Expression of Multiple Chemokine Genes by a Human Mast Cell Leukemia*

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The chemokines are a large group of cytokines that are recognized to be important mediators of inflammation. In this study we show that the human mast cell leukemia line HMC-1 is a source of multiple chemokines, including I-309, monocyte chemoattractant protein 1, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, RANTES, and interleukin-8. I-309 and MCP-1 transcripts are expressed at low levels in unstimulated HMC-1. However, phorbol ester treatment up-regulates these and other chemokine transcript levels and also up-regulates chemokine protein synthesis and secretion. Induction of chemokine transcripts in HMC-1 requires de novo protein synthesis. We compared the effects of anti-inflammatory glucocorticoids on the expression of chemokine genes in HMC-1 to their effects in activated T-cells. We find that methylprednisolone reduces MCP-1 but not other chemokine transcripts in HMC-1, even though there are distinct and more general effects on chemokine transcripts in activated T-cells. These effects are attributed to inhibition of transcription rather than transcript stability. Our results suggest that human mast cells may be a source of multiple chemokines, that glucocorticoids may inhibit the expression of only a subset of these chemokines, and that mast cells and T-cell chemokine expression may occur via distinct regulatory pathways.

Chemokines are small secreted proteins that play essential roles in the recruitment and activation of leukocytes and other cells at sites of inflammation (1). The members of this family are all structurally related and display four highly conserved cysteine residues, yet can be segregated into either of two sub-families (C-C or C-X-C) depending upon the presence or absence of an intervening amino acid between the first two conserved cysteine residues. The human C-C subfamily is comprised of at least six distinct molecules: I-309, MCP-1,1 MIP-1α, MIP-1β, RANTES, and HC14. The human C-X-C subfamily includes IL-8, PF4, platelet basic protein and its derivatives (connective tissue-activating peptide (CTAP), β-thromboglobulin, neutrophil activating peptide-2 (NAP-2), γIP-10, and the GRO family of molecules (α, β, and γ). Examination of the expression of these chemokines in various systems reveals that some are expressed by a wide range of cells, whereas others are expressed in a highly restricted fashion. For example, MCP-1, MIP-1α, MIP-1β, IL-8, and GRO molecules can be expressed upon stimulation of hematopoietic cells, fibroblasts, endothelial cells, epithelial cells, keratinocytes, and chondrocytes (1, 2). On the other hand, the only known source of PF4 is platelets, and the only known source of I-309 is activated T-cells (3, 4).

Murine mast cells have recently been recognized as a source of cytokines that are also produced by activated T-cells, including IL-3, IL-4, IL-5, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) (5–7). Among chemokines, the murine CC molecules TCA3, MIP-1α, MIP-1β, and MCP-1/IE were found to be produced by growth factor-dependent and -independent mast cell lines (8). Only a limited number of reports are available on the expression of cytokines by human mast cells, primarily because of difficulty in obtaining sufficient quantities of purified cells for analysis. However, it has recently been shown that dispersed mast cells from human foreskin and respiratory tract express TNFα and IL-4 proteins upon stimulation with anti-IgE (9, 10).

The human mast cell leukemia HMC-1 is a cell line that was established from the peripheral blood of a patient with mast cell leukemia and exhibits many characteristics of immature mast cells (11). Notably, these cells contain low levels of histamine, are stained metachromatically by toluidine blue, and contain chloroacetate esterase, aminocaproate esterase, and tryptase activities. However, they do not express cell surface FceR, a property that they share with mucosal mast cells from Trichinella spiralis-infected mice (12), primary human mast leukemia cells (13), and immature mast cells established from human fetal liver (14). Although in the absence of FceR HMC-1 cannot be activated by antigen, the cells can still be activated by treatment with phorbol esters and calcium ionophore, as can normal or transformed murine FceR+ mast cell lines (8). These cells can therefore serve as a useful system to begin an examination of mast cell expression of human chemokines. We show in this study that upon stimulation, HMC-1 cells produced an array of chemokines that is broader than that produced by stimulated human T lymphocytes. We further compared the effects of anti-inflammatory glucocorticoids on chemokine gene expression in HMC-1 and T lymphocytes and observed differential sensitivity in the two cell types. Our data suggest that glucocorticoids may inhibit the expression of only a subset of mast cell-derived chemokines and argue that chemokine genes are differentially regulated in mast cells and T-cells.

