A Novel Role for the Glucocorticoid Receptor in the Regulation of Monocyte Chemoattractant Protein-1 mRNA Stability*

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Monocyte chemoattractant protein-1 (MCP-1) plays an important role in attracting monocytes to sites of inflammation and is the dominant mediator of macrophage accumulation in atherosclerotic plaques. We have previously shown that glucocorticoids inhibit the secretion of MCP-1 in arterial smooth muscle cells (SMC) by markedly decreasing MCP-1 mRNA stability. We now report that the destabilization of MCP-1 mRNA is mediated by the glucocorticoid receptor (GR). The GR antagonist, RU486, blocked the effect of the glucocorticoid dexamethasone (Dex) on MCP-1 mRNA stability in SMC culture. Using a previously reported in vitro mRNA gel shift and stability assay, antibodies to the GR blocked the ability of cytoplasmic extracts from Dex-treated SMC to decay MCP-1 mRNA. Recombinant human GR (rhGR) bound in a concentration-dependent manner to in vitro transcribed MCP-1 mRNA, whereas other members of the steroid hormone receptor family did not. Binding of GR to MCP-1 mRNA was specific as it was not found to bind other mRNAs. Immunoprecipitation of GR in extracts from Dex-treated SMC followed by real-time reverse transcription-PCR demonstrated that endogenous GR was bound specifically to MCP-1 mRNA. The addition of exogenous rhGR blocked the ability of extracts from Dex-treated SMC to degrade MCP-1 mRNA, suggesting that exogenous rhGR can compete with an endogenous GR-containing degradative complex. These data suggest a novel role for the GR in binding to and facilitating mRNA degradation. These results provide novel insights into GR function and may provide a new approach to attenuate the inflammatory response mediated by MCP-1.

Monocyte chemoattractant protein-1 (MCP-1; also known as CCL2) is a chemokine secreted by endothelial cells, vascular smooth muscle cells, fibroblasts, and monocytes/macrophages. MCP-1 and its rodent analog, JE, are not normally present in the arterial media or intima but have been found in human, primate, and rabbit atherosclerotic plaques. In addition, MCP-1 mRNA and protein are induced within hours in the media and neointima in rat and porcine models of balloon arterial injury. Recent studies employing mice lacking MCP-1 or its receptor, CCR2, crossed into an atherosclerotic background (e.g., apoE−/− or LDLR−/− mice), have established that MCP-1 plays a dominant role in attracting monocyte/macrophages to developing atherosclerotic plaques. Rupture of unstable atherosclerotic plaques, with exposure of thrombogenic material, plays a major role in acute coronary thrombotic events associated with unstable angina, myocardial infarction, and sudden death. In addition, MCP-1 and/or CCR2 appear to mediate intimal hyperplasia in rodent and primate models of arterial injury. Therefore, MCP-1 appears to be an important target for attenuating arterial inflammation.

Glucocorticoids are clinically important agents that possess a wide variety of anti-inflammatory and anti-proliferative properties. Glucocorticoid treatment is associated with decreased macrophage accumulation in a wide variety of disease states and with decreased intimal hyperplasia in animal models of arterial injury. Long-term glucocorticoid treatment has been reported to decrease macrophage accumulation and plaque size during the development of atherosclerosis in cholesterol-fed rabbits. Short-term treatment with dexamethasone (Dex) inhibited MCP-1 expression and macrophage accumulation after femoral arterial injury in cholesterol-fed rabbits. In addition, methyl prednisolone blocked the induction of MCP-1 expression in a rat model of renal ischemia. Glucocorticoids have been shown to inhibit MCP-1 synthesis in a variety of cell types including arterial SMC, airway SMC, human leukemia cells, hyperoxia-stimulated U937 monocytic cells, and fibroblasts. We previously showed that Dex, at doses as low as 0.01 μM, completely blocked the serum- or PDGF-induced accumulation of MCP-1 mRNA and active protein in rat aortic SMC. This effect was seen with other glucocorticoids but not with mineralocorticoids, estrogen, progesterone, or testosterone. Of note, the effect of Dex on MCP-1 mRNA accumulation in rat aortic SMC was due predominantly to a decrease in mRNA stability and not to changes in MCP-1 transcription. As determined by in vivo and in vitro decay assays, Dex reduced the half-life (t1/2) of MCP-1 mRNA in the presence of PDGF or serum from ~3 h to ~30 min. The effect of glucocorticoids...
involved the binding of a protein complex to the 5′ end (nucleotides 1–224) of the MCP-1 mRNA (26). These data suggest that part of the anti-inflammatory effect of glucocorticoids may be mediated by their regulation of MCP-1 mRNA stability.

