normal donor following 24 and 48 h in culture. These early time points were chosen to minimize artifacts associated with difficulty in detaching firmly-adherent cells from tissue-culture wells. The results of this experiment, shown in Fig. 3, demonstrate that CCR2 staining on CD14 positive cells was reduced with increased time in culture. An experiment with PBMCs from a different normal donor showed similar results (not shown).

3.4. CCR2-positive monocytes release less MMP-9 than do CCR2-negative

Fig. 4 shows pro-MMP-9 activity in the supernatants of CD14 unseparated (total); CD14/CCR2− (CCR2−) and CD14/CCR2+ (CCR2+) monocytes from three normal donors. Flow cytometric analysis showed that 83±8% of those cells in the CCR2+ fraction were CD14+/CCR2+ while 11.5±9.3% of those in the CCR2− fraction were similarly labeled. Each row represents the results of an experiment using monocytes from a different normal donor. As expected, cytokine responsiveness of monocytes varied among different donors. Also, as compared to either total or CCR2-negative monocyte populations, CCR2-positive cells released similar or lesser amounts of pro-MMP-9 (arrow). For each of the three zymograms, the average pixel intensity of individual bands from the photographed gels, scanned in using Adobe Photoshop at 1200 dpi, 8 bits per pixel and assigned values of 0 (black)–256 (white), differed at $P<0.01$ between untreated CCR2+ versus untreated CCR2− using a two tailed *t* test.

4. Discussion

MCP-1 is critical to monocyte recruitment in a variety of host defense and pathological conditions (Karpus and Lukacs, 1996; Lu et al., 1998). In addition, it has been demonstrated that those monocytes which respond to MCP-1 exhibit a ‘pro-inflammatory’ phenotype, which includes increased expression of serine proteases (Owen et al., 1994). In the present study we report that monocytes which are naïve, as determined by lesser time in culture as well as CCR2 expression, secrete less MMP-9 than do more differentiated CCR2-negative cells.

The ability of monocytes to release increased quantities

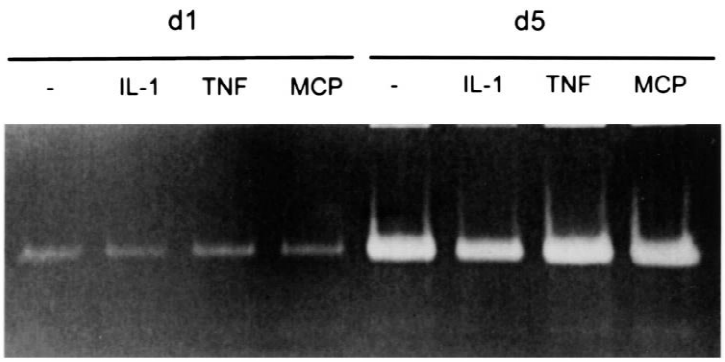

Fig. 2. Zymographic analysis of supernatants from unstimulated and cytokine stimulated monocytes obtained from a normal donor through elutriation. Again, the predominant band represents pro-MMP-9 activity while the uppermost band, which is visible in the day 5 supernatants, is likely to represent MMP-9 dimer activity.

