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IL-1 and TNF-α induction of IL-8 and monocyte chemotactic and activating factor (MCAF) mRNA expression in a human astrocytoma cell line

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SUMMARY

In order to elucidate the role of inflammatory cytokines in the central nervous system (CNS), we examined whether IL and TNF-α induce cells in the CNS to produce two newly identified leucocyte chemo-attractants, IL-8 and monocyte chemotactic and activating factor (MCAF). Several human astrocytoma and glioblastoma cell lines expressed high levels of IL-8 and MCAF mRNA *in vitro* upon stimulation with IL-1 and TNF-α. In particular, an astrocytoma cell line U373MG subclone responded markedly to IL-1 with high expression levels of IL-8 and MCAF mRNA as well as IL-6 mRNA. Both IL-8 and MCAF mRNA expression depended on the dose of IL-1 and appeared as early as 30 min to 1 hr after IL-1 stimulation, confirming that these are early inducible genes. The production of IL-8 and MCAF in the U373MG cell culture supernatants was confirmed by a competitive radioimmunoassay (RIA) as well as chemotactic activities on human neutrophils and monocytes. IL-1-induced IL-8 and MCAF mRNA expression appeared to occur at least at the transcriptional level as revealed by a nuclear run-off assay. Moreover, IL-1 treatment increased the half-life of IL -8 and MCAF mRNA markedly, suggesting that increased mRNA stability was also responsible for the enhanced gene transcription. These data suggest that IL-1 and TNF-α induce astrocytes to produce IL-8 and MCAF transcriptionally and post-transcriptionally, both of which may be responsible for leucocytosis seen in inflammation of the CNS.

INTRODUCTION

Purification and subsequent molecular cloning of a major monocyte-derived neutrophil chemotactic factor (MDNCF)[1,2] revealed that this factor belongs to a supergene family of related factors including GRO/MGSA, platelet factor-4, β-thromboglobulin[2,3] and murine homologue of KC and MIP-2.[4] MDNCF is revealed to be identical to other neutrophil chemo-attractants such as neutrophil chemotactic factor (NCF)[5] and neutrophil activating peptide (NAP),[6] which is now renamed IL-8. IL-8 is produced not only by monocytes but also by other cell types, including fibroblasts, [6-9] keratinocytes,[7,8] vascular endothelial cells[9] or hepatocytes.[10] IL-8 has chemotactic activity on neutrophils, basophils and T cells, but not on monocytes.[7] Concomitantly, another novel inflammatory cytokine, termed monocyte chemotactic and activating factor (MCAF), was purified and molecularly cloned.[11,12] MCAF, a human homologue of JE, belongs to another IL-8-related supergene family which includes RANTES, LD78/PAT464, G-26 and MIP-1α, β.[3,13] MCAF is also produced by a variety of cells stimulated with lipopolysaccharide (LPS) or cytokines such as IL-1 or TNF-α. It is chemotactic only for monocytes and appears to act as a macrophage activating factor but not as a neutrophil activating factor.[14]

Since both IL-1 and TNF-α lacked leucocyte chemotactic activity, we examined whether both factors induce cells in the central nervous system (CNS) to produce IL-8 and MCAF, both of which are chemotactic for leucocytes. Recently, Ramilo *et al.*[15] reported that IL-1 and TNF-α caused meningitis accompanied by leucocytosis in spinal fluid when they were given intraciternally, which may be mediated through IL-8 and MCAF. Astrocytes appear to play important auxiliary func-

Abbreviations: CHX, cycloheximide; CNS, central nervous system; FMLP, formyl-methionyl-leucyl-phenylalanine; MCAF, monocyte chemotactic and activating factor: NCF, neutrophil chemotactic factor; PMN, polymorphonuclear; RIA, radioimmunoassay.

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tions in immune response in the CNS as antigen presenting cells with major histocompatibility antigen expression[16-19] and release of various cytokines including glial maturation factor.[18] Accumulated data indicate that astrocytes produce cytokines or express cytokine genes such as IL-1,[20] IL-3,[21] IL-6[22] and GM-CSF.[23] Production of these inflammatory cytokines, particularly IL-1 and IL-6, seemed to be of particular interest in the infection and inflammatory response in the CNS.

In this study, we demonstrated that a human astrocytoma cell line, U373MG, expressed high levels of IL-8 and MCAF mRNA as well as IL-6 mRNA in response to IL-1 and TNF-α. In addition, the mechanism of IL-1-induced IL-8 and MCAF gene expression was studied.