Although there is considerable information on the mechanism underlying glucocorticoid-mediated transcription activation or inhibition, much less is known about the effects of glucocorticoids on mRNA stability. We now report that the effect of Dex on MCP-1 mRNA stability is mediated by the glucocorticoid receptor (GR) and involves a novel mechanism in which the GR binds directly to MCP-1 mRNA and facilitates its degradation. These results provide new insights into GR function and to the mechanisms regulating MCP-1 mRNA stability.

EXPERIMENTAL PROCEDURES

Reagents—rhGR (product number G1542), recombinant human estrogen receptor (rHER; E1528), and RU486 (M8046) were purchased from Sigma-Aldrich. Human retinoic acid receptor (RAR; product number sc-4088), human mineralocorticoid receptor (MCR; sc-4419), and protein A/G PLUS-agarose beads (sc-2003) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies recognizing amino acids 245–259 of hGR were obtained from Affinity BioReagents (Golden, CO) (PA1–512) and Abcam (Cambridge, UK) (antibody number Ab3579). Antibodies recognizing the carboxyl terminus (sc-1002) and amino terminus (amino acids 395–411 (sc-12763) and 121–421 (sc-8992)) of hGR were obtained from Santa Cruz Biotechnology. Antibody to the estrogen receptor (ER) (product number MAI-310) was obtained from Affinity BioReagents.

Cell Culture—Rat aortic SMC were isolated from the thoracic aortas of 200–300-g male Sprague-Dawley rats by enzymatic dissociation and culture as described previously (24). The response to Dex has been consistently seen with cells as early as passage 5 and as late as passage 23 (26). All experiments were performed at confluence (2×10⁶ cells/100-mm-diameter dish) 36 h after the addition of fresh Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum.

In Vitro Decay Assays—The MCP-1 constructs were generated by PCR using the full-length human MCP-1 cDNA (GenBank™ accession number S71513) as template. Linearized cDNAs were transcribed in vitro with T3 RNA polymerase (Roche Applied Science) in the presence of [α-32P]UTP (800 Ci/mmol, PerkinElmer Life Sciences) as described previously (24). Capped transcript was generated using a 1:1 ratio of m7G5′ÅpppG (Roche Applied Science) to GTP. Tissue factor (TF; Genbank accession number U07619), c-fos (X06769), Mena (mammalian homologue of Drosophila enabled, accession number BC062927), and pBluescript KS (Stratagene) mRNAs were also in vitro transcribed from their linearized full-length cDNAs. Probes were purified on 4% polyacrylamide gels. Cytosolic (S-100) extracts were prepared from SMC as described previously (26) and stored at −80 °C. Protein concentrations (generally 250–500 ng/μl) were determined by Bradford assay (Bio-Rad) (31). In vitro RNA gel shifts and decay assays were performed and analyzed on polyacrylamide gels as described previously (26).

Immunoprecipitation—Co-immunoprecipitation of MCP-1 mRNA from SMC extracts with antibody to GR was performed using the method of Tenenbaum et al. (32). Following several washes with ice-cold phosphate-buffered saline, SMC were harvested by scraping with a rubber policeman on ice. The cells were then centrifuged at 2000 × g for 5 min at 4 °C. The pellet was resuspended in polysome lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 10 mM NaF, 1 mM Na3VO4, 1 mM dithiothreitol, 1% Triton X-100, and 0.1% SDS) supplemented with RNase inhibitor (Roche Diagnostics) and protease inhibitor (protease inhibitor mixture P8340, Sigma-Aldrich). Cells were then washed through a 29-gauge needle, mixed several times with a hand pipettor, placed on ice for 5 min, and then stored at −80 °C. Prior to use, lysates were thawed and centrifuged at 14,000 × g for 10 min at 4 °C, and the supernatant was collected. Protein A/G PLUS-agarose beads were swollen in NT2 buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM MgCl2, and 0.05% Nonidet P-40) supplemented with 5% bovine serum albumin to a final ratio of ~1:5 (v/v) for at least 1 h prior to use. Beads (100 μl) were incubated for 16 h at 4 °C with 6 mg of antibody, washed five times with NT2 buffer, and then resuspended in 850 μl of NT2 buffer containing 200 units of RNase OUT (Roche Applied Science), 1 μM vanadyl ribonucleoside complexes, 1 mM dithiothreitol, and 15 mM EDTA. The beads were then mixed with 100 μl of the cell lysate and incubated with shaking at room temperature for 2 h. The beads were washed six times with ice-cold NT2 buffer and resuspended in 100 μl of NT2 buffer supplemented with 0.1% SDS and 30 μg of proteinase K at 55 °C for 30 min. The immunoprecipitated RNA was isolated by phenol-chloroform-isooamyl alcohol extraction and ethanol precipitation.