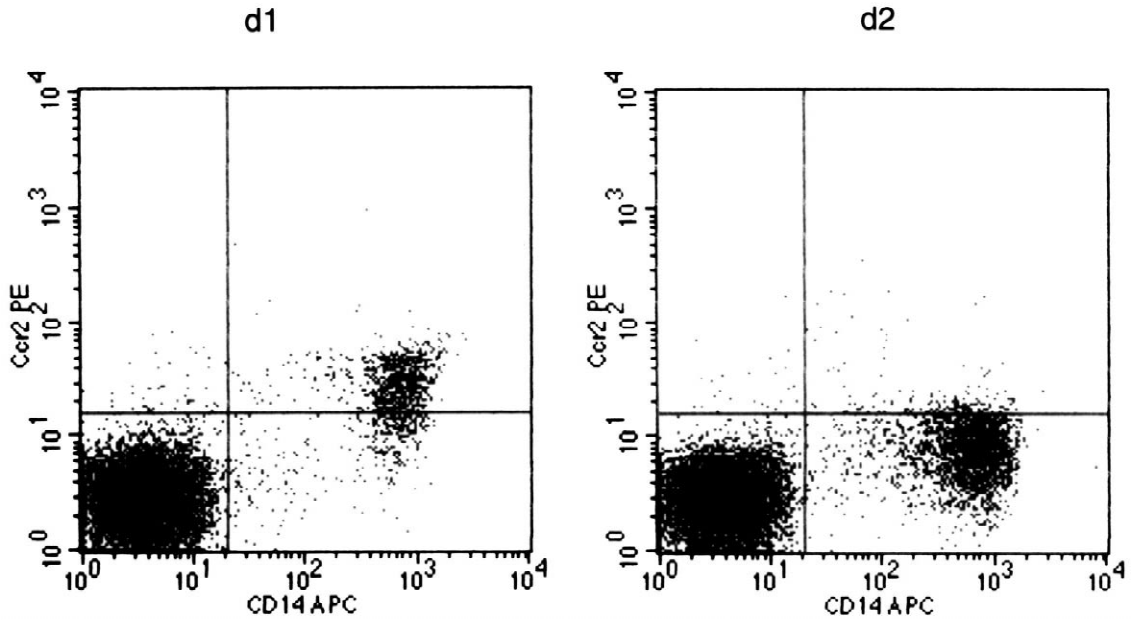

Fig. 3. Flow cytometric analysis of monocytes from a normal donor following 1 or 2 days in culture as indicated. Cells were labeled using fluorescein conjugated anti-CD14 and phycoerythrin conjugated anti-CCR2. Analysis was performed using a FACSTAR^{PLUS} machine and CellQuest software.

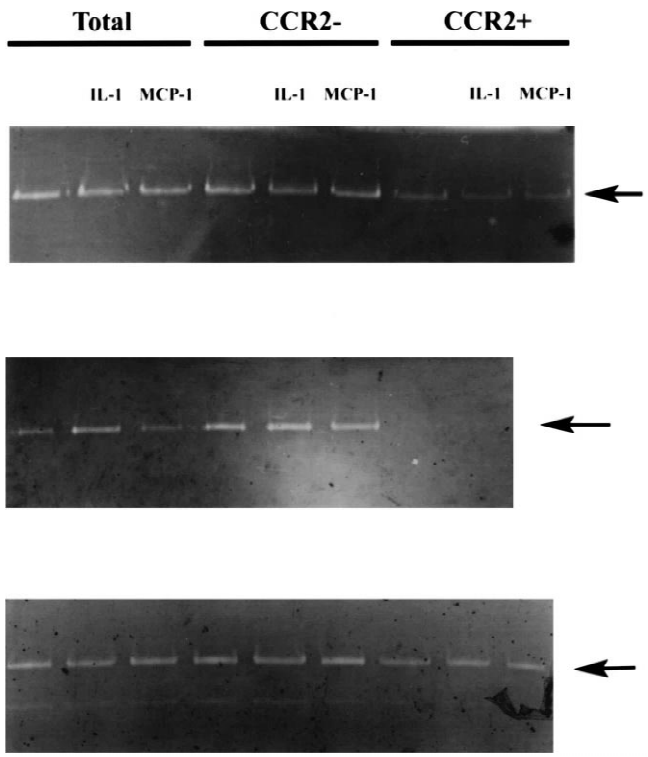

Fig. 4. Zymographic analysis of supernatants from Total, CCR2−, and CCR2+ monocyte populations from three normal donors. Supernatants from each donor are shown in a single row. Cells were either unstimulated or stimulated with IL-1β or MCP-1 as indicated. The predominant band, indicated by an arrow, represents pro-MMP-9 activity. The very faint band which runs faster than pro-MMP-9 and can be seen in the third row most likely represents pro-MMP-2.

