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Glucocorticoid-induced MIF Expression by Human CEM T cells

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Abstract

Macrophage migration inhibitory factor (MIF) is an upstream activator of the immune response that counter-regulates the immunosuppressive effects of glucocorticoids. While MIF is released by cells in response to diverse microbial and invasive stimuli, evidence that glucocorticoids in low concentrations also induce MIF secretion suggests an additional regulatory relationship between these mediators. We investigated the expression of MIF from the human CEM T cell line, which exists in two well-characterized, glucocorticoid–sensitive (CEM-C7) and glucocorticoid-resistant (CEM-C1) variant clones. Dexamethasone in low concentrations induced MIF secretion from CEM-C7 but not CEM-C1 T cells by a bell-shaped dose response that was similar to that reported previously for the release of MIF by monocytes/macrophages. Glucocorticoid stimulation of CEM-C7 T cells was accompanied by an MIF transcriptional response, which by promoter analysis was found to involve the GRE and ATF/CRE transcription factor binding sites. These data support a glucocorticoid-mediated MIF secretion response by T cells that may contribute to the regulation of the adaptive immune response.

Keywords

glucocorticoid receptor; inflammation; T cell; promoter

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Introduction

Macrophage migration inhibitory factor (MIF) is one of the first cytokine activities to be described 19, yet its upstream role in regulating the immune response has emerged relatively recently 13. MIF circulates normally in plasma and its levels increase during physiologic stress, infection, or cancer 9;13. Experimental studies employing recombinant MIF, anti-MIF, or MIF knockout (MIF-KO) mice have established that this cytokine contributes to the immunopathogenesis of such disorders as endotoxemia and sepsis 9;16, arthritis 5;34;37, inflammatory bowel disease 20, asthma 42, and tumor progression 39. Certain of the pro-inflammatory properties of MIF, whether secreted locally or systemically, have been attributed to its ability to antagonize the immunosuppressive effects of glucocorticoids 14. MIF regulates glucocorticoid action by several mechanisms; these include inducing sustained ERK1/2 MAP kinase activation, which leads to an increase in the translation of cytokine mRNAs 41;57, inhibiting glucocorticoid-induced expression of the NFκB inhibitor, IκB 18, and inhibiting glucocorticoid induction of MAP kinase phosphatase (MKP-1), which down-regulates the responses of the ERK1/2, p38, and JNK MAP kinases 1;24;52.

MIF is released by macrophages, synoviocytes, and neurons upon stimulation with low, physiologic levels of glucocorticoids14;55;61, and these data are supported by studies of tissue MIF content after adrenalectomy or glucocorticoid administration 23. Much of the MIF release response appears to be due to the secretion of protein from preformed, cytoplasmic stores 9; 23;36;45. The observation that glucocorticoids, which are immunosuppressive 50, induce the release of a counter-regulating mediator such as MIF has focused attention on the role of this pathway in modulating the immune response 14;24. Of note, MIF normally circulates at its counter-regulating concentrations (2–10 ng/ml) and blood levels follow a circadian rhythm that is similar to the diurnal variation in plasma glucocorticoids 48. Whether MIF secretion from immune cells, the anterior pituitary gland, or other diurnally responsive tissues account for this circadian rhythm remains unknown 15;29;59; nevertheless, these observations have led to the concept that MIF and glucocorticoids may act in a regulatory “dyad” to control the setpoint and the magnitude of the immune response 13;14;24. That MIF functions as a physiological regulator of glucocorticoid action also has prompted pharmacologic approaches at targeting MIF for steroid-dependent, chronic inflammatory conditions 12;35;43.

Prior reports of a specific MIF secretion response from monocytes/macrophages stimulated with low, physiologic levels of glucocorticoids 14 together with increasing evidence that MIF influences the differentiation of the adaptive T cell response 8;42 prompted us to examine more closely MIF production by human T cells. We studied MIF protein release and mRNA expression in the two closely related human T cell lines, CEM-C1 and CEM-C7, which differ in their sensitivity to glucocorticoid-dependent responses downstream of the glucocorticoid receptor 58.

Materials and Methods

Cell Lines

The CEM-C1 (clone 15) and CEM-C7 (clone 14) human T cell lines were kindly provided by Betty H. Johnson (University of Texas, Galveston) and bear the characteristics originally described by Norman and Thompson46 and Zawydiwski *et al.*63. The CEM-C7 cells are diploid, with 11,200±2400 glucocorticoid receptor (GR) sites per cell with a K_d of 13±10 nM, and are sensitive to killing by 1 μM dexamethasone. The CEM-C1 cells are diploid, with 11,200 ±2400 GR sites per cell with a K_d of 12±3 nM, and are resistant to killing by 1 μM dexamethasone. Cells were maintained in culture with RPMI and 10% FBS.

Stimulation Studies and MIF Expression Analyses

For stimulation studies, T cells were enumerated, centrifuged, and re-suspended in 48 well plates at 1×10^5 cells/ml in RPMI supplemented with 1% heat-inactivated, glucocorticoid-depleted FBS, as assessed by immunoassay14. Dexamethasone (Sigma, St. Louis, MO) diluted in RPMI/1% FBS was added at the indicated concentrations and the supernatants removed at intervals for analysis by MIF ELISA as described previously 4. For RNA analysis, the T cells were collected by centrifugation and the RNA isolated using RNAeasy (Qiagen, Valencia, CA). cDNA was synthesized using the ProFirst Strand RT-PCR kit (Stratagene, La Jolla, CA) at a concentration of 100 ng RNA/μl cDNA 31. The specific primers and reaction conditions were as follows: MIF forward, 5'-CGGACAGGGTCTACATCAA-3'; MIF reverse, 5'-CTTAGGCGAAGGTGGAGTT-3'; 18S forward, 5'-GCAATTATTCCCCATGAACG-3'; 18S reverse, '-TGTACAAAGGGCAGGGACTT-3'. The reactions employed SYBR Green Supermix (Invitrogen, Carlsbad, CA) and the conditions were: 1 cycle of 95°C for 3 min; 40 cycles of 95°C for 15 sec, followed by 60°C for 1 min; 80 cycles of increasing temperature by 0.5°C, starting at 55°C. The specific amplification of the desired target gene was verified by the appearance of a single peak in the melting curve at the predicted temperature, and the appearance of a single band of the predicted length upon gel electrophoresis. Data were analyzed using the $\Delta\Delta C_T$method for relative quantification 33.

Cell Death Detection

Cell death was quantified by ELISA for cytoplasmic histone-associated DNA fragments (Roche Diagnostics, Indianapolis, IN) 56. Cells were collected at intervals and the cell pellets lysed by addition of lysis buffer provided in the Roche kit, followed by incubation at 25°C for 30 mins. The lysates were centrifuged at 1500 rpm for 10 min and 20 μl was added to the ELISA and OD_{405} recorded. MIF immunoneutralization studies employed the neutralizing anti-MIF murine IgG_1, NIH3D9 20

Construction of a Luciferase Reporter Plasmid Containing the MIF promoter

A 1177 bp long DNA sequence comprising the 5' flanking sequence upstream of the transcription start site of the human MIF gene47 was amplified by PCR using the following oligonucleotides: 5'-CTGCAGGAACCAATACCCAT-3' and 3'-CCAGGAAGACGGTAGTACGG-5'. The resultant amplification product was gel-purified using Geneclean II (BIO 101, La Jolla, CA) and sub-cloned into the Xho/Hind II sites of the luciferase reporter vector pGL2-Basic (Promega, Madison, WI). The fidelity of construction of the *MIF-luciferase* construct was confirmed by DNA sequencing 5.

