Human T-Cell Leukemia Virus Type I Tax Activates Transcription of the Human Monocyte Chemoattractant Protein-1 Gene through Two Nuclear Factor-κB Sites

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ABSTRACT

Infection by human T-cell leukemia virus type (HTLV) I leads to adult T-cell leukemia and is also associated with the neurodegenerative disease HTLV-I-associated myelopathy/tropical spastic paraparesis. Leukocytes are attracted to sites of inflammation by chemokines. One such chemokine is monocyte chemoattractant protein (MCP)-1, a member of the C-C subfamily of chemokines. We investigated whether HTLV-I infection causes up-regulation of MCP-1, which may in turn cause recruitment of leukocytes to HTLV-I-infected areas. We now report that MCP-1 mRNA levels are elevated in HTLV-I-infected T-cell lines, when compared with uninfected ones. We further confirmed secretion of MCP-1 by HTLV-I-infected T-cell lines. MCP-1 mRNA was also expressed in leukemic cells from patients with adult T-cell leukemia. The 5' transcriptional regulatory region of the *MCP-1* gene was activated by the HTLV-I-encoded transactivator Tax in the human T-cell line Jurkat, in which endogenous MCP-1 is induced by Tax. By using site-specific point mutations, we have identified two closely spaced nuclear factor (NF)-κB sites, A1 and A2, to be important for Tax-mediated transactivation of the *MCP-1* gene. Through the use of an electrophoretic mobility shift assay, we demonstrated that Tax induced NF-κB binding to both MCP-1 κB sites. This is the first report to demonstrate that Tax can transactivate the *MCP-1* gene through the induction of NF-κB. Our results thus reveal how Tax disrupts the normally regulated *MCP-1* gene and leads to its constitutive expression in HTLV-I-infected cells. These findings may have important implications for our understanding of HTLV-I-associated diseases.

INTRODUCTION

HTLV-I[2] is the etiological agent of ATL (1, 2). HTLV-I may also be associated with several chronic inflammatory diseases of presumed autoimmune etiology, such as HAM/TSP (3, 4), HTLV-I-associated arthropathy (5), uveitis (6), and Sjögren's syndrome (7). A characteristic feature of these HTLV-I-associated diseases is infiltration of lymphocytes, including virus-infected cells, into affected tissues. The possible roles of abnormal immune activation, adhesion molecules, and cytokine or chemokine overproduction in the pathogenesis of diseases caused by HTLV-I have been suggested, but the mechanism by which they cause these diseases remains unknown.

Chemokines are thought to play a major role in the migration of cells from one tissue to another, thereby controlling cell migration during inflammation. MCP-1 is a member of the C-C chemokine family (8, 9). MCP-1 and other chemokines are typically expressed in tissues during inflammation and are induced in a variety of cell types *in vitro* by the proinflammatory mediators tumor necrosis factor-α, IL-1, and endotoxins (9, 10). MCP-1 appears to be important for activation of leukocyte subsets and triggers their adhesiveness and transmigration through the endothelial layer. Inhibition of induced MCP-1 expression is associated with reduced transmigration of monocytes (11) as well as with diminished recruitment of T cells (12). Accordingly, neutralizing antibodies against MCP-1 have been shown to inhibit T-cell recruitment and cutaneous delayed-type hypersensitivity (13), thus suggesting the importance of MCP-1 at the onset of inflammatory processes.

Recent studies revealed that MCP-1 was expressed on perivascular infiltrating cells as well as on vascular endothelium in the spinal cord lesions of HAM/TSP patients (14). These findings suggest that MCP-1 may play a pathological role in HTLV-I-associated diseases. However, little is known about the molecular mechanisms of constitutive MCP-1 expression in HTLV-I-infected T cells. The virally encoded transcriptional activator Tax has multiple functions. Tax has been shown to transform cell lines and primary T cells *in vitro,* whereas transgenic mice expressing Tax develop nonlymphoid tumors, leukemia, and various inflammatory diseases *in vivo*. Tax activates the transcription of not only the HTLV-I LTR but also of a number of cellular genes in *trans*. This transactivation by Tax is mediated through interaction with transcription factors such as cyclic AMP-responsive element binding protein (15) in the case of the HTLV-I LTR, NF-κB (16), and serum-responsive factor (CArG box binding protein; Ref. 17). To activate NF-κB, Tax has been suggested to target certain cellular kinases that induce IKKs, NIK, and mitogen-activated protein/extracellular signal-regulated kinase kinase 1 (18–24). In the present study, we report that Tax is capable of inducing the MCP-1 chemokine gene in T cells. Furthermore, we analyzed the mechanism of Tax-induced transcriptional regulation of MCP-1. We demonstrate that the activation of NF-κB is required for Tax activation of MCP-1. Tax-induced MCP-1 transactivation was mediated through two NF-κB binding sites located ∼2.6 kb from the transcription initiation site.

MATERIALS AND METHODS

Cell Lines. Human T-cell lines, Jurkat, MOLT-4, and CCRF-CEM, and HTLV-I-infected T-cell lines OMT (25), C5/MJ (26), MT-2 (27), and HPB-ATL-O were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 50 units/ml penicillin G, and 50 μg/ml streptomycin in a humidified incubator containing 5% CO_2. JPX-9 and JPX-9/M (kindly provided by Dr. M. Nakamura, Tokyo Medical and Dental University, Tokyo, Japan) is a subclone of Jurkat cells expressing Tax and nonfunctional Tax mutant under the control of the metallothionein promoter (28, 29).