MATERIALS AND METHODS

Cell lines

A human astrocytoma cell line, U373MG, was obtained from ATCC (HTB17, Rockville, MD, U.S.A.) and clones with high proliferative response to rIL1-α and rIL-1β were subcloned by a limiting dilution method as described elsewhere.[22] One of the subclones designated U373MG-B23 exhibited the highest proliferative response to IL-1 and was used throughout this study. Glioblastoma cell lines, T98G and A172, and neuroblastoma cell lines, IMR-32 and GOTO, were obtained from the JCRB Cell Bank (Kamiyoga, Tokyo, Japan). These cell lines were maintained in tissue culture dishes (100 × 20 mm, Corning 25020) in RPMI-1640 medium containing 7% foetal calf serum.

Reagents

Human rIL-1α and TNF-α with each specific activity of 1–2×10^7 U/mg protein and rIL-8 (recombinant NCF; a half maximal neutrophil chemotactic activity at 0·5–1 ng/ml or a specific activity of 2×10^6 U/mg with endotoxin content being less than 50 pg/mg protein, ref. 27) were generous gifts from Dainippon Pharmaceutical Co. (Suita-shi, Osaka, Japan). Human MCAF was purified as described elsewhere.[11,12] Human rMCAF expressed in *E. coli* was provided by Pepro Tec. Inc., NJ; this contained 10^5 U/mg of a half maximal monocyte chemotactic activity. Protein content was measured by a Bio-Rad protein assay kit (Tokyo, Japan) using bovine serum albumin as a standard. Formyl-methionyl-leucyl-phenylalanine (FMLP), cycloheximide (CHX) and actinomycin D were purchased from Sigma Chemicals Co. (St Louis, MO).

cDNA probes of cytokines

Human IL-6 cDNA probe was provided by Professor T. Hirano and T. Kishimoto, Osaka University[24] and *Taq*I–*Bam*HI fragment of 0·45 kb was used as a probe. IL-8 cDNA was prepared by cutting off the 0·4-kb insert of *Eco*R1 fragment which was subcloned into PUC19.[2] MCAF cDNA was prepared by digesting out the 0·4-kb insert of the Pst-1 fragment, which was inserted into PUC19.[12] Chicken β-actin cDNA probe was used as a 2-kb *Hind*III fragment. These fragments were labelled with [^{32}P]-dCTP (> 3000 Ci/mmol) by the Multiprime DNA labelling kit (Amersham, Bucks, U.K.; RPN1601Y).

Northern blot and dot blot analysis

Total RNA was extracted from the cultured cells by 4 M guanidine thiocyanate and centrifuged on a 5·35 M CsCl gradient, sedimenting at 35 K for 16 hr. Total RNA for the dot blot was extracted by solubilization with hypotonic buffer containing 10 mM vanadyl complex and lysing buffer containing 1·2% Triton X-100 as described elsewhere.[22,25] Northern and dot blot analysis was performed as described previously.[22,25] To study the mRNA stability, cells were stimulated with IL-1α (20 U/ml) for 2 hr, followed by incubation in the presence of 10 μg/ml actinomycin D. Total RNA was separated at various incubation periods and dot blot hybridization was performed. Density of each dot in the whole area was quantitatively measured by the Image Analyzer (Model TIF-64, Immuno-Medica, Tokyo, Japan) and expressed as the intensity compared to that of an unstimulated control.

Neutrophil isolation

Polymorphonuclear (PMN) cells were separated from peripheral blood from normal donors by Ficoll–Hypaque centrifugation, followed by sedimentation on a gelatin solution (2·5% w/v in 0·9% NaCl) to remove red blood cells, as described by Schroder *et al.*[6,9] PMN-rich fractions were collected and contaminating erythrocytes were lysed with lysing solution (Ortho, Raritan, NJ) by incubation for 5 min at 25°. The purity of PMN cells was > 98% with more than 95% neutrophils.

Neutrophil and monocyte chemotaxis assay

Neutrophil and monocyte chemotaxis was assayed in multiwell chambers (48-wells, Neuroprobe Inc., Yeda Chemicals, Tokyo, Japan) with 5 μm (for neutrophils) and 8 μm (for monocytes) polyvinylpyrrolidone-free polycarbonate chemotaxis filters as described elsewhere.[7,14,26,27] Prior to the chemotaxis assay, some of the samples were passed through a heparin–Sepharose column to remove stimulants.[8] Briefly, supernatant (2 ml) was applied on a 1-ml heparin-Sepharose column (Pharmacia-LKB, Tokyo, Japan) which was equilibrated with 50 mM phosphate buffer pH 7·5, washed with the same buffer (10 ml) and eluted with 1 ml of the same buffer containing 1 M NaCl. Eluates were then dialysed against the same buffer containing 0·14 M NaCl and were used at 1:10 or 1:20 dilution. This procedure resulted in the complete removal of IL-1 and TNF-α and a recovery of IL-8 and MCAF of more than 90%. Neutrophils or monocytes placed in each well numbered 1×10^4. Cells that had migrated for 60 min at 37° were counted after the filter was dried, fixed and stained with Giemsa (for neutrophils) or with a non-specific esterase stain (for monocytes).