Real-time PCR—First strand cDNA was synthesized from 500 ng of total RNA using the SuperScript first-strand synthesis system (Invitrogen). Assays were performed in triplicate using the Applied Biosystems Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA). The 20-μl reaction mixture was comprised of 2 μl of cDNA, 900 nM forward primers, 900 nM reverse primer, 1X SYBR Green master mix (Applied Biosystems), and DNase-free water. Amplification conditions were: 10 min at 95 °C, 40 cycles of 20 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C. The primers for rat MCP-1 spanned nucleotides 76–320 and, for TF, nucleotides 301–800.

RNA Blot Analysis—Isolation of total RNA and RNA blot hybridization with the full-length rat JE cDNA were performed as described previously (24). Equal loading of total RNA was verified by ethidium bromide staining of the 18 S and 28 S ribosomal RNA.

RESULTS

RU486 Blocks the Inhibitory Effect of Dex on MCP-1 mRNA Accumulation in SMC—We have previously shown that Dex inhibits MCP-1 mRNA accumulation in rat aortic SMC by enhancing its decay (24, 26). To elucidate the role of the GR in this process, SMC were treated for 3 h with 20 ng/ml PDGF alone or in the presence of 1 μM Dex, and MCP-1 mRNA abundance was analyzed by blot hybridization. As shown in Fig. 1, A and B, Dex completely blocked the accumulation of MCP-1 mRNA. RU486, an inhibitor of the GR, blocked the effect of Dex.
in a concentration-dependent manner. RU486 had no effect on control or PDGF-inducible levels of MCP-1 mRNA.

GR Antibody Blocks Dex-mediated MCP-1 mRNA Degradation—We have previously reported an in vitro mRNA decay assay using RNA gel shifts in which the rates of decay of MCP-1 mRNA in the presence of PDGF and Dex were similar to those measured in cell culture (26). In these assays, we observed at very early incubation times (e.g. 5 min) that the gel shift with Dex extract was greater than the gel shift seen with control extracts, suggesting that additional proteins bind to the MCP-1 mRNA in the Dex extract, perhaps facilitating its degradation (Fig. 1C). Interestingly, extracts from PDGF-treated cells produce a shift lower than control and Dex-treated extracts, suggesting that PDGF treatment might interfere with the binding of proteins that normally contribute to MCP-1 mRNA degradation (33). To elucidate further the role of GR in the Dex-mediated decay of MCP-1 mRNA, RNA decay assays were performed in the presence of an antibody to GR. As shown in Fig. 1D, cytoplasmic extracts from SMC left untreated (C) or treated with 1 μM Dex (D) for 3 h were incubated with radiolabeled MCP-1 mRNA for 5 min and analyzed by non-denaturing PAGE. The shift was not due to a non-GR contaminant, supershifts were generated more and higher shifted bands at higher concentrations, suggesting GR multimerization, mRNA multimerization, or the recruitment of additional molecules (Fig. 2C). The same shift was observed at 30 and 60 min (data not shown), suggesting that Dex alone does not degrade the MCP-1 mRNA and requires the activity of a ribonuclease. The shift with Dex extracts was substantially higher than with rhGR alone, suggesting that it is produced by a GR-containing ribonuclease complex (Fig. 2A). The GR-mediated shift of MCP-1 mRNA was concentration-dependent and generated more and higher shifted bands at higher concentrations, suggesting GR multimerization, mRNA multimerization, or the recruitment of additional molecules (Fig. 2B).