Patient Samples. The study protocol was approved by the Human Ethics Review Committees of the participating institutions. Leukemic cells from six patients diagnosed with acute-type ($n = 3$; patients 2, 4, and 6) and chronic-type ATL ($n = 3$; patients 1, 3, and 5) were analyzed. The diagnosis was based on clinical features, hematological findings, and serum anti-HTLV-I antibodies. Monoclonal HTLV-I provirus integration into the DNA of leukemic cells was confirmed by Southern blot hybridization in all cases (data not shown). Mononuclear cells were isolated by Ficoll/Hypaque (Pharmacia LKB, Uppsala, Sweden) density gradient centrifugation and washed with PBS.

RT-PCR. Total RNA was extracted with Trizol (Life Technologies, Inc., Gaithersburg, MD) according to the protocol provided by the manufacturer. First-strand cDNA was synthesized in a 20-μl reaction volume using an RNA-PCR kit (Takara Shuzo, Kyoto, Japan) with random primers. Thereafter, cDNA was amplified for 35 and 28 cycles for MCP-1 and β-actin, respectively. The oligonucleotide primers used were as follows: for MCP-1: sense, 5'-TCGCTCAGCCAGATGCAATCAATGC-3' and antisense, 5'-CCCAGGGGTAGAACTGTGGTTCAA-3' (30); and for β-actin: sense, 5'-GTGGGGGCGCCCCAGGCACCA-3' and antisense, 5'-CTCCTTAATGTCACGCACGATTTC-3'. Product sizes were 479 bp for MCP-1 and 548 bp for β-actin. Cycling conditions were as follows: denaturing at 94°C (30 s), annealing at 60°C (30 s), and extension at 72°C (60 s for MCP-1 and 90 s for β-actin). The PCR products were fractionated on 2% agarose gels and visualized by ethidium bromide staining.

Northern Blot Analysis. Total RNA (20 μg) was electrophoresed through a formaldehyde-agarose gel and transferred onto a nylon filter. Filters were prehybridized (in 0.5 M sodium phosphate, 0.1% BSA, 7% SDS, 100 μg/ml salmon testis DNA, and 100 μg/ml yeast RNA) for 2 h at 65°C and then hybridized overnight with the following: α-^{32}P-radiolabeled probes: cDNA of HTLV-I Tax (31) and glyceraldehyde-3-phosphate dehydrogenase (32). Radiolabeled probes were generated using a Megaprime DNA Labeling system (Amersham, Arlington Heights, IL).

ELISA for MCP-1. Cells were suspended in fresh culture medium at a concentration of 5×10^5 cells/ml and cultured for 72 h. After centrifugation, MCP-1 concentrations were measured in the culture supernatants using a commercially available ELISA kit (Biosource International, Inc., Camarillo, CA).

Plasmids. The LUC reporter constructs contained the proximal promoter region and distal enhancer region of the human *MCP-1* gene and have recently been described in detail (33). The HTLV-I LTR-LUC reporter was generated by introducing the full-length HTLV-I LTR into the pGL2-Basic vector (Promega Corp., Madison, WI). κB-LUC (34), containing five tandem repeats of an NF-κB binding site from the *IL-2Rα* gene, was kindly provided by Dr. J. Fujisawa (Kansai Medical University, Osaka, Japan). Plasmids pH2Rneo and pH2R40M, containing Tax, have been described previously (35). Plasmids containing wild-type Tax (pβMT-2Tax) and a mutant Tax gene (pβTaxM22), under the control of a β-actin promoter and its control plasmid (pH βAPr-1-neo), have been described elsewhere (36, 37). Wild-type Tax encoded in pβMT-2Tax is effective at activating NF-κB, CArG, and CRE sites. On the other hand, the Tax mutant encoded by pβTaxM22 can activate CArG and CRE sites but not NF-κB (38). IκB$\alpha\Delta$N (39) and IκB$\beta\Delta$N (40; kindly provided by Dr. D. W. Ballard, Vanderbilt University School of Medicine, Nashville, TN) are deletion mutants of IκBα and IκBβ lacking the NH$_2$-terminal 36 amino acids and 23 amino acids, respectively. The kinase-deficient K44M IKKα, K44A IKKβ, and KK429/430AA NIK mutants have been described previously (21).

Cell Transfection and LUC Assays. Transfections were performed by electroporation (41). In all cases, a renilla LUC expression vector, pRL-TK (Toyo Ink Co., Tokyo, Japan), was cotransfected to correct for transfection efficiency. After 24 h of incubation, transfected cells were lysed in lysis reagent (Toyo Ink Co.), and LUC activity was measured according to the protocol provided by the manufacturer. Each assay was independently repeated at least three times.

EMSA. NF-κB binding activity to κB elements was examined by EMSA as described previously (42). In brief, 5 μg of nuclear extracts were preincubated in a binding buffer containing 1 μg of poly(deoxyinosinic-deoxycytidylic acid). They were then incubated with ~50,000 cpm of α-^{32}P-labeled oligonucleotide probes containing κB elements for 15 min at room temperature. The DNA-protein complexes were separated on a 4% polyacrylamide gel and visualized by autoradiography. To examine the specificity of the κB element