Radioimmunoassay (RIA) for the quantification of human IL-8

Competitive RIA for the quantification of IL-8 was developed using ^{125}I-labelled rIL-8 and polyclonal rabbit anti-human rIL-8 antibody. rIL-8 was radioiodinated by the Bolton Hunter reagent (Amersham-Japan, Tokyo). Each 100 μl of ^{125}I-IL-8 ($1{\cdot}5 \times 10^4$ c.p.m.), sample and antiserum (1:800) were mixed, and incubated at 4° overnight. Then magnetic goat anti-rabbit IgG (Advanced Magnetics Inc., Cambridge, MA) was added; the mixture was further incubated for 2 hr at 4°, spun down and the radioactivity in the pellet was counted. Competitive RIA for MCAF was developed similarly, using ^{125}I-labelled MCAF and rabbit anti-MCAF antiserum (1:4000 dilution). By these procedures, 0·05 ng/tube (0·5 ng/ml) of IL-8 and MCAF was correctly evaluated (Ko *et al.*, manuscript submitted for publication).

Nuclear run-off assay

Nuclear run-off assay was carried out as described by Sica *et al.*[27-29] with some modification. In brief, cells were stimulated with IL-1α for 2 hr, nuclei were isolated by extraction buffer (Tris-HCl 10 mM, pH 7·4, $MgCl_2$ 3 mM, NaCl 10 mM, NP-40 0·5%), and resuspended in 250 μl ice-cold freezing buffer (Tris-HCl, pH 8·3, glycerol 40%, $MgCl_2$ 5 mM, 0·1 mM EDTA), stored at −70°. Then 60 μl of run-off buffer 5× (25 mM Tris, pH 8·0, $MgCl_2$ 12·5 mM, KCl 750 mM and 1·25 mM each of GTP, CTP and ATP) and 100 μCi [α-^{32}P]-UTP (6000 Ci/mmol; ICN, CA, U.S.A.) were added to 230 μl of nuclei suspension and incubated at 30° for 30 min. Labelled RNA was separated using a guanidine/caesium chloride procedure adding 50 μg of yeast tRNA as a carrier. The RNA pellet was resuspended in 180 μl ice-cold TNE (Tris-HCl 100 mM, EDTA 10 mM, pH 8·0, NaCl 1 M) and denatured by adding 20 μl of NaOH 2 N on ice for 10 min. The solution was neutralized by the addition of 50 μl HEPES, pH 7·2. RNA was then precipitated by adding 880 μl of ethanol; the pellet was resuspended in 100 μl hybridization buffer (TES 10 mM, SDS 0·2%, EDTA 10 mM, NaCl 300 mM and 10 μg salmon DNA). RNA was hybridized to nylon membrane filters, on which IL-8 or MCAF cDNA-containing plasmids or control PUC19 plasmid (15–20 μg) were immobilized. Hybridization was performed at 65° for 40 hr and washed twice with 0·2× SSC containing 0·1% SDS.

RESULTS

Induction of IL-6 and IL-8 mRNA expression in various brain tumour cell lines

We have previously found that subclones of a human astrocytoma cell line, U373MG, responded very well to IL-1 (IL-1α or IL-1β) to proliferate and produce a high level of IL-6 activity.[22] Northern blot analysis revealed that U373MG and an additional human glioblastoma cell line, T98G, also expressed both IL-8 and MCAF mRNA in response to IL-1 and TNF-α (Fig. 1a, b) with a considerable level of IL-6 mRNA expression (data not shown). No significant IL-8 or MCAF mRNA expression

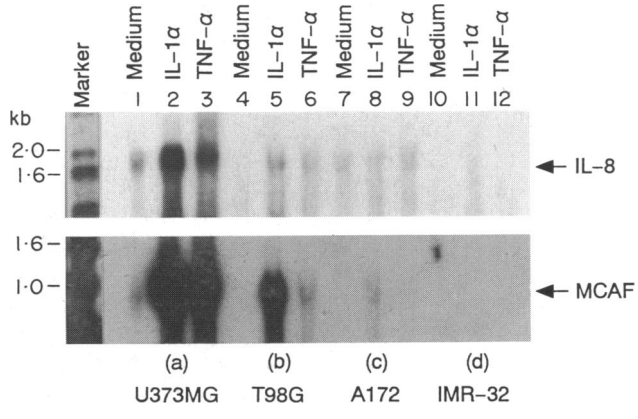

Figure 1. Induction of IL-8 and MCAF mRNA expression in astrocytoma (U373MG), glioma (T98G and A175) and neuroblastoma (IMR-32) cell lines. Cells were incubated with IL-1α (20 U/ml) or TNF-α (40 U/ml) for 4·5 hr, and total RNA was extracted by 4 M guanidine thiocyanate and CsCl sedimentation. Twenty μg of RNA were electrophoresed, and hybridized with IL-8 or MCAF cDNA probes, respectively.