MATERIALS AND METHODS

Cell Culture—Peripheral blood mononuclear cells were isolated from Leukopaks obtained from the Red Cross blood bank (Charlotte, NC) by centrifugation through Ficoll-Hypaque (Lymphocyte Separation Me-
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dium; Organon Teknika Corp., Durham, NC). Cells were washed and cultured at an initial density of 2 × 10^6 cells/ml in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Irvine Scientific, Santa Ana, CA), 2 mM L-glutamine, 10 mM HEPES (pH 7.3), and antibiotics (penicillin at 50 units/ml and streptomycin at 50 μg/ml; Life Technologies, Inc.) and were stimulated with 1 μg/ml αCD3 antibody 64.1 (Bristol-Meyers Squibb, Seattle WA) and 50 ng/ml PMA (Sigma). After 3 days of primary activation, the cells were expanded at an initial density of 1 × 10^6 cells/ml in IL-2 (Genzyme Corp., Cambridge, MA) or treated with PHA for 4 h. The polyclonal T-cell line LPL-1 was either untreated or treated with PHA for 4 h. Northern blots of total RNA samples were sequentially hybridized with 32P-labeled cDNA probes as indicated.

RESULTS

Expression of Chemokine Transcripts by Human Mast Cell Leukemia HMC-1—We used Northern blot analysis to examine the expression of chemokine transcripts in the mast cell line HMC-1 (Fig. 1). Unstimulated HMC-1 cells express moderate levels of I-309 and MCP-1 transcripts and very low levels of RANTES transcripts, but do not express detectable levels of MIP-1α, MIP-1β, and IL-8 transcripts. The detected basal level of I-309 and MCP-1 transcripts in part reflects serum responsiveness, since both of these transcripts are moderately elevated by incubation of HMC-1 in fresh serum containing medium (data not shown).

HMC-1 cells were stimulated with the phorbol ester PMA and the calcium ionophore ionomycin alone and in combination (Fig. 1). PMA was found to up-regulate I-309, MCP-1, and RANTES transcripts and to induce the expression of MIP-1α, MIP-1β, and IL-8 transcripts. In all instances, de novo protein synthesis was required for transcript induction, as induction was abolished by pretreatment with cycloheximide (data not shown). Ionomycin alone had no effect on chemokine transcript expression and did not potentiate the effects of PMA. Although chemokine transcripts were readily detected in resting and stimulated HMC-1 cells, IL-2 transcripts were not detected under any condition tested (data not shown). This result is consistent with previous data analyzing other human and murine mast cell samples, which identified transcripts encoding a number of cytokines, but not IL-2.

The observed sizes for the various chemokine transcripts in HMC-1 were consistent with previous reports examining expression in other cell types (1, 2). Notably, two different size classes of I-309 transcripts were detected, an abundant class of 0.55 kb and a less abundant class of 2.4 kb. Previous charac-
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FIG. 2. Secretion of I-309 and MCP-1 chemokines by the human mast cell leukemia line HMC-1. HMC-1 cells were stimulated with PMA as indicated, were metabolically labeled, and culture supernatants were harvested. Culture supernatants were immunoprecipitated using the indicated antibodies, and immunoprecipitates were resolved by SDS-polyacrylamide gel electrophoresis.

**Secretion of Chemokine Proteins by Human Mast Cell Leukemia HMC-1**—Because HMC-1 could express high levels of chemokine transcripts, we sought to determine whether these cells were sources of secreted chemokine peptides. HMC-1 cells that were either unstimulated or stimulated for 4 or 8 h were metabolically labeled with a 2-h pulse of [35S]methionine and [35S]cysteine, and culture supernatants were harvested and immunoprecipitated using anti-I-309 and anti-MCP-1 antibodies (Fig. 2). Low levels of 16- and 8-kDa I-309 peptides, and low levels of 15- and 11-kDa MCP-1 peptides were detected in supernatants of unstimulated cells. The 16- and 8-kDa I-309 species are the expected sizes of glycosylated and nonglycosylated I-309, respectively (22). The two forms of MCP-1 are presumed to represent differentially glycosylated forms of this molecule, which would be consistent with previous studies (23, 24).