probes, unlabeled competitor oligonucleotides were preincubated with nuclear extracts for 15 min before incubation with probes. The probes or competitors used were prepared by annealing the sense and antisense synthetic oligonucleotides as follows: NF-κB element A1 in the *MCP-1* gene, 5'-gatcGATCTGGGAACTTCCAAAGC-3'; A1 mutant (MA1), 5'-gatc-GATCTaGaAACTTCCAAAGC-3'; NF-κB element A2 in the *MCP-1* gene, 5'-gatcAGAGTGGGAATTTCCACTCA-3'; A2 mutant (MA2), 5'-gatcA-GAGTGGGAATTcggACTCA-3'; and a typical NF-κB element from the *IL-2Rα* gene, 5'-gatcCGGCAGGGGAATCTCCCTCTC-3'. Underlined sequences represent the NF-κB binding site, and mutations are indicated in lowercase. To identify NF-κB/Rel proteins in the DNA-protein complex revealed by EMSA, antibodies specific for various NF-κB/Rel family proteins, including RelA/p65, p50, c-Rel, and NF-κB2 p52 (Santa Cruz Biotechnology, Santa Cruz, CA), were used to elicit a supershift and/or to inhibit DNA-protein complex formation. These antibodies were incubated with the nuclear extracts for 45 min at room temperature, before incubation with radiolabeled probes.

RESULTS

Constitutive Expression of MCP-1 mRNA in T-Cell Lines Infected with HTLV-I. To determine whether MCP-1 expression in T cells correlates with HTLV-I infection, MCP-1 mRNA levels from four HTLV-I-infected and three uninfected human T-cell lines were assessed by RT-PCR using MCP-1-specific primers. Expression of HTLV-I mRNA was confirmed in all four HTLV-I-infected T-cell lines by Northern blot analysis. The MCP-1 transcript was detected
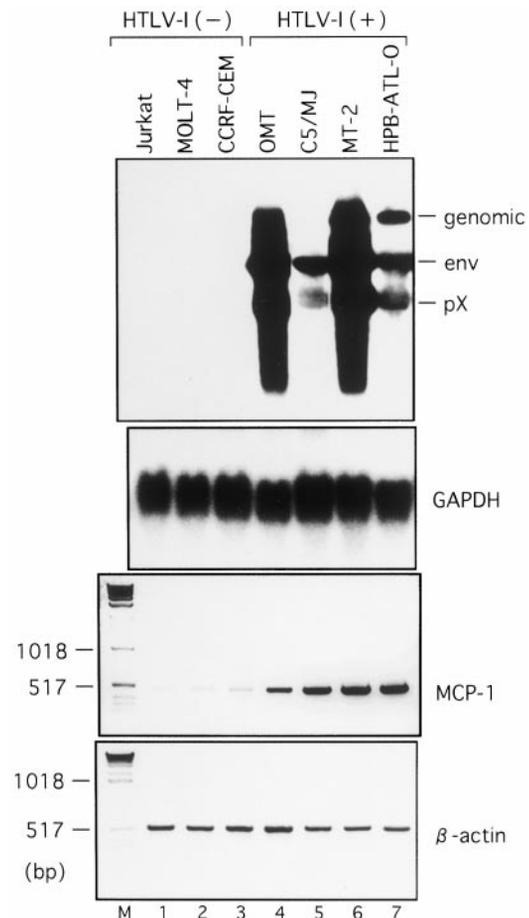

Fig. 1. Determination of the expression of HTLV-I mRNA by Northern blot analysis and MCP-1 mRNA by RT-PCR analysis in various HTLV-I-infected and uninfected human T-cell lines. Total RNA samples were prepared from the indicated T-cell lines. Predominant 2.1-, 4.2-, and 8.5-kb HTLV-I mRNA species were detected in OMT, C5/MJ, MT-2, and HPB-ATL-O cell lines (*Lanes 4–7*). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and β-actin expression served as controls.
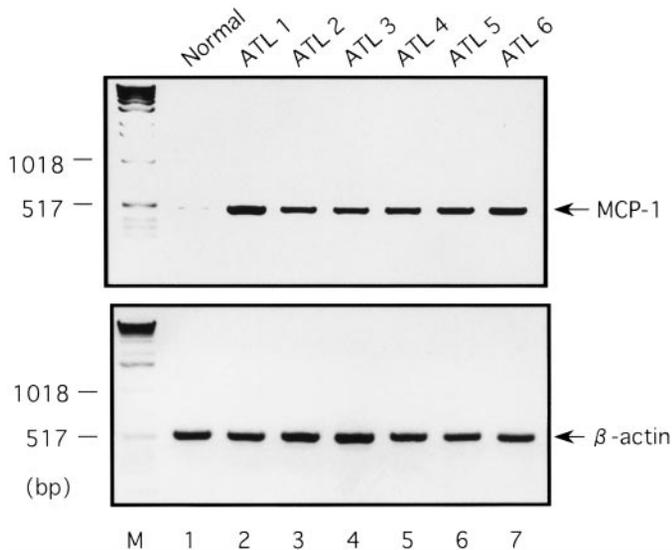
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Fig. 2. Detection of MCP-1 mRNA in leukemic cells obtained from ATL patients by RT-PCR analysis. *Lane 1*, normal PBMCs RNA; *Lanes 2–7*, PBMCs RNA samples from patients with ATL. *Bottom*, ethidium bromide staining of the RT-PCR performed with β-actin primers. *Arrows*, position of the specifically amplified DNA.
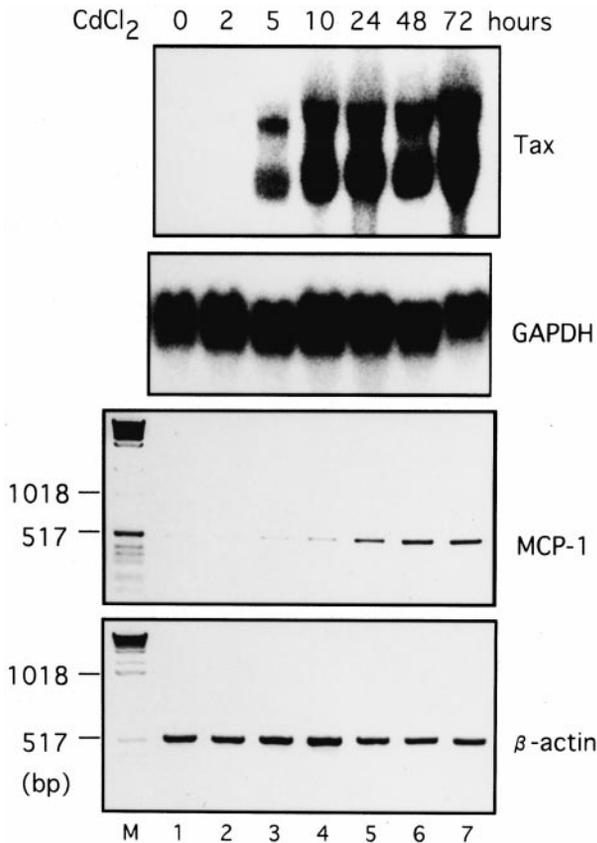