Site-Directed Mutagenesis

The ATF/CRE binding site (TGGCGTCA, at −116 to −109 bases from the MIF start codon) and the GRE consensus sequence (AGATGGTCCCC, at −847 to −837 bases) were deleted from the *MIF-luciferase* expression plasmid by using the Transformer Site-Directed Mutagenesis Kit (Clontech, Mountain View, CA). The mutagenic primer, which is homologous to the wild-type sequence except for the deleted binding site was: ATF/CRE, 5'-GGCCTGGCGCCGGCGGCAAAAGGCGGGACCAC-3'; and for GRE, 5'-GATATGCCTGGCACCTGCTGAGTTTACCATTAGTGG-3'. The selection primer, which converts a single Bam HI site within the vector into a single restriction site for Aat II was: 5'-CTTATCATGTCTGACGTCGTCGACCGATGC-3'). The primer plasmid annealing reaction was performed at 100°C for 3 min and 0°C for 5 min in a 20 μl reaction volume containing 0.1 μg pGL2B-MIF DNA, 0.1 μg of the mutagenic primer, 0.1 μg of the selection primer, and 2 μl of 10× annealing buffer. For the synthesis of the mutant strand, 2–4 units of T4 DNA polymerase, 4–6 units of T4 DNA ligase, 3 μl of 10× synthesis buffer, and 5 μl H_2O were added to the annealing reaction and incubated at 37ʙC for 2.5 hrs. The synthesized DNA was

purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA). To reduce the proportion of the parental DNA (>95% in the total reaction), the DNA then was digested with BamHI, which linearizes the non-mutated plasmids. Plasmids with an altered unique restriction site are resistant to digestion by the original enzyme and have a high probability of containing the desired mutation (the transformation efficiency of the circular mutated plasmid is at least a 100× higher than that of the linearized parental plasmid). For the restriction digestion, the purified DNA was incubated with Bam HI, and 10 μl of the restriction digest then were transformed into competent *E. coli* BMH 71-18 *mut*S cells. The plasmid DNA was isolated with the Qiagen Plasmid Mini Kit. To select for the mutant plasmids, 100 ng of the purified DNA (total volume 20 μl) was incubated with 20 units Bam HI (NEB), 2 μl 10× NEB react buffer U, and 0.2 μl 100× BSA for 2 hrs at 37ʙC. Ten units of fresh enzyme then were added followed by an additional incubation at 37ʙC for 1 hr. Five μl of this reaction were transformed into 100 μl of *E.coli* DH5α MAX efficiency chemically competent cells (Invitrogen, Carlsbad, CA). The fidelity of construction was confirmed by bidirectional sequencing of the plasmid DNA.

Transient Transfection of CEM-C7-cells

The *MIF-luciferase* reporter plasmid was introduced into CEM-C7 or CEM-C1 T cells by liposome mediated transfection using the DMRIE-C Reagent (Invitrogen) in OPTI-MEM I reduced serum medium5. For each transfection, 0.5 ml of media were mixed with 6 μl of DMRIE-C reagent (2mg/ml) in a polystyrene 6-well plate. After adding plasmid DNA (*MIF-luciferase* or vector control) in 0.5 ml OPTI-MEM per well, the plates were incubated at room temperature for 45 min to allow formation of the lipid-DNA complexes. 2×10^6 cells in 0.2 ml OPTI-MEM then were added and the plates were incubated for 6 hrs at 37ʙC in a 5% CO_2 incubator. 2.5 ml of cultivation media was added per well and the cells were incubated for another 16 hrs before commencing the induction experiments.

For induction of the *MIF* promoter, the transiently transfected cells were plated in 6-well plates at a density of 2×10^6 cells/well in 2 ml of normal growth media and incubated for 12 hrs with the following agents: dexamethasone (Sigma), phorbol-12-myristate-13-acetate (PMA, give source), ionomycin (Calbiochem), phytohemagglutinin (PHA), anti-CD3 plus anti-CD28 antibody4, and recombinant human MIF10. Co-transfection studies employing the *MIF-luciferase* plasmid and the human glucocorticoid receptor (GR) expression plasmid HGO27 were performed in CEM-C7 T cells at different plasmid ratios (GR expression plasmid : MIF-luciferase plasmid).

Luciferase Assay

Cells were washed twice in PBS and resuspended in 35 μl of Passive Lysis Buffer (Promega) per well. After incubation at room temperature for 15 min, the cells were vortexed for 15 sec and microcentrifuged for 5 min at 12,000×g. The supernatants (*i.e.* cell lysates) were transferred into a fresh tube and the luciferase activity measured as follows: 20 μl of the cell lysate was mixed with 100 μl of Luciferase Assay Reagent (Promega) and the light output measured immediately with a TD-20/20 luminometer (Promega) with a delay time of 5 sec and an integration time of 20 sec. The protein concentration of the cell lysates was measured by using the Bio-Rad DC Protein Assay.

Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts were prepared and analyzed by EMSA as previously reported 54. Two μg of nuclear extracts were incubated for 15 min at room temperature with radio-labeled probes. Reaction mixtures were electrophoresed through 6% non-denaturing polyacrylamide gels, and the gels were dried and exposed to X-ray films. The sequence (5'->3') of the probes were as follow: ATF/CRE, GGCGCCGGCGGTGGCGTCACAAAA; GRE,

GGCACCTGCTAGATGGTCCCCGA; ATF/CRE mutant, GGCGCCGGCGGTGTGTTCACAAAA; and GRE mutant GGCACCTGCTATATTTTCCCCGA. The EMSA studies were replicated in independent experiments and the gel data quantified by densitometric analysis as previously described 32.

MIF mRNA Stability

MIF mRNA stability was assessed in CEM-C7 T cells incubated for 4 hrs with dexamethasone (10^{-8} M) before the addition of actinomycin D (10 μg/mL) for 0, 1, 3, 5, 7, 9 and 11 hrs. Total RNA was extracted and processed as previously described 54. MIF-specific mRNA signals were quantified using an Instant Imager 2024 (Packard, Meriden, CT) and reported relative to ribosomal RNA level.

Results

MIF Expression by Human CEM-C7 and CEM-C1 T Cells

Glucocorticoids in low physiologic concentrations induce the release of MIF from monocytes/macrophages14 and other cell types 55;61, and prior studies have shown an increase in MIF expression in primary T cells and in T cell lines after activation with PHA or anti-CD3 4. To address whether glucocorticoids also induce MIF expression in T lymphocytes, we studied the two human T cell lines, CEM-C1 and CEM-C7, which differ in their sensitivity to glucocorticoids. The CEM-C1 and CEM-C7 cell lines are closely related genetically as they were cloned from the same individual, but they differ in their responsiveness to glucocorticoids downstream of the action of the glucocorticoid receptor (GR) 58. The glucocorticoid-sensitive, CEM-C7 T cell clone undergoes glucocorticoid-dependent apoptosis while the glucocorticoid-resistant, CEM-C1 T cell clone tolerates concentrations of up to 10^{-5}M of the synthetic glucocorticoid, dexamethasone.

The CEM-C7 and CEM-C1 T cells were incubated with dexamethasone in the concentration range of 10^{-16}–10^{-8}M and MIF production assessed by ELISA analysis of the conditioned medium. As shown in Figure 1, the glucocorticoid-sensitive CEM-C7 T cells but not the glucocorticoid-resistant CEM-C1 T cells responded to dexamethasone by releasing MIF in a time-dependent fashion, and increased levels were evident by 2 hrs. MIF release follows a bell-shaped response curve with respect to the stimulating glucocorticoid concentration, with MIF detectable at 10^{-8} M (at 4 hrs), peaking at 10^{-12} M (at 2 hrs), and then decreasing. These results agree with data reported in murine monocytes/macrophages, which showed both a similar threshold and bell-shaped response profile for MIF release when assessed by Western blotting 14.

The rapid release of MIF from CEM-C7 T cells suggested the possibility that MIF was released from cytoplasmic pools, which exist in several cell types 3;7;15, including T cells, where intracellular concentrations of 170 fg/cell have been documented in the mouse LBRM T-cell line 4. Glucocorticoids induce T cell apoptosis 50, which could lead to MIF release; however, we considered apoptosis to be an unlikely mechanism for MIF appearance in supernatants given the low concentrations of dexamethasone employed and the longer time course (>20 hrs) required for apoptosis in CEM-C7 cells 58. We detected low levels of apoptosis (<12%) under our experimental conditions, which did not correspond to the observed, glucocorticoid-dependent increases in MIF release (Fig. 1B). ELISA measurement revealed an intracellular MIF content of ~300 fg/cell in unstimulated CEM-C7 T cells. By calculation therfore, the glucocorticoid-induced release of MIF from pre-formed intracellular pools may account for ≥85% of the MIF detectable in supernatants, assuming the absence of *de novo* protein translation.

Since MIF is known to protect from activation-induced apoptosis in macrophages 40 and to promote the survival of B lymphocytes 26, we further assessed whether the neutralization of secreted MIF influenced baseline apoptotic responses (Fig. 1C). The addition of a neutralizing anti-MIF monoclonal antibody did not affect the viability of the glucocorticoid-sensitive, CEM-C7 T cell line after 20 hrs of culture, suggesting that endogenous MIF release conferred no appreciable effect on cell survival.