of MMP-9 with differentiation could be significant with respect to select pathological conditions. For example, MMPs produced within the brain parenchyma may facilitate CNS entry of peripheral blood leukocytes (Rosenberg et al., 1992; Anthony et al., 1998). MMPs produced within the brain may also help cells to migrate through the parenchymal ECM. In addition, such MMPs could play a role in pathogen clearance and/or host cell injury. With respect to potential cytotoxicity, MMPs may activate potentially neurotoxic cytokines such as IL-1β (Schonbeck et al., 1998). Also, it has been demonstrated that select ECM proteins may facilitate host cell survival (Nichol et al., 1995; Bozzo et al., 1997; Libby et al., 1999). Moreover, extracellular matrix proteins may aid localization of those neurotrophins with which they are associated (Houweling et al., 1998; Gruenbaum and Carew, 1999).

The ability of select cytokines, including TNF-α and IL-1β, to increase the release of MMP-9 and other effectors (Kitagawa et al., 1996) from monocytes may also contribute to the pathogenesis of CNS inflammation. With respect to HIV dementia, select cytokines may be elevated in the peripheral blood (Penton-Rol et al., 1998) leading to immune activation in the CNS (Herkenham et al., 1998) and/or to activation of peripheral blood monocytes (Pulliam et al., 1997). Moreover, activated peripheral blood monocytes may more easily enter the CNS and/or stimulate immune activation once there. Immune activation within the CNS could in turn be associated with increased production of MCP-1 by astrocytes and a consequent selective recruitment of CCR2-positive monocytes (Conant et al., 1998; Cinque et al., 1998; Kelder et al., 1998). And while this scenario is of course speculative, it would at

least suggest that immune activation within the CNS may be prevented, at least in some part, by prevention of peripheral immune activation (Gartner, 2000).

In summary, the finding that CCR2-positive, naïve monocytes produce lesser amounts of MMP-9 than do cytokine stimulated or more differentiated monocytes is consistent with the possibility that this key pro-inflammatory enzyme plays a role in macrophage effector functions. Inhibiting MMP-9 activity may exert neuroprotective effects in conditions characterized by infiltration of the CNS by activated monocytes.

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References

Anthony, D.C., Miller, K.M., Fearn, S., Townsend, M.J., Opdenakker, G., Wells, G.N., Clements, J.M., Chandler, S., Gearing, A.J., Perry, V.H., 1998. Matrix metalloproteinase expression in an experimentally-induced DTH model of multiple sclerosis in the rat CNS. J. Neuroimmunol. 87, 67–72.

Boring, L., Gosling, J., Chensue, S.W., Kunkel, S.L., Farese, R.V., Broxmeyer, H.E., Charo, I.F., 1997. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. J. Clin. Invt. 100, 2552–2561.

Bozzo, C., Bellomo, G., Silengo, L., Tarone, G., Altruda, F., 1997. Soluble integrin ligands and growth factors independently rescue neuroblastoma cells from apoptosis under nonadherent conditions. Exp. Cell. Res. 237 (2), 326–337.

Cinque, P., Vago, L., Mengozzi, M., Torri, V., Ceresa, D., Vicenzi, E., Transidico, P., Vagani, A., Sozzani, S., Mantovani, A., Lazzarin, A., Poli, G., 1998. Elevated cerebrospinal fluid levels of monocyte chemotactic protein-1 correlate with HIV-1 encephalitis and local viral replication. AIDS 12 (11), 1327–1332.

Conant, K., Garzino-Demo, A., Nath, A., McArthur, J.C., Halliday, W., Power, C., Gallo, R.C., Major, E.O., 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.

Dawson, T.C., Kuziel, W.A., Osahar, T.A., Maeda, N., 1999. Absence of CC chemokine receptor-2 reduces atherosclerosis in apolipoprotein E-deficient mice. Atherosclerosis 143 (1), 205–211.