Fig. 3. Induction kinetics of the *MCP-1* gene in JPX-9 cells treated with CdCl$_2$. Total RNA samples were prepared from CdCl$_2$-treated JPX-9 cells at the indicated time points. The expression of Tax and MCP-1 in the extracted RNA was analyzed by Northern blot and RT-PCR analysis, respectively.

strongly in all four HTLV-I-infected cell lines (Fig. 1, *Lanes 4–7*), whereas it was hardly detectable in the uninfected lines (Fig. 1, *Lanes 1–3*). β-Actin served as the internal control for each sample. This result demonstrates that the *MCP-1* gene is constitutively expressed in HTLV-I-infected T-cell lines.

Expression of MCP-1 mRNA in Cells Derived from ATL Patients. Expression of the *MCP-1* gene in leukemic cells derived from patients with ATL was assessed by RT-PCR. In all cases, we observed significant expression of MCP-1 mRNA (Fig. 2), indicating that this gene is up-regulated in cells derived from ATL patients. In contrast, MCP-1 was hardly expressed in PBMCs from normal donors.

Tax Induces Expression of the *MCP-1* Gene. Because HTLV-I encodes a strong transcriptional transactivator, Tax, implicated previously in deregulation of host genes, we strongly suggest that Tax was up-regulating *MCP-1* gene transcription. To test whether Tax transactivates the *MCP-1* gene, we next used the inducible Jurkat Tax transfectant, JPX-9, which generates Tax after the addition of CdCl$_2$ (28). The level of expression of Tax mRNA in these cells was determined by Northern blot analysis, and expression of the *MCP-1* gene was assayed by RT-PCR (Fig. 3). The addition of CdCl$_2$ (20 μM) to the culture medium of JPX-9 cells induced the expression of Tax within 5 h, which persisted until 72 h after treatment. A concomitant increase in the expression of MCP-1, within 24 h of treatment with CdCl$_2$, was observed in JPX-9 cells. This increase in MCP-1 mRNA levels was further enhanced 72 h after the treatment. The induction of MCP-1 was attributable to Tax but not by CdCl$_2$ treatment, because it was not observed in JPX-9/M expressing the nonfunctional Tax protein after treatment with CdCl$_2$ (data not shown). These results indicate that Tax alone is capable of causing elevated expression of the *MCP-1* gene in Jurkat T cells.

Elevated MCP-1 Levels in Culture Supernatants from HTLV-I-infected T Cells. We next measured production of MCP-1 in the culture supernatants from HTLV-I-infected and uninfected T-cell lines by ELISA. As shown in Fig. 4*A*, MCP-1 was produced at low
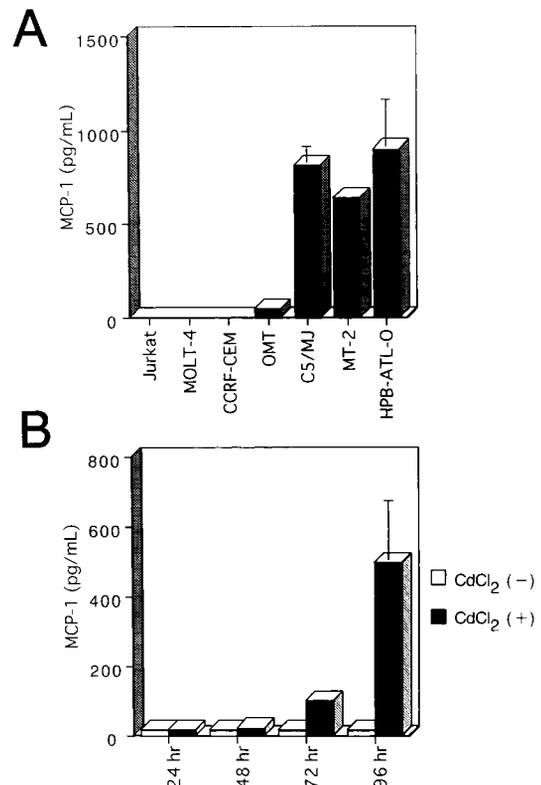

Fig. 4. *A*, measurements of MCP-1 secreted in the culture supernatants of human T-cell lines by ELISA. The indicated cell lines were plated at 5×10^5/ml, and culture supernatants were collected after 72 h. *B*, induction of MCP-1 in JPX-9 cells after CdCl$_2$ induced Tax expression. JPX-9 cells inoculated at 5×10^5/ml were treated with or without 20 μM CdCl$_2$ for the indicated durations. MCP-1 secreted in the culture supernatants was quantified by using ELISA. *Bars*, SD.