We next examined whether the T cell release of MIF was accompanied by a change in steady state MIF mRNA levels. Using quantitative PCR, and as control, the transcript for long-lived 18S ribosomal RNA, glucocorticoid stimulation was found to result in a significant, dose-dependent increase in the level of MIF mRNA in the sensitive CEM-C7 T cells but not in the resistant CEM-C1 T cells (Fig. 2). The highest levels of MIF mRNA were observed at 2 hrs and at the same dexamethasone concentration (10^{-12} M) that corresponded with the highest levels of MIF protein production (Fig. 1A). These data are consistent with the interpretation that glucocorticoids at low doses lead to a specific induction of both MIF mRNA and protein expression in the human CEM-C7 T cell line. We also measured the intracytoplasmic content of MIF in dexamethasone-stimulated T cells as a function of time. While the intracellular content of MIF of CEM-C7 T cells appeared to fall by 22.4% at 2 hrs, this decrease was not statistically significant when compared to baseline (0 hrs) or to the MIF content of CEM-C1 T cells (CEM-C7- 0 hrs: 4.9+0.97 μgMIF/g cellular protein vs. 2 hrs: 3.8+0.39 μgMIF/g cellular protein, P=NS). We conclude that any loss of MIF from intracellular stores is likely counterbalanced by repletion from newly translated protein.

Glucocorticoid Regulation of MIF Transcription

To begin to investigate regions within the *MIF* promoter that may be responsible for glucocorticoid-inducible expression, we examined the promoter sequence for potential transcription factor binding sites that might be regulated by glucocorticoids. There is a consensus glucocorticoid responsive element (GRE) 837 nucleotides upstream of the *MIF* transcription start site that may interact with the glucocorticoid receptor (GR) DNA binding domain, and an activating transcription factor/cAMP-response element (ATF/CRE) at position −109 (Fig. 3A). This later site is influenced by the ATF/CREB family of transcription factors, whose activity may be regulated by the direct binding of the glucocorticoid receptor to c-Jun complexes 30 or by glucocorticoid suppression of JNK/SAPK, which phosphorylates c-Jun and ATF-2 57. We prepared a transfectable reporter plasmid in which the 1177 bp upstream segment of the *MIF* promoter was ligated 5' to the coding region of the luciferase gene. Luciferase reporter plasmids employing this region of the *MIF* promoter have been shown to respond to different transcriptional activators 5;62, and these studies have revealed potential cell-specific effects 2. A MIF-reporter plasmid (*MIF-luciferase*) was transfected into several different human T cell lines to first assess the responsiveness of this construct to the standard T cell activating stimulus, PMA/ionomycin. Among the different T cell lines tested (Jurkat, SupT, CEM-C7), the CEM-C7 cell line showed the highest level of induction of luciferase expression (>5-fold over control, *data not shown*). Glucocorticoid addition also induced the expression of luciferase by the *MIF* promoter in this transfectable, reporter system (Fig. 3B). Expression levels peaked at 55% over unstimulated controls at a dexamethasone concentration of 10^{-8} M. The concentration of dexamethasone that induced optimal expression of the *MIF-luciferase* plasmid was higher than that observed for the activation of the endogenous MIF gene. This discrepancy likely reflects the requirement of additional promoter or enhancer elements that are not present in the promoter construct. The glucocorticoid-induced expression of luciferase nevertheless followed a bell-shaped dose-response curve, with expression decreasing at high glucocorticoid concentrations ($>10^{-8}$ M). These data support the interpretation that transcription through the *MIF* promoter is a control point in the regulation of *MIF* expression by T cells and possibly other cell types 55;61.

To confirm a role for the GR in the transcriptional activation of the *MIF* promoter, we also performed co-transfection experiments utilizing the *MIF-luciferase* plasmid and a human GR expression plasmid (GR). CEM-C7 T cells co-transfected with an equivalent amount of *MIF-luciferase* and GR expression plasmid showed a >25% higher induction of luciferase expression when compared to cells transfected with *MIF-luciferase* alone (Fig. 3C). This enhancement in *MIF-luciferase* expression increased significantly when the ratio of the GR expression plasmid to the MIF promoter/luciferase plasmid was increased to ≥10:1.

Role of the GRE and ATF/CRE Sites in the Transcriptional Regulation of the MIF promoter

We synthesized two additional *MIF-luciferase* reporter plasmids in which the consensus sequences for the 8 bp ATF/CRE (TGGCGTCA) or the 11 bp GRE (AGATGGTCCCC) binding sites were selectively deleted (Fig. 4A). Human CEM-C7 T cells transfected with the reporter construct bearing the ATF-site deletion (*MIF-ΔATF/CRE luciferase*) showed a 15% reduction in baseline gene expression and a complete loss of glucocorticoid inducible expression (Fig. 4B). The reporter construct with the GRE-site deletion (*MIF-ΔGRE luciferase*) also showed a 10–15% reduction in baseline expression, and a marked suppression of glucocorticoid inducibility. While a low level of inducible expression may be detected at dexamethasone concentrations of $>10^{-6}$ M (versus 10^{-4} M), this effect was not statistically significant. These data indicate that the putative ATF/CRE and GRE sites play a role in glucocorticoid-regulated *MIF* expression in CEM-C7 T cells.

Using electrophoretic mobility shift assays (EMSA), we observed that the DNA-binding activity to the ATF/CRE site increased upon dexamethasone treatment of CEM-C7 T cells (Figs. 4C, D). This finding is in agreement with the observation that dexamethasone increased *MIF* promoter activity in an ATF/CRE site-dependent fashion (Fig. 4B). The GRE site did not show modulation of DNA binding activity under these experimental conditions, however this result does not preclude possible effects on the endogenous *MIF* promoter at the level of chromatin organization 11.

Glucocorticoids also influence gene expression by regulating the degradation of mRNA 50. One well-characterized mechanism is by the glucocorticoid regulation of RNA binding proteins that interact with AU-rich elements that are present in the 3' UTR of many cytokine transcripts 17;28. MIF lacks such an element 38;47; nevertheless, we assessed the stability of MIF mRNA under glucocorticoid induction by measuring mRNA levels after addition of the RNA polymerase inhibitor, actinomycin D. As shown in Fig. 5, dexamethasone did not influence the turnover of MIF mRNA in T cells under these experimental conditions.

Discussion

The cloning of murine MIF from corticotrophic pituitary cells9 together with the observation that the circulating level of this cytokine follows a circadian rhythm49 that is influenced by physiologic stressors14;23 supports the concept that MIF has a close regulatory relationship with glucocorticoids24. Several of MIF's pro-inflammatory actions have been attributed to its ability to counter-regulate the immunosuppressive action of glucocorticoids; among these regulatory mechanisms are sustained ERK1/2 MAPK activation and inhibition of cytoplasmic phospholipase A241, prevention of glucocorticoid induction of the NF-κB inhibitor, IκBα18, and suppression of the glucocorticoid-induced expression of MAPK phosphatase (MKP-1) 52;60. Mice genetically-deficient in MIF also have reduced glucocorticoid levels, which may be due to the development of these mice in the absence of a physiologic, glucocorticoid counter-regulator (*i.e.* MIF) 31.

While glucocorticoids normally suppress the expression of pro-inflammatory cytokines51, high levels of circulating glucocorticoids have been found to induce MIF expression in the

thymus and the spleen, which are rich in T cells23. *In vitro* studies have shown that several cell types, including monocytes/macrophages, secrete MIF in response to low levels of glucocorticoids14;55;61. MIF secretion followed a bell-shaped dose-response curve but is suppressed at high, anti-inflammatory concentrations of glucocorticoids ($>10^{-6}$M) 14. The regulated production of MIF at low physiologic levels of glucocorticoids has suggested the possibility that these steroids exert an important influence on baseline MIF expression, at least in certain cell types 14;23.

In the present report, we examined MIF production from human CEM T cells, which exist in two closely related variant clones that are distinguished by glucocorticoid sensitivity (CEM-C7) and resistance (CEM-C1) 58. Both CEM-C7 and CEM-C1 T cells produce similar concentrations of the glucocorticoid receptor and show a similar ability to metabolize dexamethasone. The two cell lines also respond to glucocorticoids by inducing glutamine synthethase, however CEM-C1 T cells are resistant to dexamethasone-induced apoptosis. It has been concluded that this cell line manifests a block in a distinctive set of glucocorticoid-responsive genes, and recent work has revealed specific changes in gene families related to proliferation, differentiation, and apoptosis 58. We observed that glucocorticoids in low concentrations ($<10^{-8}$M) induced MIF secretion as quickly as 2 hours from the sensitive (CEM-C7) but not the resistant (CEM-C1) T cells. The pattern of MIF secretion also was reminiscent of the bell-shaped dose-response reported previously for the release of MIF from human or mouse monocytes/macrophages 14. Of importance, the release of MIF by CEM-C7 T cells was not accompanied by apoptosis, which could lead to the release of MIF from pre-formed, intracellular pools 15. These observations suggest a specialized, glucocorticoid-dependent pathway for MIF release from CEM-C7 T cells.