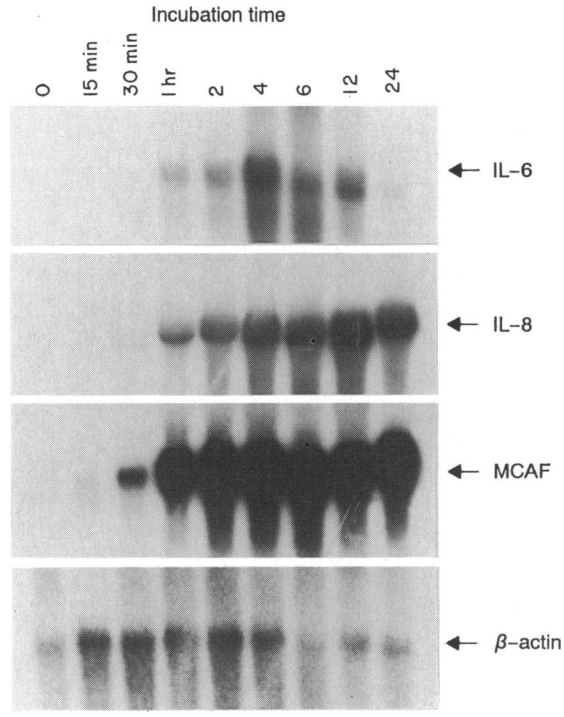

Figure 2. Kinetics of IL-8 and MCAF mRNA expression in IL-1α-induced U373MG cells. Confluent cells in each two plastic dishes (2×10^7 cells) were incubated with IL-1α (20 U/ml) for the indicated periods and 20 μg total RNA were electrophoresed and hybridized with each cDNA probe.

was induced in another glioblastoma cell line, A172, and two neuroblastoma cell lines including IMR-32 (Fig. 1c, d) and GOTO (data not shown) with IL-1 or TNF-α stimulation. Expression of IL-6 and IL-8 mRNA in the U373MG cell line was induced similarly by stimulation with IL-1β, and TNF-α, but not with other cytokines including IL-6 (data not shown).

Induction and kinetics of IL-8 and MCAF mRNA expression in an U373MG subclone

We focused on an U373MG subclone (B23) with high IL-8 mRNA expression to scrutinize how the inflammatory cytokine gene expression was regulated. Figure 2 shows that both IL-8 (~2-kb transcript) and MCAF mRNA (~0·9-kb transcript) expression were time-dependent and appeared as early as after 0·5 to 1 hr of incubation, reaching maximal levels at 2–4 hr, and maintained constant levels for up to 24 hr. These results indicate that these inflammatory cytokines are of the early inducible gene transcripts.

Induction of IL-6, IL-8 and MCAF mRNA expression by IL-1α was seen to occur in a dose-dependent manner with maximal expression at 10 to 100 U/ml (Fig. 3). Generally, TNF-α was less potent in inducing this cytokine mRNA expression than IL-1α. Both IL-8 and MCAF mRNA induction occurred concomitantly and no segregation of expression was observed.

Neutrophil and macrophage chemotactic activity in U373MG cell culture supernatants

In addition, we examined whether the IL-1-stimulated U373MG supernatants contained IL-8 and MCAF biological

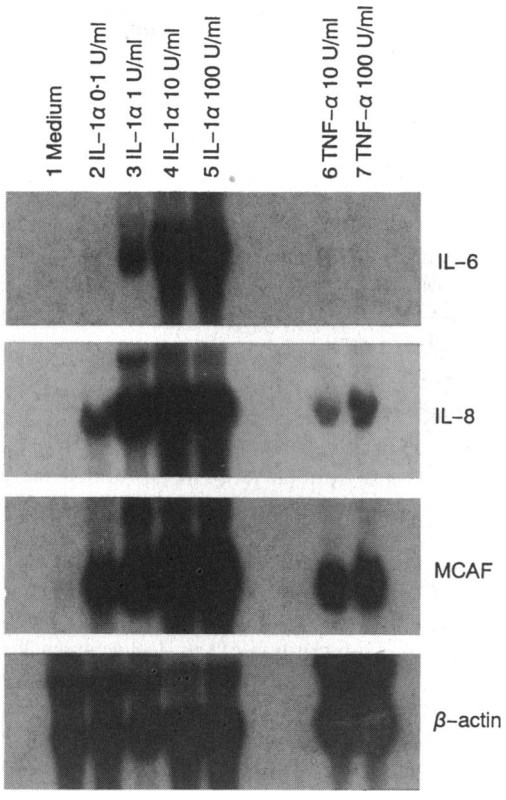

Figure 3. Dose-response of IL-8 and MCAF mRNA expression in U373MG cells. Cells were incubated with IL-1α or TNF-α for 4·5 hr and total RNA was electrophoresed and hybridization was done using IL-6, IL-8, MCAF and β-actin cDNA as probes.