4941

Fig. 5. Transactivation of the MCP-1 promoter by wild-type and mutant Tax. Jurkat cells were cotransfected with 0.2 μg of either pGLM-ENH or pGLM-PRM and with 5 μg of either a wild-type (*WT*) or mutant (*M22*) Tax expression vector and 0.4 μg of pRL-TK as an internal control. LUC activities were monitored 24 h later. The results correspond to the means of three individual transfections and are expressed as fold induction relative to the basal level measured in cells transfected with the empty vector (pHβAPr-1-neo); *bars,* SD. For comparison, data showing the inducibility of κB (κ*B-LUC*) and HTLV-I LTR (*LTR-LUC*) reporter plasmids by Tax and M22 Tax have been added.
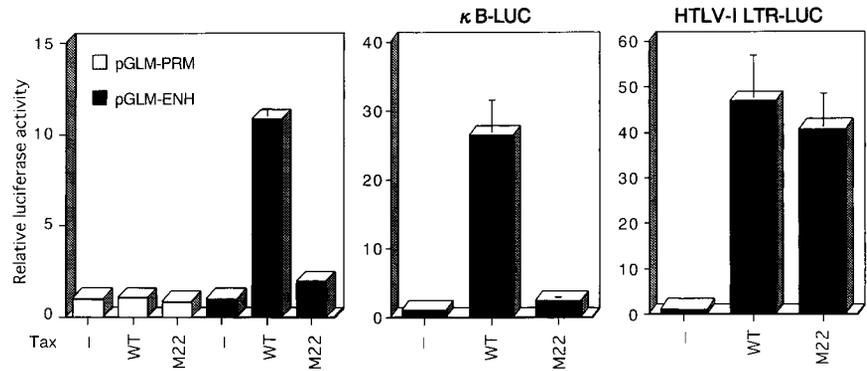
levels by OMT cells and at very high levels by C5/MJ, MT-2, and HPB-ATL-O. MCP-1 was not produced in uninfected cell lines. MCP-1, thus, was not only expressed but also secreted by HTLV-I-infected T-cell lines. Furthermore, we measured production of MCP-1 in culture supernatants of JPX-9 with or without CdCl$_2$ treatment (Fig. 4*B*). Thus, consistent with the ability of Tax to induce the transcription of the *MCP-1* gene, the actual MCP-1 chemokine can be produced and secreted by Tax-expressing cells.

Transactivation of the MCP-1 Promoter by Tax. To examine whether the MCP-1 promoter/enhancer responds to Tax, we cotransfected Tax together with LUC reporters under the control of the MCP-1 5′ regulatory sequences. Structural features of the human MCP-1 promoter have been described recently (33, 43, 44). Although basal promoter activity is dependent on a proximal promoter region containing an SP-1 site, cytokine-inducible promoter activity is mainly mediated by a distal enhancer region (Fig. 7). Two NF-κB binding sites (A1 and A2), which are crucial for enhancer activity, have been identified in the distal enhancer region (33, 43, 44). Therefore, we used an MCP-1 promoter/enhancer LUC construct, pGLM-ENH, containing the enhancer (between −2742 and −2513) and promoter regions (between −107 and +60) of the human MCP-1 promoter to test for Tax responsiveness [Fig. 5 and see Fig. 7 and Ueda *et al.* (33) for details]. As shown in Fig. 5, wild-type Tax caused an 11-fold stimulation of the pGLM-ENH reporter construct in Jurkat cells. Because the pGLM-PRM reporter, containing only the proximal MCP-1 promoter region, was not responsive to Tax, we conclude that the Tax-responsive target sites are localized in the MCP-1 distal enhancer. Importantly, the Tax M22 plasmid, which is defective for NF-κB activation, failed to activate pGLM-ENH (Fig. 5). This mutant of Tax maintained the ability to transactivate the HTLV-I LTR (Fig.

5). These results indicated that Tax most likely activates the MCP-1 distal enhancer through the NF-κB pathway.

Tax-mediated Activation of the MCP-1 Distal Enhancer Can Be Blocked by Dominant Interfering Signaling Components of the NF-κB Pathway. We next examined whether blocking of the NF-κB pathway can affect Tax-induced transactivation of the MCP-1 distal enhancer by using dominant interfering mutants of IκBα and IκBβ and kinase-deficient mutants of IKKα, IKKβ, and NIK. As controls for these experiments, the effects of these mutants on Tax-mediated activation of the HTLV-I LTR and a multimerized NF-κB site from the IL-2Rα promoter (κB-LUC) were evaluated in parallel. Consistent with previous findings (20, 21), transfection of these mutant vectors resulted in inhibition of Tax activation of κB-LUC activity (Fig. 6). In contrast, these mutants produced no significant inhibition of Tax-induced HTLV-I LTR LUC activity. Finally, these mutants were cotransfected along with the wild-type Tax plasmid and the pGLM-ENH. As shown in Fig. 6, the elevated LUC activity in response to Tax was markedly suppressed by cotransfection with the dominant interfering mutants. These results confirmed that the NF-κB pathway was involved in Tax-induced transactivation of the MCP-1 distal enhancer.