Epstein, L.G., Gendelman, H.E., 1993. Human immunodeficiency virus type-1 infection of the nervous system:pathogenetic mechanisms. Ann. Neurol. 33, 429–436.

Fantuzzi, L., Borghi, P., Ciolli, V., Pavlakis, G., Belardelli, F., Gessani, S., 1999. Loss of CCR2 expression and functional response to monocyte chemotactic protein (MCP-1) during the differentiation of human monocytes: role of secreted MCP-1 in the regulation of the chemotactic response. Blood 94 (3), 875–883.

Gartner, S., 2000. AIDS: HIV infection and dementia. Science 287, 602–604.

Gartner, S., Popovic, M., 1990. Virus isolation and production. In: Aldovini, A., Walker, B.D. (Eds.), Techniques in HIV Research. Stockton Press, NY, Chapter 3.

Glass, J.D., Fedor, H.S., Wesselingh, S.L., McArthur, J.C., 1995. Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. Ann. Neurol. 38, 755–762.

Gruenbaum, L.M., Carew, T.J., 1999. Growth factor modulation of substrate-specific morphological patterns in Aplysia bag cell neurons. Learn Mem. 6, 292–306.

Gu, L., Okada, Y., Clinton, S.K., Gerard, C., Sukhova, G.K., Libby, P., Rollins, B.J., 1998. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol. Cell 2 (2), 275–281.

Herkenham, M., Lee, H.Y., Baker, R.A., 1998. Temporal and spatial patterns of c-fos mRNA induced by intravenous interleukin-1: a cascade of non-neuronal cellular activation at the blood–brain barrier. J. Comp. Neurol. 400 (2), 175–196.

Houweling, D.A., Lankhorst, A.J., Gispen, W.H., Bar, P.R., Joosten, E.A., 1998. Collagen containing neurotrophin-3 (NT-3) attracts regrowing injured corticospinal axons in the adult rat spinal cord and promotes partial functional recovery. Exp. Neurol. 153, 49–59.

Karpus, W.J., Lukacs, N.W., 1996. The role of chemokines in oral tolerance. Abrogation of nonresponsiveness by treatment with anti-monocyte chemotactic protein-1. Ann. N.Y. Acad. Sci. USA 778, 133–134.

Kelder, W., McArthur, J.C., Nance-Sproson, T., Lanier, R., Griffin, D.E., 1998. β-chemokines MCP-1 and RANTES are increased in cerebrospinal fluid of patients with HIV-associated dementia. Ann. Neurol.

Kitagawa, S., You, A., Yagisawa, M., Azuma, E., Yoshida, M., Furukawa, Y., Takahashi, M., Masuyama, J., Takaku, F., 1996. Activation of human monocyte functions by tumor necrosis factor: rapid priming for enhanced release of superoxide and erythrophagocytosis, but no direct triggering of superoxide release. Exp. Hematol. 24, 559–567.

Kurihara, T., Warr, G., Loy, J., Bravo, R., 1997. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. J. Exp. Med. 186, 1757–1762.

Kuroiwa, T., Lee, E.G., Danning, C.L., Illei, G.G., McInnes, I.B., Boumpas, D.T., 1999. CD40 ligand-activated human monocytes amplify glomerular inflammatory responses through soluble and cell-to-cell contact-dependent mechanisms. J. Immunol. 163 (4), 2168–2175.

Kuziel, W.A., Morgan, S.J., Dawson, T.C., Griffin, S., Smithies, O., Ley, K., Maeda, N., 1997. Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. Proc. Natl. Acad. Sci. USA, 12503–12508.

Leppert, D., Waubant, E., Burk, M.R., Oksenberg, J.R., Hauser, S.L., 1996. Interferon beta-1b inhibits gelatinase secretion and in vitro migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis. Ann. Neurol. 40 (6), 846–852.