Table 1. Chemotactic activity in U373 cell culture supernatants

Samples	Concentration	Chemotactic activity* Neutrophils /1 × 10^4	Chemotactic activity* Monocytes /1 × 10^4
None		<5	<5
FMLP	10^{-8} M	225±34	128±22
FMLP	10^{-9} M	122±22	ND
rIL-8	500 ng/ml	48±12	ND
rIL-8	100 ng/ml	34±6	<5
Purified MCAF	100 ng/ml	<5	33±8
U373 supernatant stimulated with			
Medium		9·5±3	6·3±3
IL-1α	20 U/ml	64±20 (140±15)†	42±18 (105±14)†
TNF-α	20 U/ml	32±10	55±9
IL-1α	20 U/ml without cells	12±4	8·5±4

* Chemotactic activity was assayed using a microwell chamber with 45 μl cells in upper chamber and 25 μl samples in the lower chamber, incubated at 37° for 1 hr. Cells were stained with Giemsa (neutrophils) or non-specific esterase (monocytes). Data are expressed as mean±SE from three experiments.

† Eluates from heparin-Sepharose column were tested.

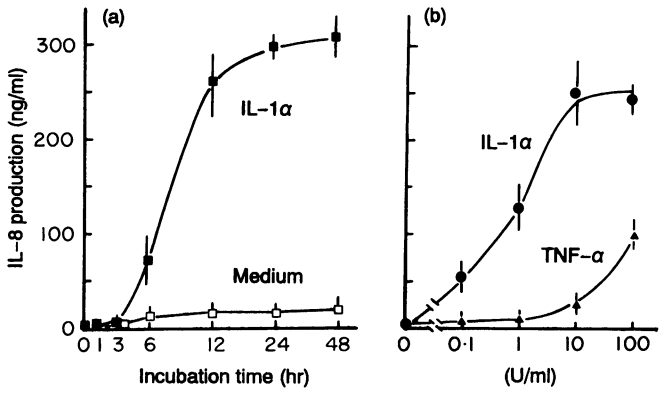

Figure 4. IL-8 production by IL-1α-stimulated U373MG cells. IL-8 levels were determined by the competitive RIA. (a) Kinetics of IL-8 production, when stimulated with 20 U/ml of IL-1α. (b) Dose dependency of IL-1α and TNF-α, measured after 24-hr of incubation. Data are shown as mean±SE from three independent experiments.

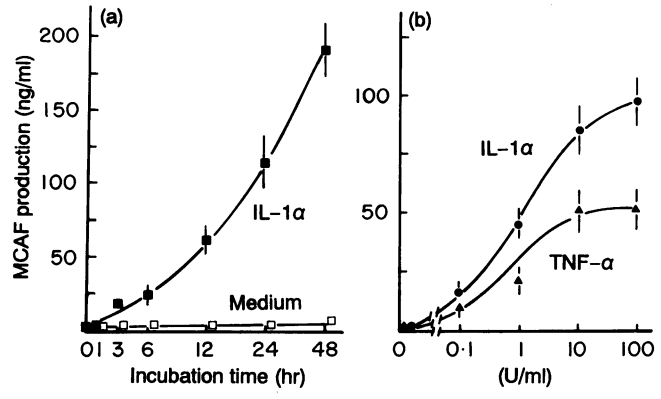

Figure 5. MCAF production by IL-1α-stimulated U373MG cells. MCAF levels were determined by the competitive RIA. (a) Kinetics of MCAF production. (b) Dose dependency of IL-1α and TNF-α, measured after 24 hr of incubation. Data are shown as mean±SE from three independent experiments.

activity, i.e. chemotaxis on human neutrophils and macrophages, using a multiwell chemotaxis chamber. As shown in Table 1, IL-1α- and TNF-α-stimulated U373MG supernatants, crude supernatants or eluates from heparine-Sepharose column had significant chemotactic activity both on human neutrophils and macrophages as compared with rIL-8 or purified MCAF standard, respectively, while IL-8 and MCAF chemotactic activity was lower than 10^{-9} M FMLP.