RU486, by itself, did not interfere with the binding of the GR to MCP-1 mRNA (Fig. 2C). In addition, Dex alone did not interfere with the gel shift (data not shown), suggesting that the interaction between GR and MCP-1 mRNA did not involve the steroid binding domain of GR. The interaction between GR and MCP-1 mRNA was seen with multiple lots of rhGR from two different sources (Sigma and Panvera). To further establish that the shift was not due to a non-GR contaminant, supershifts were performed with five different antibodies to GR generated against amino acids 245–259 (Ab1 and Ab5), 395–411 (Ab2), carboxyl terminus (Ab3), and 121–421 (Ab4). Supershifts were involved in MCP-1 mRNA degradation. To determine whether there was any physical interaction between MCP-1 and GR, in vitro transcribed 32P-labeled full-length human MCP-1 mRNA was incubated directly with rhGR and analyzed on non-denaturing gels. The rhGR produced a shift in MCP-1 mRNA after only 5 min of incubation (Fig. 2A). The same shift was observed at 30 and 60 min (data not shown), suggesting that GR alone does not degrade the MCP-1 mRNA and requires the activity of a ribonuclease. The shift with Dex extracts was substantially higher than with rhGR alone, suggesting that it is produced by a GR-containing ribonuclease complex (Fig. 2A). The GR-mediated shift of MCP-1 mRNA was concentration-dependent and generated more and higher shifted bands at higher concentrations, suggesting GR multimerization, mRNA multimerization, or the recruitment of additional molecules (Fig. 2B).
observed with all GR antibodies (Fig. 2D). No supershift was observed with antibody to the ER, another member of the steroid receptor family. Of note, a supershift was also seen when Dex extracts were incubated with antibodies to the GR for 5 min (Fig. 2E). This supershift was not seen after a 30-min incubation (Fig. 1D), suggesting that in the presence of antibody to GR, the high molecular weight complex is not stable. It is of interest that different antibodies produced different shifts, both with rhGR and with Dex extracts. This may be due to differences in valence (information not available from the manufacturers) or differences in protein configuration on the non-denaturing gels.

Specificity of the MCP-1 mRNA-GR Interaction—To examine whether the MCP-1 RNA-GR interaction exhibits hormone receptor specificity, human and rat MCP-1 mRNA were incubated with other members of the steroid hormone receptor superfamily, including the ER, MCR, and RAR. Only rhGR produced a shift with MCP-1 mRNA (Fig. 3A). This result is consistent with our previous findings in SMC culture and in gel shifts showing that the inhibition of MCP-1 mRNA accumulation was glucocorticoid-specific (24, 26). A similar shift was seen when rhGR was incubated with full-length rat JE (Fig. 3B) and a 1–224-nucleotide fragment from the 5’ end of the JE mRNA (Fig. 3C), which we have previously identified as the minimum region required to maintain Dex sensitivity (26).

To examine whether the MCP-1 RNA-GR interaction exhibits mRNA specificity, rhGR was incubated with three other in vitro transcribed mRNAs, including c-fos, Mena, and TF. None of the mRNA produced a shift with rhGR (Fig. 4).

Exogenous GR Blocks the Effect of Dex Extracts on MCP-1 mRNA Stability—We previously found that the addition of Dex to cytoplasmic extracts from untreated or PDGF-treated SMC did not alter their effects on MCP-1 mRNA stability, whereas extracts from cells treated with Dex markedly decreased MCP-1 mRNA stability (26). This suggested that Dex treatment caused a critical modification that either increased the activity or allowed for the formation of a stable degradative complex. The above data suggest that this complex includes the GR. We therefore sought to determine whether exogenous rhGR would be able to compete with this putative endogenous active degradative complex. As shown in Fig. 5, the addition of rhGR to extracts from Dex-treated SMC blocked the ability of the extracts to degrade MCP-1 mRNA in a concentration-dependent manner. In contrast, the addition of exogenous rhER had no effect. These results suggest that rhGR may act as a competitive inhibitor of a GR-containing degradative complex.

Endogenous MCP-1 mRNA Binds to GR—The above studies demonstrate that MCP-1 mRNA and GR interact in vitro. Western blot analysis demonstrated that GR was present, at similar levels, in extracts from control and Dex-treated SMC.
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**TABLE 1**

| Sample                      | Cycles | Sample                      | Cycles | Sample                      | Cycles |
|-----------------------------|--------|-----------------------------|--------|-----------------------------|--------|
| MCP-1 mRNA                  |        | TF mRNA                     |        | TF mRNA                     |        |
| MCP-1 mRNA/GR               | 20.7   | 10.000                      | 20.6   | 10.000                      |
| MCP-1 mRNA/GR/GR Ab         | 28.8   | 36.4                        | 37     | 0.1                         |
| MCP-1 mRNA/ER               | 35.4   | 0.4                         | 40     | <0.1                        |
| MCP-1 mRNA/ER/ER Ab         | 37.3   | 0.1                         | 38.9   | <0.1                        |
| Control extract/GR Ab       | 26.5   | 179.5                       | 37.6   | <0.1                        |
| Dex extract/GR Ab           | 30     | 15.9                        | 38.9   | <0.1                        |
| Control extract/ER Ab       | 35.2   | 0.4                         | 36.9   | 0.1                         |
| Dex extract/ER Ab           | 36.4   | 0.2                         | 35.2   | 0.4                         |