Further investigations showed that the stimulation of CEM-C7 T cells by glucocorticoids is associated with a MIF transcriptional response that peaks at 2 hours. Glucocorticoid induction of MIF expression also was evident by plasmid reporter analysis, with highest expression observed at a concentration 10^{-8}M dexamethasone. A role for the glucocorticoid receptor in this transcriptional response was confirmed by co-transfection of a GR expression plasmid, which enhanced *MIF* promoter activity in a dose-dependent fashion. We further examined the role of two potential glucocorticoid-regulatory sites in the transcriptional activation of the *MIF* promoter, a consensus GRE site at −837 and an ATF/CRE site at −109. Each site was deleted and the resulting *MIF-luciferase* reporter constructs re-studied for MIF expression. Removal of either the GRE or the ATF/CRE site resulted in a 10–15% reduction in baseline *MIF* transcriptional activity and a loss of glucocorticoid inducibility, with the ATF/CRE site possibly contributing more to glucocorticoid responsiveness than the GRE site. We also observed that the DNA-binding activity to the *MIF* ATF/CRE site, but not the GRE site, increased significantly upon dexamethasone treatment of T cells. Although the GRE site did not show increased DNA binding activity, we cannot rule out its possible involvement by epigenetic mechanisms or changes in chromatin organization 11. We also did not observe a significant affect of glucocorticoids on the stability of MIF mRNA transcripts.

These data provide first insight into the influence of glucocorticoids on *MIF* transcription, and they add to the emerging characterization of the *MIF* promoter. To date, the only response elements that have been functionally evaluated are a cAMP response element that mediates constitutive 53 and corticotrophin releasing factor-induced *MIF* transcription in murine pituitary cells45;62, and a hypoxia response element that mediates transcriptional activation by HIF-16.

These data, which were obtained in a well-characterized, glucocorticoid-sensitive human T cell line, support prior observations that have shown a role for glucocorticoids in the production of MIF protein from different tissues and cell lines 14;23;55;61. The present findings, as well

as prior reports, are remarkable in that the release of MIF is observed at concentrations of glucocorticoids that are at the very low end of glucocorticoid receptor occupancy. This level of sensitivity may be due in part to the glucocorticoid-free culture conditions employed prior to stimulation, and it supports an important role for the glucocorticoid receptor in cellular MIF production. The reporter studies also indicate that *MIF* transcription is enhanced by increasing the intracellular content of the glucocorticoid receptor; nevertheless, our data do not rule out a possible contribution of non-genomic mechanisms for MIF release 50.

MIF is expressed constitutively in many cell types 23. *In vivo*, high levels of glucocorticoids increase both circulating MIF concentrations and the MIF content of tissues such as the spleen and thymus 14;22;49. While prior studies have attributed changes in the MIF content of different organs to post-transcriptional regulatory mechanisms, the present observations that MIF release is accompanied by transcriptional activation is noteworthy and point to a role for glucocorticoids in both constitutive and inducible MIF expression in lymphoid tissue. Our data thus support two potential mechanisms for the glucocorticoid induced release of MIF. First, dexamethasone exerts a direct and rapid effect on MIF release from pre-formed, cellular pools. It is noteworthy that MIF, which lacks an N-terminal leader sequence 10, is released by a specialized, non-conventional pathway that is mediated by the Golgi-associated protein, p115 36. Activated glucocorticoid receptor complexes thus may initiate MIF secretion by interaction with components of this pathway. Second, glucocorticoids induce mRNA transcription over a slightly longer time course by an indirect effect on the GRE.

An increasing literature supports the value of cytokine blockade in different inflammatory and immunologic diseases21. In the case of MIF, which acts in large measure to counter-regulate the immunosuppressive properties of glucocorticoids 13;24, it has been hypothesized that pharmacologic inhibition of MIF may be especially beneficial in those inflammatory conditions, such as asthma or rheumatoid arthritis, that become refractory to steroid treatment 35;44. Given the observation that MIF expression is itself induced by glucocorticoids14;23 it is noteworthy that a clinical study in the autoimmune disease systemic lupus erythematosus has reported a positive correlation between circulating MIF levels and steroid dosage 25. Thus, the identification of glucocorticoid congeners that retain immunosuppressive but not MIF stimulatory activity may offer a novel pharmacologic approach for the more efficacious treatment of inflammatory diseases.

Abbreviations

ATF/CRE, activating transcription factor/cAMP-response element; EMSA, electrophoretic mobility shift assay; ERK1/2, extracellular-signal-regulated kinase; GRE, glucocorticoid responsive element; MAPK, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; *MIF*, human gene for MIF; PHA, phytohemagluttinin; PMA, phorbol myristate acetate..

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References

1. Aeberli D, Yang Y, Mansell A, Santos L, Leech M, Morand EF. Endogenous macrophage migration inhibitory factor modulates glucocorticoid sensitivity in macrophages via effects on MAP kinase phosphatase-1 and p38 MAP kinase. FEBS Letters 2006;580:974–981. [PubMed: 16442105]

2. Alourfi Z, Donn RP, Stevens A, Berry A, McMaster A, Ray DW. Glucocorticoids suppress macrophage migration inhibitory factor (MIF) expression in a cell-type-specific manner. Journal of Molecular Endocrinology 2005;34:583–595. [PubMed: 15821118]

3. Bacher M, Meinhardt A, Lan HY, Mu W, Metz CN, Chesney JA, Calandra T, Gemsa D, Donnelly T, Atkins RC, Bucala R. Migration inhibitory factor expression in experimentally induced endotoxemia. Am J Pathol 1997;150:235–246. [PubMed: 9006339]

4. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, Gemsa D, Donnelly T, Bucala R. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proc Natl Acad Sci USA 1996;93:7849–7854. [PubMed: 8755565]

5. Baugh JA, Chitnis S, Donnelly SC, Monteiro J, Lin X, Plant BJ, Wolfe F, Gregersen PK, Bucala R. A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. Genes Immun 2002;3:170–176. [PubMed: 12070782]

6. Baugh JA, Gantier M, Li L, Byrne A, Buckley A, Donnelly SC. Dual regulation of macrophage migration inhibitory factor (MIF) expression in hypoxia by CREB and HIF-1. Biochemical and Biophysical Research Communications 2006;347:895–903. [PubMed: 16854377]

7. Benigni F, Atsumi T, Calandra T, Metz C, Echtenacher B, Peng T, Bucala R. The proinflammatory mediator macrophage migration inhibitory factor (MIF) induces glucose catabolism in muscle. J Clin Invest 2000;106:1291–1300. [PubMed: 11086030]

8. Bernhagen J, Bacher M, Calandra T, Metz CN, Doty SB, Donnelly T, Bucala R. An essential role for macrophage migration inhibitory factor in the tuberculin delayed-type hypersensitivity reaction. J Exp Med 1996;183:277–282. [PubMed: 8551232]

9. Bernhagen J, Calandra T, Mitchell RA, Martin SB, Tracey KJ, Voelter W, Manogue KR, Cerami A, Bucala R. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature 1993;365:756–759. [PubMed: 8413654]

10. Bernhagen J, Mitchell RA, Calandra T, Voelter W, Cerami A, Bucala R. Purification, bioactivity, and secondary structure analysis of mouse and human macrophage migration inhibitory factor (MIF). Biochemistry 1994;33:14144–14155. [PubMed: 7947826]

11. Bowen H, Kelly A, Lee T, Lavender P. Control of cytokine gene transcription in Th1 and Th2 cells. Clinical and Experimental Allergy 2008;38:1422–1431. [PubMed: 18647314]

12. Bucala R, Lolis E. MIF: A Critical Component of Autoimmune Inflammatory Diseases. Drug News Perspect 2005;18:417–426. [PubMed: 16362080]

13. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol 2003;3:791–800. [PubMed: 14502271]

14. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, Cerami A, Bucala R. MIF as a glucocorticoid-induced modulator of cytokine production. Nature 1995;377:68–71. [PubMed: 7659164]

15. Calandra T, Bernhagen J, Mitchell RA, Bucala R. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J Exp Med 1994;179:1895–1902. [PubMed: 8195715]

16. Calandra T, Echtenacher B, Le Roy D, Pugin J, Metz CN, Hültner L, Heumann DMD, Bucala R, Glauser MP. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nature Medicine 2000;6:164–169.