The secretion of both I-309 and MCP-1 species were dramatically up-regulated by PMA stimulation. Additional forms of I-309 were detected, including a prominent 12-kDa form. However, the degree of I-309 protein heterogeneity varied in different experiments (data not shown). Similarly, a novel 14-kDa MCP-1 species was also secreted. These distinct I-309 and MCP-1 species are thought to represent differentially glycosylated forms of the respective proteins, but their precise structures are uncertain.

**Differential Glucocorticoid Sensitivity of Chemokine Expression in HMC-1 and T-cells**—Anti-inflammatory glucocorticoids are known to have potent down-regulatory effects on cytokine expression in T-cells and in some other cell types (25–33). To understand the effects of glucocorticoids on HMC-1 chemokine expression, cells were incubated with various corticosteroids, including the glucocorticoids methylprednisolone, hydrocortisone, and dexamethasone, and the nonglucocorticoid progesterone, with or without simultaneous PMA induction. In unstimulated cells, basal expression of I-309 and MCP-1 transcripts was assayed as a function of treatment with a range of corticosteroid concentrations (Fig. 3A). I-309 transcripts were unaffected by treatment with 0.1–10 μM doses of methylprednisolone, hydrocortisone, or dexamethasone. On the other hand, levels of MCP-1 transcripts were detectably inhibited by as little as 0.1 μM doses of these compounds and were maximally inhibited by 1 μM doses. Significant inhibition was observed with either 2 h (data not shown) or 4 h (Fig. 3A) of treatment. Progesterone, which does not possess glucocorticoid activity, failed to inhibit the induction of MCP-1 transcripts. Thus, inhibition of MCP-1 transcript levels is a specific property of glucocorticoids and is not a generalized property of corticosteroids. Of the three glucocorticoids tested, methylprednisolone and dexamethasone were more potent inhibitors than hydrocortisone; methylprednisolone was chosen for further experiments.

Glucocorticoid effects on PMA-inducible expression of chemokine transcripts in HMC-1 were investigated next (Fig. 3B). Cells were transferred into medium containing fresh serum and were stimulated with PMA in the presence or absence of 10 μM methylprednisolone for 4 or 8 h. As a control, cells were transferred into medium containing fresh serum and were harvested after 4 or 8 h with no further treatment. With fresh serum alone, both I-309 and MCP-1 transcripts are mildly and transiently induced, resulting in a decay in transcript levels between 4 and 8 h after transfer. As observed previously (Fig. 1), PMA treatment resulted in dramatic increases in I-309 and MCP-1 transcripts and induction of MIP-1α, MIP-1β, and RANTES transcripts. Although MCP-1 transcripts were still induced by PMA treatment in the presence of methylprednisolone, the induced levels were decreased by 48 and 73% after 4 and 8 h, respectively, relative to cells treated with PMA alone. In contrast, the levels of I-309, MIP-1α, MIP-1β, and RANTES mRNAs were unaffected.

To evaluate the mechanism by which methylprednisolone reduces MCP-1 mRNA levels, two experiments were performed. First, we isolated nuclear and cytoplasmic RNA fractions to analyze basal transcript levels in HMC-1 (Fig. 4A). Analysis of untreated and PMA treated control cells indicated that the fractionation protocol was effective. For example, the nuclear fractions displayed relatively low levels of 0.55-kb I-309 transcripts and higher levels of a transcript that was slightly larger than the 2.4-kb transcripts found in the cytoplasm. Whereas the cytoplasmic 2.4-kb transcript is known to represent a fully spliced form that utilizes a distal polyadenylation site, we assume that the larger nuclear form represents an unspliced I-309 transcript that utilizes the proximal polyadenylation site. Such a transcript is predicted to be 2.8 kb long based upon the I-309 genomic structure (34). Unspliced and partially spliced

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2 M. Miller, personal communication.
FIG. 3. Corticosteroid inhibition of basal and inducible MCP-1 transcript levels in HMC-1. A, total RNA was isolated from unstimulated HMC-1 cells cultured for 4 h either alone (lane 1) or in the presence of graded concentrations of methylprednisolone (MP; lanes 2–4), dexamethasone (DEX; lanes 5–7), hydrocortisone (HC; lanes 8–10), and progesterone (PROG; lanes 11–13). Concentrations of each compound tested were 10, 1, and 0.1 μM. A Northern blot of RNA samples was sequentially hybridized with the indicated 32P-labeled I-309 and MCP-1 probes. B, total RNA was isolated from HMC-1 cells that were either unstimulated or stimulated with PMA in the presence or absence of methylprednisolone (MP), as indicated. A Northern blot of RNA samples was sequentially hybridized with the indicated 32P-labeled probes.

forms of MCP-1 transcripts were also detected in the nuclear but not the cytoplasmic fractions.