Detection of IL-8 activity in IL-1-stimulated U373MG cells

In parallel with IL-8 and MCAF biological activities, we measured IL-8 and MCAF production in the U373MG culture supernatants directly by competitive RIA. We found that after a 6-hr incubation, significant levels of IL-8 were detectable, reaching a maximal level of 300 ng/ml after 12 hr of stimulation with IL-1α (Fig. 4a). IL-8 production was dose-dependent on IL-1α and TNF-α was less potent in stimulating IL-8 production

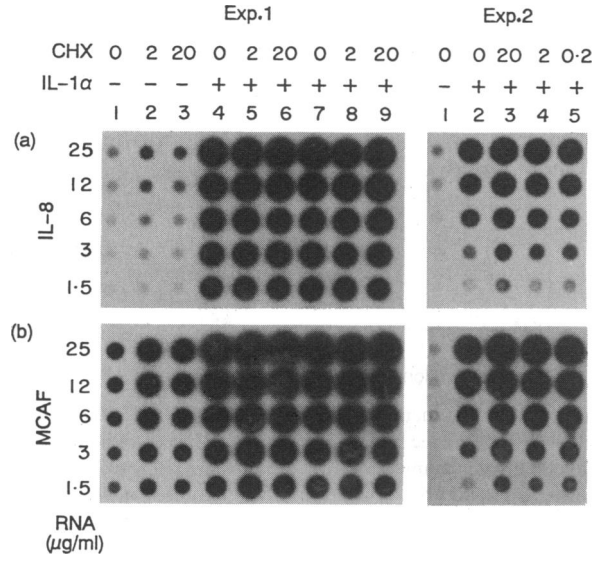

Figure 6. Effects of CHX on the IL-8 and MCAF mRNA expression in U373MG cells. Cells were incubated with CHX (0, 2 and 20 μg/ml) alone or with IL-1α (20 U/ml) for 4 hr (Exp. 1, lanes 4 to 9; Exp. 2, lanes 2 to 5). In lanes 7 to 9 of Exp. 1, cells were preincubated for 1 hr with CHX, followed with stimulation with IL-1α. Total RNA was separated and dot blot analysis was performed.

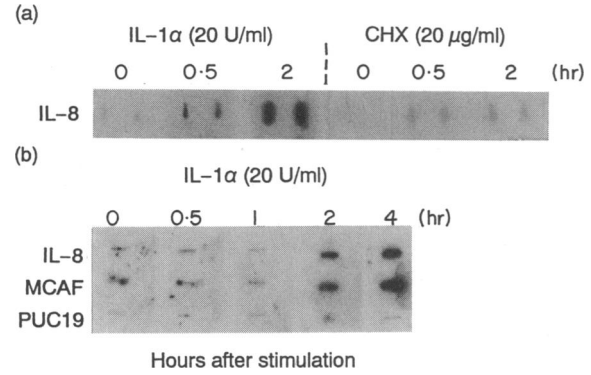

Figure 7. Nuclear run-off analysis of IL-8 and MCAF gene expression in IL-1α-stimulated U373MG cells. DNA plasmids immobilized to filters are (a) human IL-8 cDNA (20 μg/lane), (b) human IL-8, MCAF and PUC19 plasmids (each 15 μg/lane). Nuclear extracts were prepared at 0, 0·5 and 2 hr (a), and 0, 0·5, 1, 2 and 4 hr (b) and labelled *in vitro* with [^{32}P]-UTP, and ^{32}P-labelled RNA elongates were hybridized to filters for 24 hr.

than IL-1α (Fig. 4b), which was consistent with the observation of mRNA expression in Fig. 3. Similarly, significant levels of MCAF were observed after 3–6 hr of stimulation with IL-1α, reaching a maximal level around 200 ng/ml (Fig. 5). All these results demonstrate that the U373MG cell line has the capacity to produce IL-8 and MCAF being preceded by rapidly induced mRNA expression in response to IL-1α and TNF-α.

Effects of CHX on IL-8 and MCAF mRNA induction

Since CHX, an inhibitor of protein synthesis, was reported to upregulate 9E3 gene transcription, an IL-8-related gene family,[30] in a human fibrosarcoma cell line,[31] we next examined whether it induced this gene expression by itself or in combination with IL-1. The effect of CHX was, however, not as drastic as shown in Fig. 6a and 6b. CHX alone at 0·2 to 10 μg/ml induced IL-8 mRNA only minimally, but rather high MCAF mRNA expression. Similarly, CHX had an enhancing effect particularly on MCAF mRNA expression but not so markedly on IL-8 mRNA, indicating that new protein synthesis was not required for this gene expression.

Transcriptional regulation of IL-8 mRNA

In order to determine whether enhanced IL-8 mRNA expression occurred at the transcriptional level, we performed nuclear run-off experiments. Figure 7 demonstrates that nuclear extracts from IL-1α-stimulated cells prepared at 0, 0·5 and 2 hr (Fig. 7a) or 0, 0·5, 1, 2 and 4 hr (Fig. 7b) contained high newly transcribed IL-8 and MCAF mRNA as compared to an unstimulated control (incubation time = 0), or compared to a PUC19 plasmid control (Fig. 7b). Transcription of the IL-8 gene reached more than 10-fold after 2 hr of incubation with IL-1α. CHX enhanced low but significant IL-8 mRNA transcription as well. The above results indicated that IL-8 and MCAF mRNA accumulation was ascribable at least to the increased IL-8 mRNA transcription in this cell line.