17. Chesney J, Mitchell R, Benigni F, Bacher M, Spiegel L, Al-Abed Y, Han JH, Metz C, Bucala R. An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: Role in tumor cell glycolysis and the Warburg effect. Proc Natl Acad Sci USA 1999;96:3047–3052. [PubMed: 10077634]

18. Daun JM, Cannon JG. Macrophage migration inhibitory factor antagonizes hydrocortisone-induced increases in cytosolic IκBα. Am J Physiol 2000;279:R1043–R1049.

19. David J. Delayed hypersensitivity *in vitro*: Its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc.Natl.Acad.Sci.USA 1966;56:72–77. [PubMed: 5229858]

20. de Jong YP, Abadia-Molina AC, Satoskar AR, Clarke K, Rietdijk ST, Faubion WA, Mizoguchi E, Metz CN, Sahli MA, ten Hove T, Keates AC, Lubetsky JB, Farrell RJ, Michetti P, van Deventer SJ,

Lolis E, David JR, Bhan AK, Terhorst C. Development of chronic colitis is dependent on the cytokine MIF. Nat.Immunol 2001;2:1061–1066. [PubMed: 11668338]

21. Feldmann M. Many cytokines are very useful therapeutic targets in disease. J.Clin.Invest 2008;118:3533–3536. [PubMed: 18982159]
22. Fingerle-Rowson G, Angstwurm M, Andreesen R, Ziegler-Heitbrock HW. Selective depletion of CD14+ CD16+ monocytes by glucocorticoid therapy. Clin Exp Immunol 1998;112:501–506. [PubMed: 9649222]
23. Fingerle-Rowson G, Koch P, Bikoff R, Lin X, Metz CN, Dhabhar FS, Meinhardt A, Bucala R. Regulation of macrophage migration inhibitory factor expression by glucocorticoids *in vivo*. Am J Pathol 2003;162:47–56. [PubMed: 12507889]
24. Flaster H, Bernhagen J, Calandra T, Bucala R. The macrophage migration inhibitory factor-glucocorticoid dyad: Regulation of inflammation and immunity. Molecular Endocrinology 2007;21:1267–1280. [PubMed: 17389748]
25. Foote A, Briganti EM, Kipen Y, Santos L, Leech M, Morand EF. Macrophage migration inhibitory factor in Systemic Lupus Erythematosus. J Rheumatol 2004;31:268–273. [PubMed: 14760795]
26. Gore Y, Starlets D, Maharshak N, Becker-Herman S, Kaneyuki U, Leng L, Bucala R, Shachar I. Macrophage migration inhibitory factor (MIF) induces B cell survival by activation of a CD74/CD44 receptor complex. J Biol Chem 2008;283:2784–2792. [PubMed: 18056708]
27. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM. Primary Structure and Expression of A Functional Human Glucocorticoid Receptor Cdna. Nature 1985;318:635–641. [PubMed: 2867473]
28. Ishmael FT, Fang X, Galdiero MR, Atasoy U, Rigby WFC, Gorospe M, Cheadle C, Stellato C. Role of the RNA-binding protein tristetraprolin in glucocorticoid-mediated gene regulation. Journal of Immunology 2008;180:8342–8353.
29. Isidori AM, Kaltsas GA, Korbonits M, Pyle M, Gueorguiev M, Meinhardt A, Metz C, Petrovsky N, Popovic V, Bucala R, Grossman AB. Response of serum macrophage migration inhibitory factor levels to stimulation or suppression of the hypothalamo-pituitary-adrenal axis in normal subjects and patients with Cushing's disease. J.Clin. Endocrinol. Metab 2002;87:1834–1840. [PubMed: 11932327]
30. Joyce DA, Gimblett G, Steer JH. Targets of glucocorticoid action on TNF-alpha release by macrophages. Inflamm.Res 2001;50:337–340. [PubMed: 11506387]
31. Kevill KA, Bhandari V, Kettunen M, Leng L, Fan J, Mizue Y, Dzuira JD, Reyes-Mugica M, McDonald CL, Baugh JA, O'Connor CL, Aghai ZH, Donnelly SC, Bazzy-Asaad A, Bucala RJ. A role for macrophage migration inhibitory factor in the neonatal respiratory distress syndrome. J Immunol 2008;180:601–608. [PubMed: 18097062]
32. Kleemann R, Hausser A, Geiger G, Mischke R, Burger-Kentischer A, Flieger O, Johannes FJ, Roger T, Calandra T, Kapurniotu A, Grell M, Finkelmeier D, Brunner H, Bernhagen J. Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. Nature 2000;408:211–216. [PubMed: 11089976]
33. Krockenberge MWC, Ossadnick M, Honig A, Hausler S, Voigt H, Becker JC, Leng L, Steinle A, Weller M, Bucala R, Dietl J, Wischhusen J. Macrophage Migration Inhibitory Factor contributes to the immune escape of ovarian cancer by down-regulating NKG2D. J Immun 2008;180:7339–7348.
34. Leech M, Metz C, Santos L, Peng T, Holdsworth SR, Bucala R, Morand EF. Involvement of macrophage migration inhibitory factor in the evolution of rat adjuvant arthritis. Arthritis & Rheum 1998;41:910–917. [PubMed: 9588744]
35. Lolis E, Bucala R. Therapeutic approaches to innate immunity. Nature Rev Drug Discovery 2003;2:635–645.
36. Merk M, Baugh J, Zierow S, Leng L, Pal U, Lee SJ, Ebert AD, Mizue Y, Trent JO, Mitchell R, Nickel W, Kavathas PB, Bernhagen J, Bucala R. The Golgi-Associated Protein p115 Mediates the Secretion of Macrophage Migration Inhibitory Factor. J Immunol 2009;182(in press)
37. Mikulowska A, Metz CN, Bucala R, Holmdahl R. Macrophage migration inhibitory factor is involved in the pathogenesis of collagen type II-induced arthritis in mice. J Immunol 1997;158:5514–5517. [PubMed: 9164975]

38. Mitchell R, Bacher M, Bernhagen J, Pushkarskaya T, Seldin MF, Bucala R. Cloning and characterization of the gene for mouse macrophage migration inhibitory factor (MIF). Journal of Immunology 1995;154:3863–3870.
39. Mitchell RA, Bucala R. Tumor growth-promoting properties of macrophage migration inhibitory factor (MIF). Seminars in Cancer Biology 2000;10:359–366. [PubMed: 11100884]
40. Mitchell RA, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, Bucala R. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. Proc Natl Acad Sci USA 2002;99:345–350. [PubMed: 11756671]
41. Mitchell RA, Metz CN, Peng T, Bucala R. Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF). Regulatory role in cell proliferation and glucocorticoid action. J Biol Chem 1999;274:18100–18106. [PubMed: 10364264]
42. Mizue Y, Ghani S, Leng L, McDonald C, Kong P, Baugh J, Lane SJ, Craft J, Nishihira J, Donnelly SC, Zhu Z, Bucala R. Role for Macrophage Migration Inhibitory Factor (MIF) in Asthma. Proc Natl Acad Sci USA 2005;102:14410–14415. [PubMed: 16186482]
43. Morand EF. New therapeutic target in inflammatory disease: macrophage migration inhibitory factor. Internal Medicine Journal 2005;35:419–426. [PubMed: 15958113]
44. Morand EF, Leech M, Bernhagen J. MIF: a new cytokine link between rheumatoid arthritis and atherosclerosis. Nature Reviews Drug Discovery 2006;5:399–410.
45. Nishino T, Bernhagen J, Shiiki H, Calandra T, Dohi K, Bucala R. Localization of macrophage migration inhibitory factor (MIF) to secretory granules within the corticotrophic and thyrotrophic cells of the pituitary gland. Molecular Medicine 1995;1:781–788. [PubMed: 8612200]
46. Norman MR, Thompson EB. Characterization of A Glucocorticoid-Sensitive Human Lymphoid-Cell Line. Cancer Research 1977;37:3785–3791. [PubMed: 269011]
47. Paralkar V, Wistow G. Cloning the human gene for macrophage migration inhibitory factor (MIF). Genomics 1994;19:48–51. [PubMed: 8188240]
48. Petrovsky N, McNair P, Harrison LC. Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. Cytokine 1998;10:307–312. [PubMed: 9617577]
49. Petrovsky N, Socha L, Silva D, Grossman AB, Metz C, Bucala R. Macrophage migration inhibitory factor (MIF) exhibits a pronounced circadian rhythm relevant to its role as a glucocorticoid counter-regulator. Immunol Cell Biol 2003;81:137–143. [PubMed: 12631237]
50. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids - New mechanisms for old drugs. New England Journal of Medicine 2005;353:1711–1723. [PubMed: 16236742]
51. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids - New mechanisms for old drugs. New England Journal of Medicine 2005;353:1711–1723. [PubMed: 16236742]
52. Roger T, Chanson AL, Knaup-Reymond M, Calandra T. Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1. Eur J Immunol 2005;35:3405–3413. [PubMed: 16224818]
53. Roger T, Ding X, Chanson AL, Renner P, Calandra T. Regulation of constitutive and microbial pathogen-induced human macrophage migration inhibitory factor (MIF) gene expression. Eur J Immunol 2007;37:3509–3521. [PubMed: 18034423]
54. Roger T, David J, Glauser MP, Calandra T. MIF regulates innate immune responses through modulation of Toll-like receptor 4. Nature 2001;414:920–924. [PubMed: 11780066]
55. Santos L, Hall P, Metz C, Bucala R, Morand EF. Role of macrophage migration inhibitory factor (MIF) in murine antigen-induced arthritis: interaction with glucocorticoids. Clinical and Experimental Immunology 2001;123:309–314. [PubMed: 11207663]
56. Shi X, Leng L, Wang T, Wang W, Du X, McDonald C, Chen Z, Murphy JW, Lolis E, Noble P, Knudson W, Bucala R. CD44 is the Signaling Component of the Macrophage Migration Inhibitory Factor-CD74 Receptor Complex. Immunity 2006;25:595–606. [PubMed: 17045821]
57. Swantek JL, Cobb MH, Geppert TD. Jun N-terminal kinase/stress activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor alpha (TNF-α) translation: glucocorticoids inhibit TNF-αtranslation by blocking JNK/SAPK. Mol Cell Biol 1997;17:6274–6282. [PubMed: 9343388]