Cooperation between the A1 and A2 Sites of the MCP-1 Distal Enhancer in Tax-induced Activation. Two NF-κB binding sites (A1 and A2) have been identified in the distal enhancer region of the *MCP-1* gene, which are crucial for enhancer activity (33, 43, 44). To investigate the role of the A1 site and a possible cooperation with the A2 site in Tax transactivation, the sequences of the A1 and the A2 sites were mutated (Fig. 7). The A1 and A2 mutant reporter constructs were cotransfected along with the Tax expression plasmid. As shown in Fig. 7, Tax-induced LUC activity was significantly reduced by

Fig. 6. Functional effects of IκBα and IκBβ mutants and kinase-deficient IKKα, IKKβ, and NIK mutants on HTLV-I Tax induction of the MCP-1 distal enhancer in Jurkat cells. Cells were transfected with 5 μg of Tax (pH2R40M), 5 μg of the IκB α mutant IκB$\alpha\Delta$N, IκBα mutant IκB$\beta\Delta$N, the K44M mutant of IKKα, the K44A mutant of IKK β, or the kinase-deficient KK429/430AA mutant of NIK, and 0.2 μg of pGLM-ENH, the κB-LUC, or the HTLV-I LTR LUC reporter plasmid. Each transfection also contained 0.4 μg of pRL-TK and was supplemented to 5 μg with the parental pCMV4 vector. LUC activity is presented as fold induction relative to the basal level measured in cells transfected with pH2Rneo. The values are means from three independent experiments; *bars,* SD.
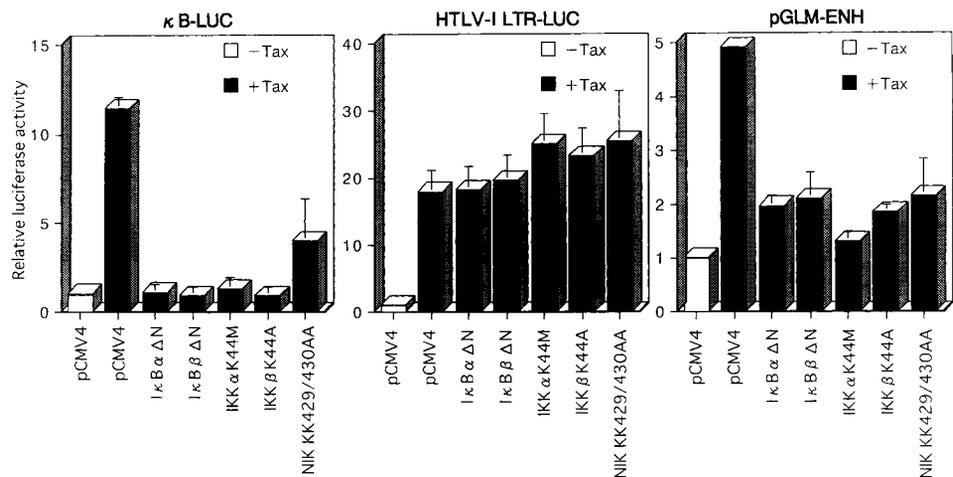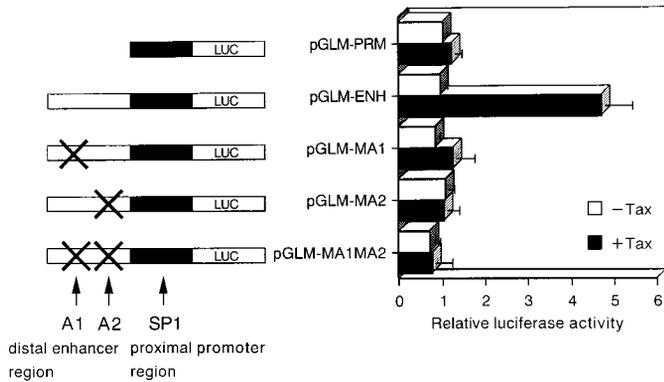
Fig. 7. Cooperation between the A1 and A2 sites of the MCP-1 distal enhancer in Tax-induced *MCP-1* gene transcription. Schematic representation of the MCP-1 reporter constructs. ■, proximal promoter region; □, distal enhancer region of the *MCP-1* gene. The A1 and A2 sites of the MCP-1 distal enhancer region were mutated (×) in some of the constructs. These constructs were transfected into Jurkat cells, and LUC activities were compared. Cells were cotransfected with either pH2R40M (+*Tax*) or the empty vector pH2Rneo. *Bars,* SD.