In vitro transcribed MCP-1 and tissue factor mRNA were incubated with rhGR and then immunoprecipitated with either GR or ER antibody. The precipitates were analyzed for MCP-1 mRNA by real-time PCR. The ER antibody failed to precipitate either mRNA. Extracts from control and Dex-treated SMC were then immunoprecipitated with GR or ER antibody, and total RNA was extracted and subjected to real-time PCR. The GR antibody precipitated 180 and 16 pg of MCP-1 mRNA from control and Dex extracts, respectively. The ER antibody did not immunoprecipitate MCP-1 mRNA. TF mRNA was not immunoprecipitated by either GR or ER antibody. These data strongly suggest that MCP-1 mRNA and GR interact endogenously.

The amount of MCP-1 mRNA immunoprecipitated from cell extracts was similar to that precipitated using exogenous rhGR, suggesting that the experiments performed with exogenous MCP-1 mRNA and rhGR described above may have been conducted under conditions that approximated concentrations found in the cell. Also of note was that the amount of MCP-1 mRNA precipitated from control extracts was ~10-fold higher than that precipitated from Dex extracts. This is likely due to the ability of the Dex extracts to more effectively degrade MCP-1 mRNA and is supported by the finding that levels of MCP-1 mRNA were ~80-fold higher in extracts from control SMC than from Dex-treated cells prior to immunoprecipitation (control extracts, 22.3 cycles; Dex extracts: 28.6 cycles).

**DISCUSSION**

Glucocorticoids are potent anti-inflammatory agents that are used in the treatment of a variety of diseases. We have previously shown that glucocorticoids decrease the expression of MCP-1 in vascular SMC by altering mRNA stability (24). In this study, we have employed in vitro mRNA gel shifts and a recently reported assay utilizing immunoprecipitation and real-time PCR to examine the mechanism underlying the effect of glucocorticoids on MCP-1 mRNA stability. Our data demonstrate that the effect is mediated by the GR and, based upon our findings utilizing exogenous recombinant GR, likely involves direct binding of the GR to MCP-1 mRNA. To our knowledge, this represents a novel mechanism for regulating mRNA stability and is the first report of a functional consequence of an interaction between the GR and mRNA. We cannot, however, rule out the possibility that in intact cells, this interaction occurs through an intermediary.

Glucocorticoids have wide-ranging effects on gene expression, which for the most part are mediated by binding to the GR and activating transcription of genes containing a glucocorticoid-response element (35). Glucocorticoids also inhibit transcription either by binding of the glucocorticoid-GR complex to inhibitory promoter elements or by binding to and interfering with the activity of transcriptional activators, such as AP1 (36). Glucocorticoids also have actions that are not mediated by direct binding of the glucocorticoid–GR complex to DNA (37). The importance of these effects is underscored by the viability of mice containing a point mutation A458T in the GR that prevents glucocorticoid-response element-dependent transcription but allows for other GR activities, such as transrepression of AP1-mediated transcription (38).

The current study focused on the role of glucocorticoids in regulating MCP-1 mRNA stability. Glucocorticoids have been found to decrease the stability of a variety of mRNAs, including those encoding cyclooxygenase (COX)-2, cyclin D3, eotaxin, granulocyte-macrophage colony-stimulating factor, interferon-β, interleukin-1, interleukin-6, inducible nitric oxide synthase, leukocyte inhibitory factor, and vascular endothelial growth factor (reviewed in Ref. 37). In addition, glucocorticoids have been shown to increase the stability of a few mRNAs, including those encoding COX-1 and fibronectin (37). The mechanisms underlying the effect of glucocorticoids on most of these mRNAs have not been elucidated. For COX-2 (39) and interferon-β (40), the effect of glucocorticoids on mRNA sta-
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The GR was found to play a role in mediating the stability of MCP-1 mRNA. In many studies examining glucocorticoid-mediated changes in mRNA stability, the role of the GR was not explored. The GR has been shown to bind directly to the MCP-1 mRNA and thus compete with other proteins that might bind to the mRNA.