58. Thompson EB, Johnson BH. Regulation of a distinctive set of genes in glucocorticoid-evoked apoptosis in CEM human lymphoid cells. Recent Progress in Hormone Research 2003;Vol 58:175–197. [PubMed: 12795419]58

59. Tierney T, Patel R, Stead CAS, Leng L, Bucala R, Buckingham JC. Macrophage migration inhibitory factor is released from pituitary folliculo-stellate-like cells by endotoxin and dexamethasone and attenuates the steroid-induced inhibition of interleukin 6 release. Endocrinology 2005;146:35–43. [PubMed: 15388650]

60. Toh ML, Yang Y, Leech M, Santos L, Morand EF. Expression of mitogen-activated protein kinase phosphatase 1, a negative regulator of the mitogen-activated protein kinases, in rheumatoid arthritis - Up-regulation by interleukin-1 beta and glucocorticoids. Arthritis and Rheumatism 2004;50:3118–3128. [PubMed: 15476200]

61. Vedder H, Krieg JC, Gerlach B, Gemsa D, Bacher M. Expression and glucocorticoid regulation of macrophage migration inhibitory factor (MIF) in hippocampal and neocortical rat brain cells in culture. Brain Research 2000;869:25–30. [PubMed: 10865055]

62. Waeber G, Thompson N, Chautard T, Steinmann M, Nicod P, Pralong FP, Calandra T, Gaillard RC. Transcriptional activation of the macrophage migration-inhibitory factor gene by the corticotropin-releasing factor is mediated by the cyclic adenosine 3',5'-monophosphate responsive element-binding protein CREB in pituitary cells. Molecular Endocrinology 1998;12:698–705. [PubMed: 9605932]

63. Zawydiwski R, Harmon JM, Thompson EB. Glucocorticoid-Resistant Human Acute Lymphoblastic Leukemic-Cell Line with Functional Receptor. Cancer Research 1983;43:3865–3873. [PubMed: 6134583]

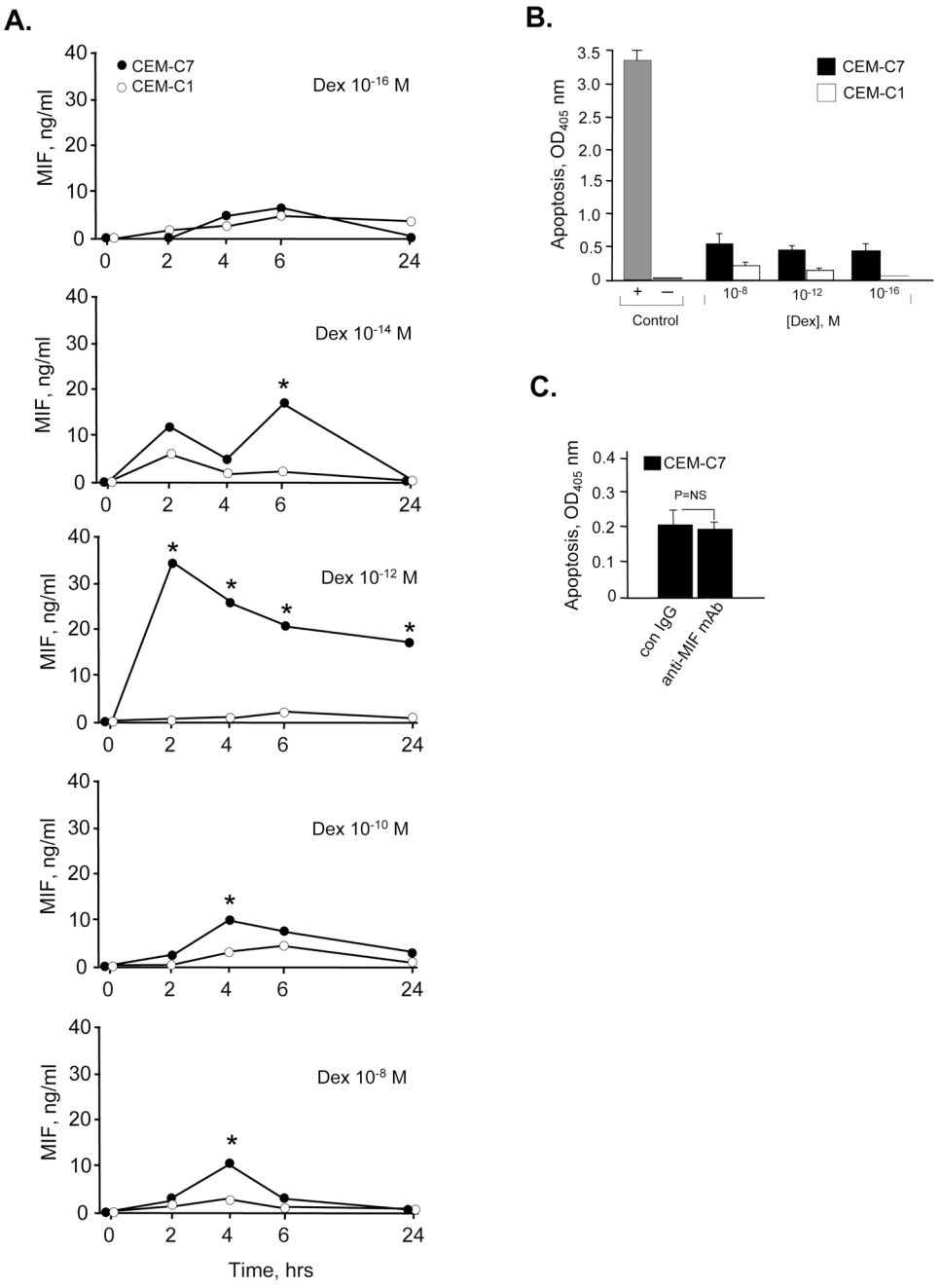

FIGURE 1.

A) Dexamethasone induces MIF release from glucocorticoid-sensitive CEM-C7 but not glucocorticoid-resistant CEM-C1 cells. The two T cell lines (1×10^5 cells/ml) were cultured in 48 well plates in RPMI and 1% heat-inactivated, glucocorticoid-depleted FBS, and dexamethasone added at the indicated concentrations. Supernatants were removed at intervals for analysis by MIF ELISA. Assays were performed in triplicate, and the results shown are representative of three experiments.

B) Low concentrations of dexamethasone show negligible effects on CEM-C7 or CEM-C1 T cell apoptosis. T cells were collected 24 hrs after stimulation with dexamethasone and apoptosis quantified by ELISA as described in the *Methods*. The positive control is the ELISA

value of a corresponding CEM-C7 T cell culture after apoptosis induction. Values shown are the mean±SD of triplicate measurements. *p<0.05.