DISCUSSION

This study has revealed that HTLV-I infection of T cells leads to a marked increase in the transcription of the *MCP-1* gene resulting from transactivation by the viral Tax protein. Consistent with this finding, HTLV-I-infected and Tax-expressing T cells secrete the MCP-1 chemokine. MCP-1 is induced by proinflammatory cytokines and growth factors (9, 10). Molecular studies from a number of laboratories have identified some of the *cis*-regulatory elements and *trans*-acting factors involved in this response. Two NF-κB binding sites (A1 and A2) located ~2.6 kb from the transcription initiation site appear to function as the critical elements in MCP-1 induction in response to IL-1β and tumor necrosis factor-α (33, 43, 44). Additional elements in proximity of these κB sites (45, 46) and a 7-bp response element situated with the 3′ untranslated region of the MCP-1 transcript (47) are also involved in the regulation of *MCP-1* gene expression, in response to platelet-derived growth factor. Thus, this distal group of
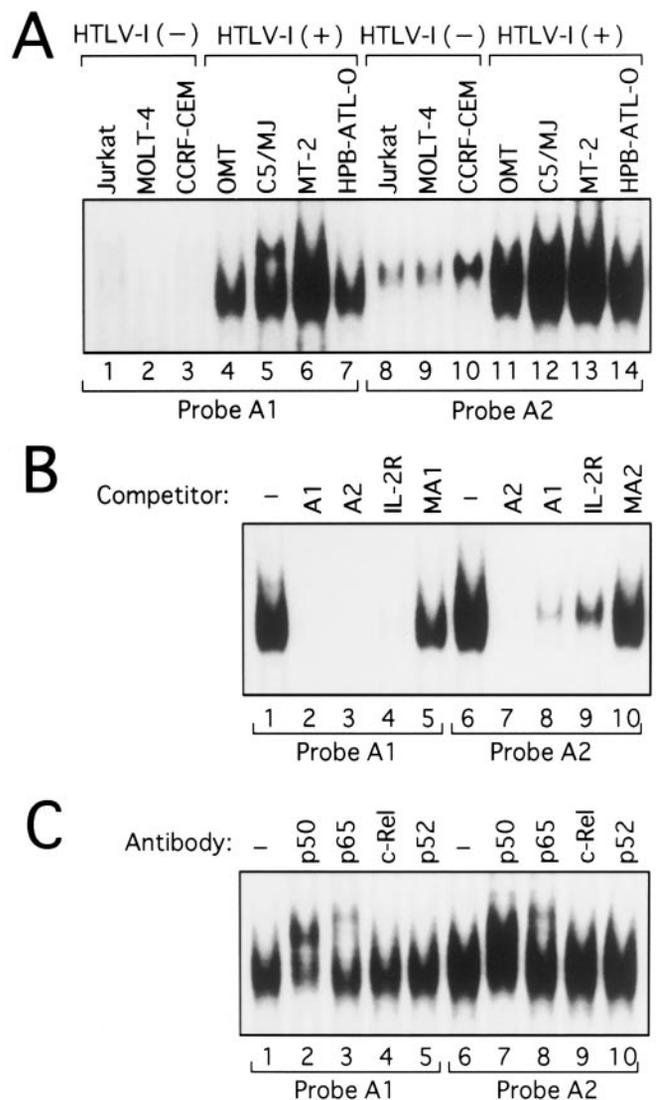
mutation in either the A1 or A2 sequences, indicating that Tax transactivation of the MCP-1 distal enhancer involves both A1 and A2 sites. These results suggest that both the A1 and the A2 sites are important for Tax-induced *MCP-1* gene transcription.

Binding of NF-κB/Rel Family Proteins to the A1 and A2 κB Elements of the MCP-1 Distal Enhancer. We next examined whether NF-κB/Rel family members can actually bind to the putative A1 and A2 κB elements identified in the MCP-1 distal enhancer by EMSA. Synthetic oligonucleotides containing the A1 site (−2640 to −2632 relative to the MCP-1 transcription start site) and the A2 site (−2612 to −2603) of the MCP-1 distal enhancer were used as probes. When the A1 site was used as a probe, a clear shifted band was observed with nuclear extracts from HTLV-I-infected T-cell lines but not with uninfected cell nuclear extracts (Fig. 8*A*, *Lanes 1–7*). This shifted complex was specific to the A1 fragment because complex formation was competed away by addition of excess cold probe and the typical NF-κB sequence of the IL-2Rα enhancer and also by probe A2 but not by a mutant sequence of A1 (Fig. 8*B*, *Lanes 1–5*). The A2 probe essentially showed the same pattern of complex formation as probe A1 (Fig. 8*A*, *Lanes 8–14,* and Fig. 8*B*, *Lanes 6–10*). To identify which NF-κB/Rel family members were binding to the A1 and A2 elements of the MCP-1 distal enhancer, we performed EMSA using antibodies specific for members of the NF-κB/Rel family. Supershifts were seen with anti-p65 and anti-p50 antibodies in complexes formed with both probes A1 and A2, illustrating that these complexes contain the p50 and p65 subunits of NF-κB (Fig. 8*C*).

To examine which NF-κB/Rel family members Tax was inducing to associate with the A1 and A2 elements of the MCP-1 distal enhancer, we performed an EMSA analysis using nuclear extracts from the Tax-inducible T-cell line, JPX-9. Induction of Tax expression resulted in induction of complex formation with probes A1 and A2 (Fig. 9*A*). This binding was competed away by the typical NF-κB sequence derived from the IL-2Rα enhancer but not by mutant sequences of the A1 and A2 elements (Fig. 9*B*). The Tax-induced complexes identified by the A1 and A2 probes were also characterized. These complexes were supershifted by the addition of anti-p50 or anti-p65 antibodies (Fig. 9*C*). Thus, Tax induces *MCP-1* gene expression, at least in part, through the induced binding of the p50 and p65 NF-κB/Rel family members to the A1 and A2 elements of the MCP-1 distal enhancer.