Leppert, D., Waubant, E., Galardy, R., Bunnett, N.W., Hauser, S.L., 1995. T cell gelatinases mediate basement membrane transmigration in vitro. J. Immunol. 154 (9), 4379–4389.

Libby, R.T., Lavallee, C.R., Balkema, G.W., Brunken, W.J., Hunter, D.D., 1999. Disruption of laminin beta2 chain production causes alterations in morphology and function in the CNS. J. Neurosci. 19 (21), 9399–9411.

Lu, B., Rutledge, B., Gu, L., Fiorillo, J., Lukacs, N.W., Kunkel, S.L., North, R., Gerard, C., Rollins, B.J., 1998. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1 deficient mice. J. Exp. Med. 187, 601–608.

Nichol, K.A., Schulz, M.W., Bennett, M.R., 1995. Nitric oxide-mediated death of cultured neonatal retinal ganglion cells: neuroprotective properties of glutamate and chondroitin sulfate proteoglycan. Brain Res 697 (1-2), 1–16.

Owen, C.A., Campbell, M.A., Boukedes, S.S., Campbell, E.J., 1994. Monocytes recruited to sites of inflammation express a distinctive proinflammatory (P) phenotype. Am. J. Physiol. 267, L786–L796.

Penton-Rol, G., Polentarutti, N., Luini, W., Borsatti, A., Mancinelli, R., Sica, A., Sozzani, S., 1998. Mantovani A Selective inhibition of expression of the chemokine receptor CCR2 in human monocytes by IFN-gamma. J. Immunol. 160 (8), 3869–3873.

Pulliam, L., Gascon, R., Stubblebine, M., McGuire, D., McGrath, M.S., 1997. Unique monocyte subset in patients with AIDS dementia. Lancet 349, 692–695.

Rosenberg, G.A., Kornfeld, M., Estrada, E., Liotta, L.A., Statler-Stevenson, W.G., 1992. TIMP-2 reduces proteolytic opening of the blood brain barrier by type IV collagenase. Brain Res. 576, 203–207.

Sasseville, V.G., Newman, W.A., Lackner, A.A., Smith, M.O., Lausen, N.C.G., Beall, D., Ringler, D.J., 1992. Elevated vascular cell adhesion molecule-1 in AIDS encephalitis induced by simian immunodeficiency virus. Am. J. Pathol. 141, 1021–1030.

Schonbeck, U., Mach, F., Libby, P., 1998. Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1beta processing. J. Immunol. 161, 3340–3346.

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 (1), 64–68.

Weber, C., Draude, G., Weber, K.S., Wubert, J., Lorenz, R.L., Weber, P.C., 1999a. Downregulation by tumor necrosis factor-alpha of monocyte CCR2 expression and monocyte chemotactic protein-1-induced transendothelial migration is antagonized by oxidized low-density lipoprotein: a potential mechanism of monocyte retention in atherosclerotic lesions. Atherosclerosis 145 (1), 115–123.

Weber, K.S.C., von Hundelshausen, P., Clark-Lewis, I., Weber, P.C., Weber, C., 1999b. Differential immobilization and hierarchial involvement of chemokines in monocyte arrest and transmigration on inflamed endothelium in shear flow. Eur. J. Immunol. 29, 700–712.

Wee Yong, V., Krekoski, C.A., Forsyth, P.A., Bell, R., Edwards, D.R., 1998. Matrix metalloproteinases and diseases of the CNS. TINS 21, 75–80.

Weiss, J.M., Downie, S.A., Lyman, W.D., Berman, J.W., 1998. Astrocyte-derived monocyte-chemoattractant protein-1 directs the transmigration of leukocytes across a model of the human blood-brain barrier. J. Immunol. 161 (12), 6896–6903.

Zhang, Y., McCluskey, K., Fujii, K., Wahl, L.M., 1998. Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-alpha, granulocyte-macrophage CSF, and IL-1 beta through and -independent mechanisms. J. Immunol. 161 (6), 3071–3076.