C) Immunoneutralization of MIF does not influence baseline apoptosis. CEM-C7 T cells (1×10^5/ml) were stimulated with $1x10^{-12}$ M dexamethasone in the presence of a neutralizing anti-MIF mAb or a control IgG1 (both added at 100 μg/ml). Apoptosis was quantified by ELISA, and data are from replicate experiments,

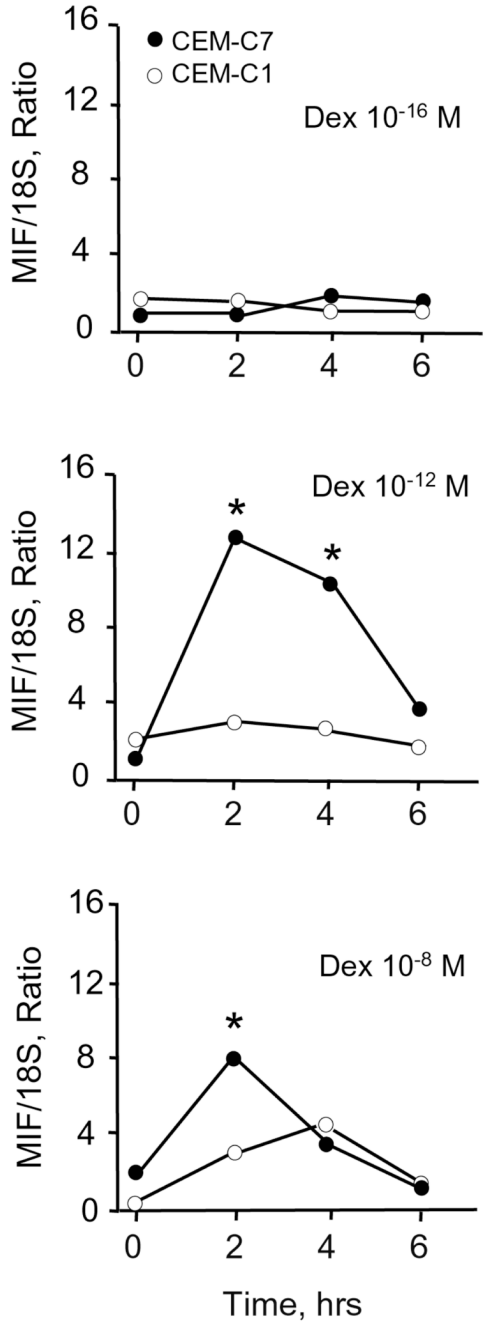

FIGURE 2.

Dexamethasone induces MIF mRNA expression from glucocorticoid-sensitive CEMC-7 but not glucocorticoid-resistant CEM-C1 cells. T cells were cultured and stimulated as in Fig. 1, and the mRNA for MIF isolated at intervals and analyzed by quantitative PCR. The RNA levels for MIF are expressed relative to 18S ribosomal RNA for the same sample as described in the *Methods*. Values are the mean of triplicate determinations, with *p<0.05.

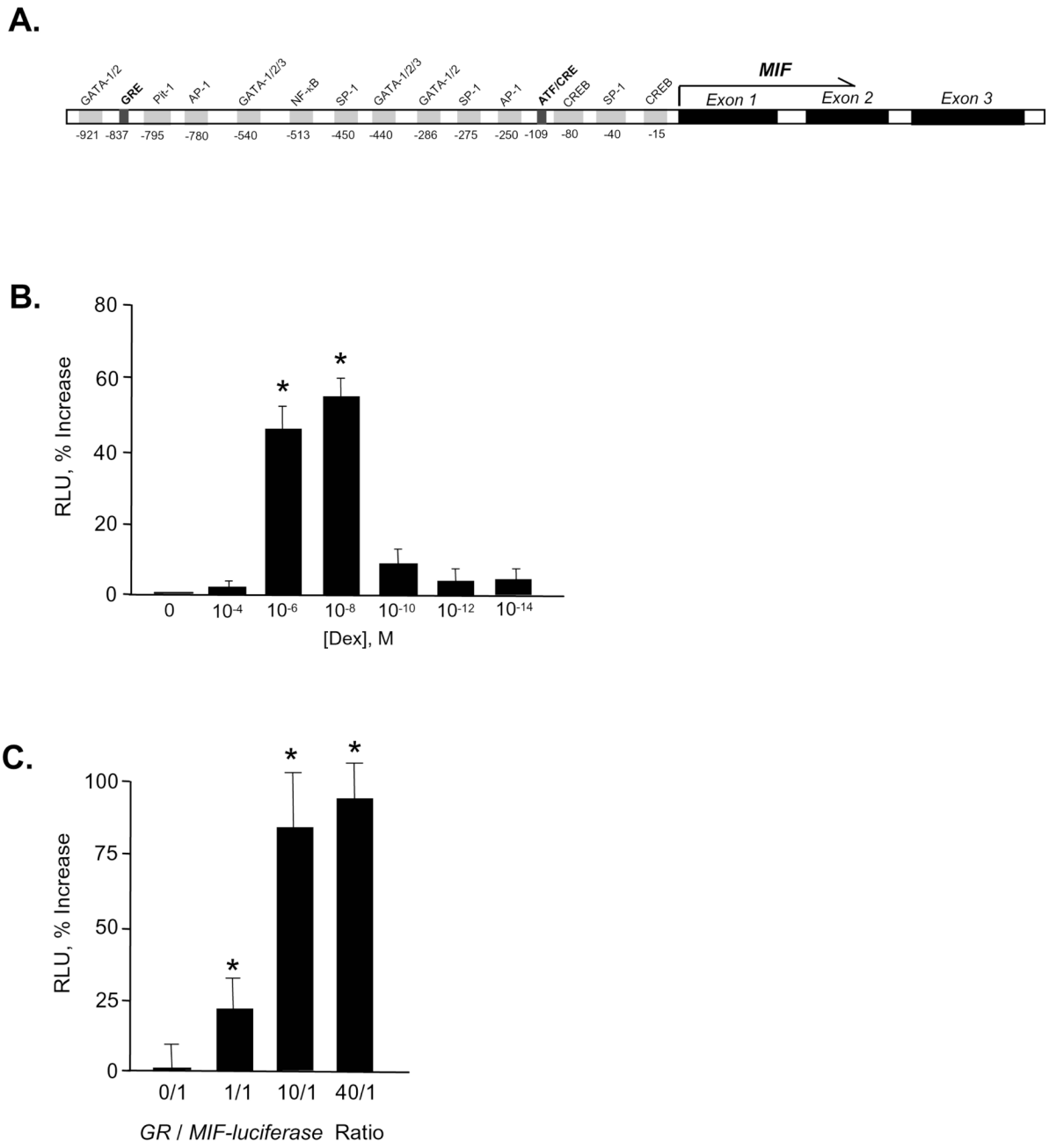

FIGURE 3.

A) Schematic diagram of the human *MIF* gene (22q11.2). The upper diagram shows the three exons, the promoter region, and putative transcription factor binding sites, including the positions of the GRE and ATF/CRE sites of interest 5.

B) Dexamethasone induces *MIF* promoter activity in CEM-C7 T cells. Cultured T cells (2×10^6 cells/well) were transfected with the *MIF-luciferase* reporter plasmid and stimulated with dexamethasone 16 hrs later. Luciferase activity was measured at 12 hrs as described in the *Methods* and expressed relative to cellular protein concentration. Data shown are the Mean ±SD from three experiments. *$p<0.05$.

C) The glucocorticoid receptor enhances the transcriptional activation of the *MIF* Promoter. Cultured CEM-C7 T cells were transfected with the *MIF-luciferase* reporter plasmid together with increasing amounts of a GR expression plasmid (GR). Luciferase expression was measured 12 hrs after the addition of 10^{-8} M dexamethasone. The values shown are expressed for the ratio of GR / *MIF-luciferase* plasmid and show the mean of triplicate experiments. *$p<0.05$.