Fig. 8. Binding of nuclear proteins from HTLV-I-infected T cells to the A1 and A2 probes derived from the MCP-1 distal enhancer. *A,* nuclear extracts were prepared from HTLV-I-infected (OMT, C5/MJ, MT-2, and HPB-ATL-O) and uninfected (Jurkat, MOLT-4, and CCRF-CEM) T cells and incubated with ³²P-labeled A1 (*Lanes 1–7*) and A2 probes (*Lanes 8–14*). *B,* sequence specificity of NF-κB binding activity in HPB-ATL-O cells. Competition assays were performed with 100-fold excess amounts of each specific competitor oligonucleotide. *C,* characterization of NF-κB/Rel proteins that bound to the A1 and A2 κB sites of the *MCP-1* gene. Nuclear extracts from HPB-ATL-O cells were preincubated with appropriate antibodies before addition of ³²P-labeled probes.
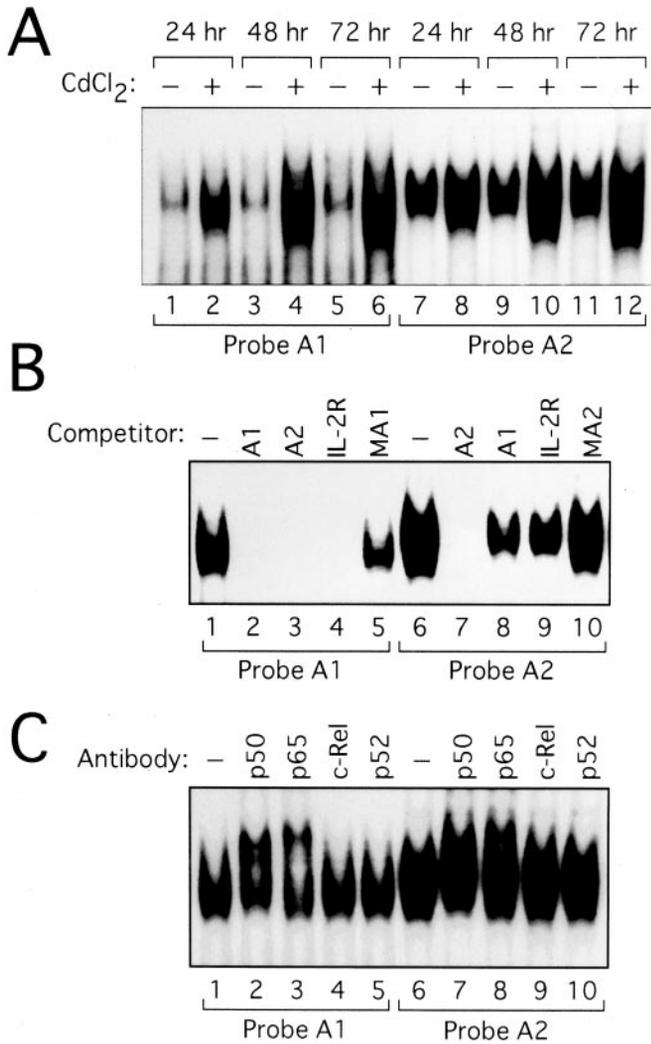
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Fig. 9. Tax-induced NF-κB binding activity to the A1 and A2 elements of the MCP-1 distal enhancer. *A,* nuclear extracts from JPX-9 cells, treated with or without CdCl₂ (20 μM) for the indicated time periods, were mixed with κB elements A1 (*Lanes 1–6*) and A2 (*Lanes 7–12*). *B,* nuclear extracts from JPX-9 cells treated with CdCl₂ for 48 h were mixed with labeled probes A1 (*Lanes 1–5*) and A2 (*Lanes 6–10*) in the absence or presence of 100-fold excess amounts of each specific competitor oligonucleotide. *C,* JPX-9 nuclear extracts obtained after 48 h of CdCl₂ treatment were incubated with the indicated antibodies, before addition of the labeled probes A1 (*Lanes 1–5*) and A2 (*Lanes 6–10*).

eases. One of the characteristic features of HTLV-I-associated diseases is prominent tissue infiltration of lymphoid cells. For example, ATL is frequently accompanied by lymphadenopathy, hepatospleno-megaly, and skin lesions. Similarly, major pathological changes in HAM/TSP are marked infiltration of HTLV-I-infected T cells around small vessels in the spinal cord. MCP-1 has been considered as an important mediator that specifically stimulates directional migration of T cells and monocytes/macrophages. MCP-1 has also been shown to modulate leukocyte adhesion molecule expression and other leukocyte functions that are necessary for leukocytes to leave the circulation and infiltrate tissues. Aberrant production of MCP-1 by leukemic cells in ATL or HTLV-I-infected T-cells in HAM/TSP may promote influx of lymphoid cells into the affected tissues.

In summary, this study allowed identification of mechanisms by which MCP-1 expression is deregulated by the pathogenic human retrovirus HTLV-I. Additional studies of the potential function of MCP-1 in HTLV-I-infected T cells may provide important insights into the pathogenesis of HTLV-I infection.

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regulatory elements plays a significant role in *MCP-1* gene transcription. Tax is known to activate NF-κB by activating IKKs and other related molecules (18–24). Our finding that a Tax mutant defective in NF-κB activation (M22) was unable to transactivate the MCP-1 distal enhancer together with the observation that dominant-negative IκBs, IKKs, and NIK expression plasmids blocked Tax transactivation of this same enhancer allowed us to conclude that the activation of the *MCP-1* gene by the HTLV-I Tax protein involves primarily the NF-κB pathway. The observation that binding of NF-κB transcription factors to both A1 and A2 sites and reduction of activity of the MCP-1 promoter by mutation in either element is consistent with Tax requiring the NF-κB pathway to transactivate the MCP-1 promoter/enhancer.

Pathological roles for MCP-1 in HTLV-I-associated diseases are not known. MCP-1 is mainly produced by monocytes and not by T cells. However, we demonstrated that aberrant MCP-1 expression was restricted to T cells expressing HTLV-I. We, therefore, speculated that deregulated MCP-1 expression in HTLV-I-infected cells may represent an important pathogenic determinant in HTLV-I-associated dis-

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