Stability of IL-8 and MCAF mRNA

Although enhanced transcription is at least partly involved in the increased accumulation of IL-8 and MCAF mRNA, mRNA stabilization may also be responsible for this process. Therefore, we determined in the blot analysis the stability of IL-8 and MCAF mRNA. When the cells were stimulated with IL-1α (20 U/ml) for 2 hr, addition of actinomycin D (10 μg/ml) completely inhibited the following new RNA synthesis by IL-1α. Under these conditions, total RNA was prepared at each incubation interval after actinomycin D treatment to look at the decline of IL-8 and MCAF mRNA. Constitutive expression of IL-8 and MCAF mRNA, though they were at a low level, declined with a half-life of approximately 6 hr, respectively (Fig. 8a, b). When stimulated with IL-1α, no significant decline of IL-8 and MCAF mRNA was observed at least during 8 hr of incubation, suggesting that IL-1 treatment greatly advantaged the stabilization of the mRNA of these cytokines.

DISCUSSION

The present study clearly indicates that a human astrocytoma cell line, U373MG, produces various cytokines including IL-8 and MCAF as well as IL-6 in response to IL-1 and TNF-α stimulation. This observation was evidenced not only by the induction of mRNA but also by biological activities and direct measurement of IL-8 and MCAF. Astrocytes are presumed to play a crucial role in injury or infection of the CNS. In the initial phase of CNS response to trauma which is termed reactive gliosis, reactive astrocytes become hypertrophied, increase their mitotic activity and increase their content of glial fibrillary acidic protein.[32] It is conceivable that various cytokines are involved during these steps.

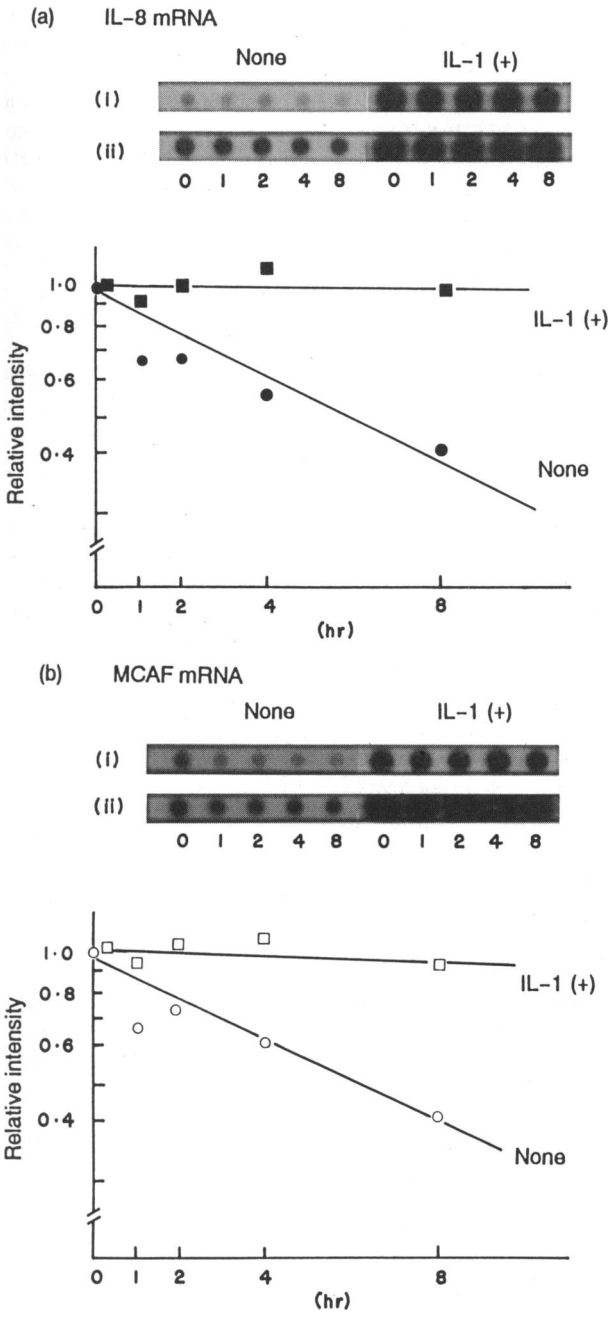

Figure 8. Stability of IL-8 and MCAF mRNA in CHX and IL-1α-stimulated U373MG cells. Cells were incubated with medium (lanes 1 to 5) or IL-1α (20 U/ml; lanes 6 to 10) for 2 hr, washed and incubated in the presence of actinomycin D (10 μg/ml). Total RNA was obtained after each incubation period of 0, 1, 2, 4 and 8 hr, and dot blot analysis was done using serial dilution of RNA. In lanes 1 to 5, a double amount (12·5 μg) of RNA was used and in lanes 6 to 10, 6·2 μg of RNA were applied. Filters were hybridized with (a) IL-8 cDNA probe, and (b) MCAF cDNA probe. Intensity of dot was measured by an Image Analyzer (Immuno-Medica) and expressed as a ratio compared with the intensity at time 0. Mean of two experiments (i, ii) is shown in the plots.