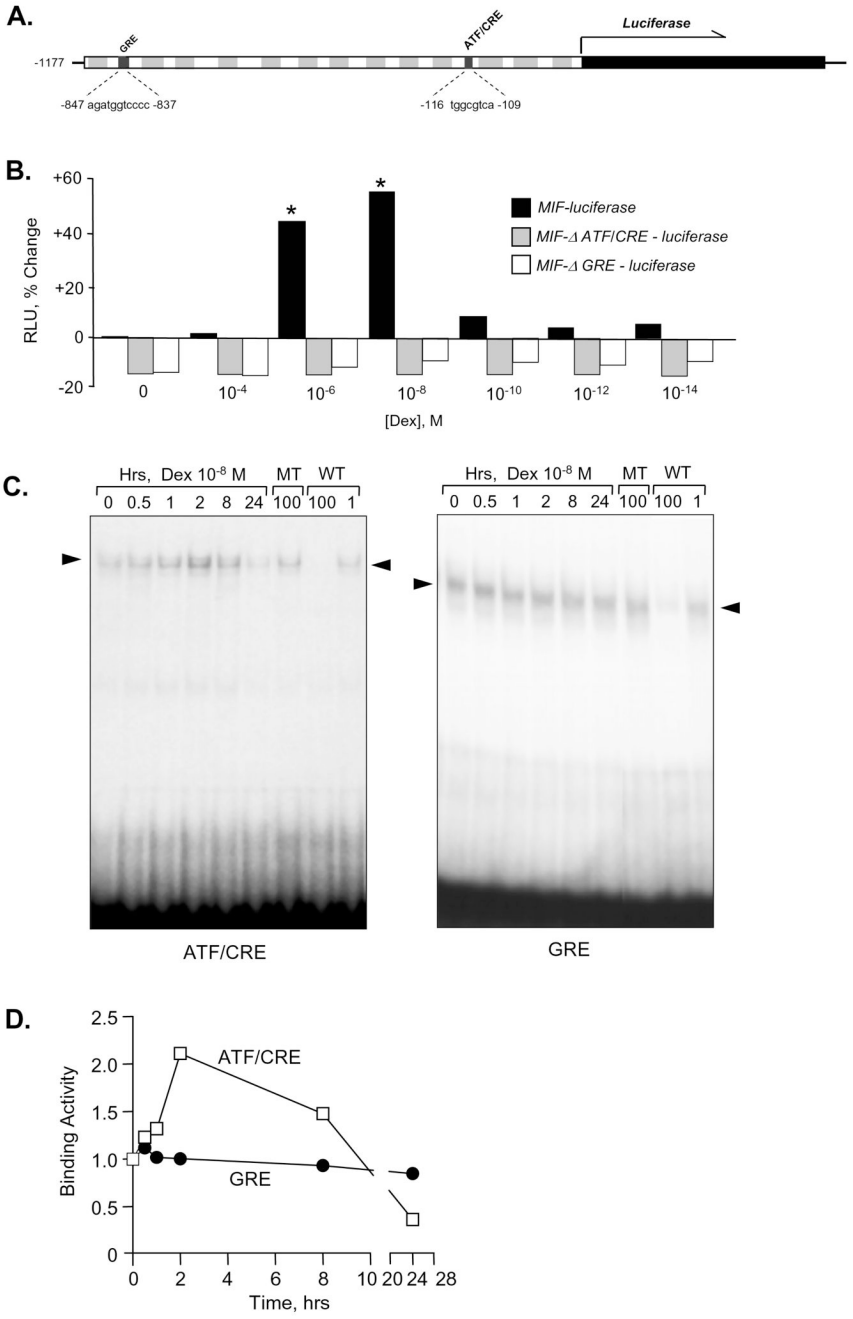

FIGURE 4.

A) Diagram showing the deleted GRE and ATF/CRE elements used to construct the *MIF-ΔATF/CRE-luciferase* and the *MIF-ΔGRE-luciferase* reporter plasmids. B) Reduced MIF promoter activity upon deletion of the ATF/CRE or GRE sites. Cultured CEM-C7 T cells were transfected with the indicated reporter plasmids and the luciferase activity measured 12 hrs later. Values are expressed relative to control plasmid DNA, and are the mean of triplicate determinations. *p<0.05.

C) Dexamethasone increases DNA binding activity to the ATF/CRE site. Nuclear extracts from CEM-C7 T cells were incubated for increasing time with 10^{-8} M dexamethasone and analyzed by EMSA using radiolabeled ATF/CRE or GRE oligonucleotides. Competition

analyses were performed by adding 100-fold or 1-fold excess of unlabeled wild-type (WT) or mutant (MT) oligonucleotides to the reaction mixture. Specific complexes are indicated by the arrowheads. Results are representative of two independent experiments.

D) Time dependent DNA binding activity at the ATF/CRE and GRE after glucocorticoid stimulation. Densitometric quantification of the EMSA analyses shown in C) and illustrating the increase in ATF/CRE but not GRE binding activity at 2 hrs after dexamethasone (10^{-8} M) addition.

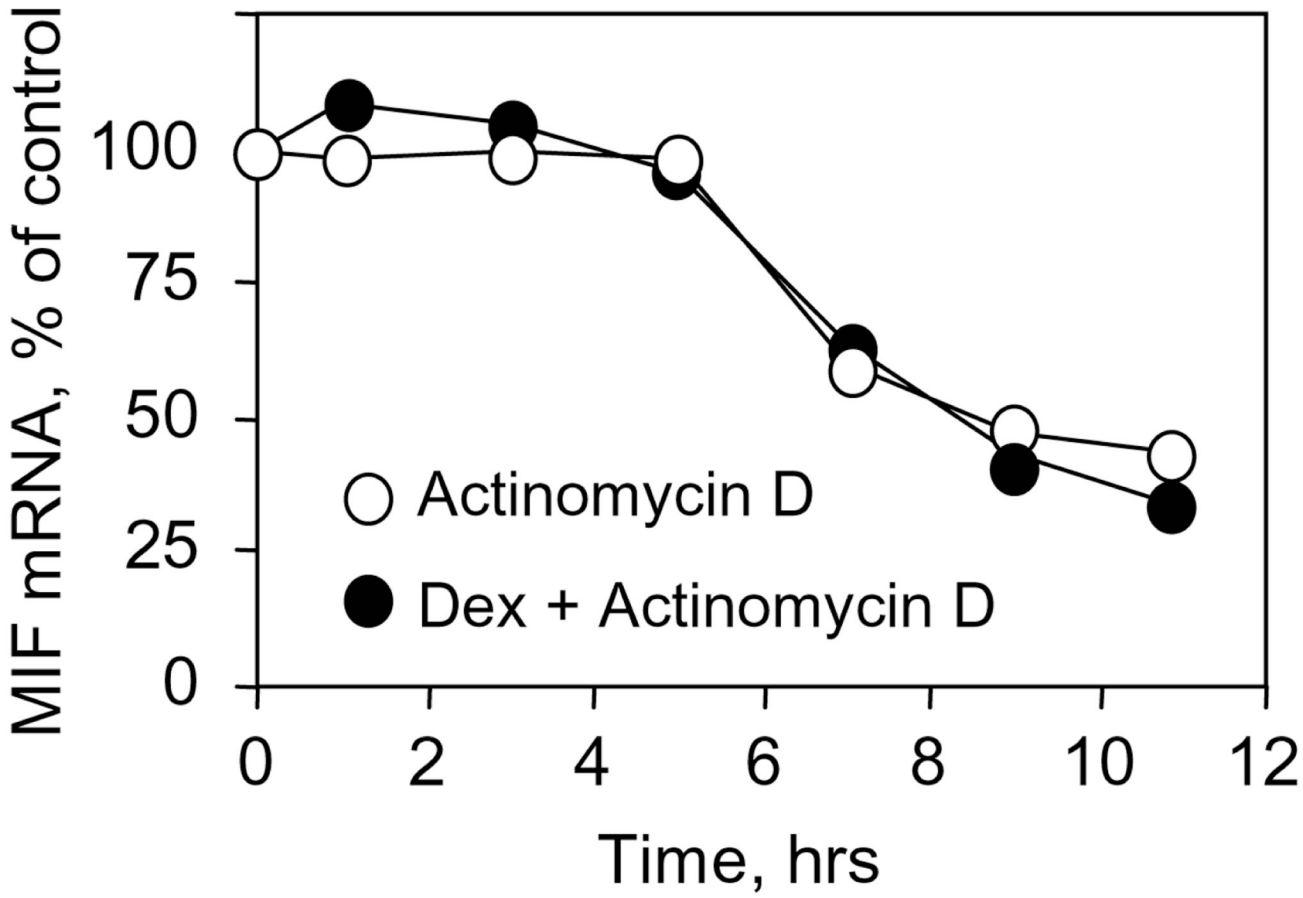

FIGURE 5.

Dexamethasone does not influence MIF mRNA stability in human T cells. MIF mRNA levels in CEM-C7 T cells incubated for 4 hrs with (✶) or without (O) 10^{-8} M dexamethasone before the addition of actinomycin D for 0, 1, 3, 5, 7, 9 and 11 hrs. MIF-specific mRNA signals were quantified relative to ribosomal RNA by image analysis, and the data are expressed as a percent of the signal present at time 0 (*i.e.* before the addition of actinomycin D).