Our current data suggest that the effect of Dex on MCP-1 mRNA stability is mediated by the GR in that RU486 blocked the effect of Dex on MCP-1 mRNA stability in cultured SMC, and antibodies to the GR blocked the ability of cytoplasmic extracts from Dex-treated cells to rapidly degrade MCP-1 mRNA. In many studies examining glucocorticoid-mediated changes in mRNA stability, the role of the GR was not explored.

The GR was found to play a role in mediating the stability of mRNAs encoding surfactant protein A (42), fibronectin (43), interleukin-1α (44), and granulocyte-macrophage colony-stimulating factor (45) in that the effect was blocked by RU486. In these cases, the effect of glucocorticoids on mRNA stability was dependent upon new transcription and protein synthesis in that they were inhibited by actinomycin D and cycloheximide, respectively. In contrast, the effect of glucocorticoids on MCP-1 mRNA in SMC was not inhibited by either actinomycin D or cycloheximide (26). This further suggests that the GR-dependent mechanism regulating MCP-1 mRNA is novel.

A major feature of this report is that the GR binds directly to MCP-1 mRNA. Similar binding was not seen with other mRNAs or with other members of the steroid hormone receptor family (e.g. ER, MCR, RAR). We also employed a recently described assay involving immunoprecipitation with the GR antibody followed by real-time PCR to demonstrate that endogenous GR was bound to MCP-1 mRNA in cell extracts. Previous studies have shown that the GR binds to various types of RNA, including rRNA (46) and tRNA (47). No functional consequence has been ascribed to this binding, although one report suggested that certain types of RNA might bind to a specific site on the GR and prevent it from binding to and transactivating DNA (48). To our knowledge, ours is the first report demonstrating that the GR binds to mRNA. Importantly, our data suggest that the binding may have functional consequences in facilitating the destabilization of MCP-1 mRNA in that antibodies to the GR or exogenous hGR blocked MCP-1 mRNA degradation.

The GR-mediated decrease in MCP-1 mRNA stability appears to require cellular signaling in that the addition of Dex directly to cytoplasmic extracts from untreated cells failed to alter their effect on MCP-1 mRNA stability (26). The effect was abrogated by treating extracts with proteinase K or heating at 95 °C, suggesting that it involves a heat labile protein. Treating cells with Dex in the presence of either cycloheximide or actinomycin D did not alter the ability of extracts to destabilize MCP-1 mRNA, suggesting that Dex sensitivity is mediated by alterations in constitutively expressed proteins. This may involve enhancing the formation or activity of a degradative complex involving the GR and a ribonuclease, perhaps by modifying the GR or the ribonuclease (Fig. 6A). Alternatively, the binding of the GR to the MCP-1 mRNA may induce a conformational change that facilitates degradation by the ribonuclease. Exogenous rhGR was as effective as GR antibody in blocking the ability of extracts from Dex-treated cells to degrade MCP-1 mRNA, further supporting the concept that Dex causes a critical modification that enhances the activity of a degradative complex. We hypothesize (Fig. 6B) that exogenous rhGR can bind directly to the MCP-1 mRNA and thus compete with the GR-containing degradative complex. The cell extracts used in this study were cytoplasmic because in our previous report, nuclear extracts from Dex- or PDGF-treated SMC failed to alter MCP-1 mRNA stability. This would argue that the action of the purported GR-containing degradative complex on MCP-1 mRNA is occurring in the cytoplasm.

Monocyte/macrophage accumulation is a key event in many inflammatory diseases, including atherosclerosis. MCP-1 appears to play the major role in macrophage accumulation in developing atherosclerotic lesions. Macrophages are the primary source of cholesterol-rich foam cells and are thought to play a key role in mediating plaque rupture (49), the cause of most acute coronary syndromes. Therefore, there has been intense interest in developing approaches to inhibit MCP-1 or block its receptor, CCR2 (50). Glucocorticoids are used to treat a variety of inflammatory diseases. Long term treatment, however, is associated with deleterious side effects, which limit their utility to only the most severe illnesses. Our current study suggests that the effect of glucocorticoids on one part of the inflammatory response, i.e. the secretion of MCP-1 protein, involves an unusual pathway that is likely to be far more restricted than the effects of glucocorticoids on transcription. Elucidation of the precise mechanism by which glucocorticoids and the GR mediate MCP-1 mRNA stability might provide new approaches to inhibiting MCP-1 and the design of agents that share some of the anti-inflammatory properties of glucocorticoids but potentially fewer side effects.
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