Microglia cells or monocytes in the brain are known to produce IL-1.[20] Since IL-1 has been shown to be a potent mitogen for rat astroglial cells, the release of IL-1 by inflammatory cells may promote the astroglial response that occurs in the damaged mammalian brain,[18,19] as well as during development of the mammalian brain.[33] Like normal astroglia, it has been shown that the U373MG cell line exhibits proliferative response to IL-1 and TNF-α.[22,34] Our study showed, using a human astrocytoma cell line, that this cell line produces many proinflammatory cytokines in response to IL-1 and TNF-α. Recently, it was reported that intrathecal injection of IL-1 or TNF-α caused meningitis in rabbits, as did intrathecal injection of lipopolysaccharide.[15] Induction of IL-8 and MCAF by IL-1 or TNF-α may be involved in this pathogenesis, since IL-8 and MCAF are both potent chemo-attractants, causing a rapid infiltration of neutrophils and macrophages and activation of these cells.

On the other hand, it was recently demonstrated that two human astrocytoma cell lines, U87MG and U373MG, could produce and express G-CSF and GM-CSF mRNA in response to IL-1α and IL-1β.[23] Since G-CSF and GM-CSF are crucial factors in the differentiation and maturation of granulocytes and macrophages, the evidence on the production of these CSF further supports the role of astrocytes for the enhanced survival and activation of neutrophils and phagocytes in the inflammatory lesions in the CNS, in addition to IL-8 and MCAF. Direct evidence must be obtained, however, e.g. by the *in situ* staining of the cells with antibodies against these cytokines or *in situ* detection of mRNA in the inflammatory lesion in the CNS.

Accumulation of IL-8 mRNA and MCAF mRNA expression by IL-1α was partly ascribed to the augmented transcription, as was demonstrated in the run-off assay. Similarly, Sica *et al.* reported that IL-1 and TNF transcriptionally activated IL-8 mRNA[35] and MCAF mRNA gene expression[28] in human endothelial cells. It has been previously shown that CHX alone could induce a high level of 9E3 mRNA, a v-src-inducible protein of an IL-8 supergene family, which was due to the increased transcription of this gene.[30] Recently, co-authors (N.M. *et al.*) have revealed IL-1 and TNF-responsive elements in the 5′-flanking region of the IL-8 gene.[35] These are two *cis* elements located between −94 and −71 bp in the 5′-flanking region: one potential binding site is proposed for an NF-κB-like factor and the other for an NF/IL-6-like factor.[36] NF-IL6, a nuclear factor newly identified by Akira *et al.*,[37] binds to an IL-1 responsive element in the IL-6 gene. It also binds to the regulatory regions for various acute-phase protein genes and several other cytokine genes such as TNF, IL-8 and G-CSF. Although it is not yet clear whether NF-κB or NF-IL6 is acting also on IL-8 and MCAF transcription in astrocytes as well, it is highly possible that these factors act as common nuclear factors to amplify the gene expression of inflammatory cytokines.

In addition to the transcriptional regulation, our data indicated that enhanced stability of these mRNAs by IL-1α treatment also contributed to the enhanced mRNA accumulation. Many inflammatory cytokines and proto-oncogens contain AT-rich sequence motifs, particularly the 8-nucleotide consequence sequence (TTATTTAT), in the 3′-untranslated region.[30,38] The presence of this octamer sequence is generally

considered to confer the instability of mRNA, resulting in a rapid degradation of the messages.[30,39,40] In fact, using various CAT constructs containing the 3′-untranslated region, induction of TNF mRNA was greatly enhanced when the octamer sequence was deleted.[41] These results clearly indicate that the octamer-containing segments are repressing the translation of the TNF mRNA. Half-lives of IL-8 and MCAF mRNA appeared to be much longer than other cytokine messages including GM-CSF or many IFN mRNAs.[38] There were only two repeats of this 8-nucleotide sequence in IL-8 mRNA and no repeats in MCAF mRNA, but there were instead several 6 or 7-nucleotide sequences (TATTTA, TTATTT, TATTTAT). The latter sequences may make IL-8 and MCAF mRNA more stable than GM-CSF or interferon mRNAs.[41] The mechanism by which IL-1 stabilizes IL-8 and MACF mRNAs remains unsolved and is still to be elucidated.

In conclusion, our present data support the idea that both transcriptional and translational mechanisms, in a co-ordinate fashion, contribute to the regulation of IL-8 and MCAF gene expression.

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