Analysis of nuclear and cytoplasmic fractions from cells treated for 2 h with 10 μM methylprednisolone showed levels of I-309 transcripts to be unchanged in both compartments. However, levels of MCP-1 transcripts in both compartments were significantly reduced (60% reduction of nuclear, 48% reduction of cytoplasmic). These results suggest that methylprednisolone-mediated inhibition of MCP-1 mRNA is primarily a nuclear effect, but do not exclude additional effects on cytoplasmic mRNA stability.

In order to directly investigate any effect of glucocorticoid on cytoplasmic mRNA stability, PMA-stimulated HMC-1 cells were incubated in the presence or absence of methylpred-
nisolone for 2 h and were then cultured further in the presence of methylprednisolone treated and untreated cells. Taken together, these results argue that the expression of individual chemokine genes in a mast cell line and in activated T-cells are differentially sensitive to glucocorticoid treatment.

DISCUSSION

Although the chemokines are now recognized as an important group of inflammatory mediators that can be produced by a variety of cell types following appropriate stimulation, mast cell expression of chemokines has not previously been evaluated in a comprehensive fashion. In this report, we showed that the human mast cell leukemia line HMC-1 is a source of a wide array of chemokines, including the CC chemokines I-309, MCP-1, MIP-1α, MIP-1β, and RANTES and the C-X-C chemokine IL-8. Our results therefore confirm and extend a recent study by Moller et al. (35), who demonstrated inducible IL-8 expression in HMC-1. These observations argue that mast cells, in addition to releasing potent mediators such as histamine and arachidonic acid metabolites immediately following stimulation, may be induced to synthesize and secrete a wide array of inflammatory cytokines at later times. Although mast cells have been shown to produce an array of cytokines generally associated with T-cells (5–8), our data suggest that the range of chemokines produced by mast cells may be broader than that produced by T-cells. Since our study examines a mast cell leukemia that does not express FceRs, it will be important to extend our results to normal human mast cells activated via FcεR cross-linking in future studies.

Previous studies have shown that anti-inflammatory glucocorticoids are ineffective at inhibiting histamine release from human lung, intestine, or skin mast cells (36). However, effects on mast cell cytokine synthesis have not been examined in detail. We find that glucocorticoids inhibit basal and inducible transcription of the MCP-1 gene in HMC-1, but fail to inhibit transcription of other C-C chemokines. MCP-1/JE expression was previously shown to be inhibited by glucocorticoids in stimulated human fibrosarcoma cells and synoviocytes (37, 38), in stimulated murine vascular smooth muscle cells and fibroblasts (39, 40), and in ischemic rat kidneys (39), but was not inhibited by glucocorticoids in stimulated human vein endothelial cells (39). This suggests that inhibition could be cell type- or stimulus-specific. Along this line, we note that glucocorticoids inhibited I-309 expression in activated T-cells, but not in HMC-1. Because MIP-1α, MIP-1β, and RANTES transcripts are unaffected by methylprednisolone in HMC-1 and are only mildly reduced in activated T-cells, we conclude that in both cell types, glucocorticoids are not globally effective inhibitors of the expression of chemokines, an important class of inflammatory cytokines.
Previous results indicate that glucocorticoid inhibition of MCP-1/JE mRNA results from mRNA destabilization in stimulated vascular smooth muscle cells (39), but from a transcriptional block in stimulated 3T3 fibroblasts (40). The effects on MCP-1 (in HMC-1 cells) and I-309 (in T-cells) transcripts noted in our experiments are likely to occur at the level of transcription. Although negative regulation of cytokine gene transcription by glucocorticoids is not well understood, a recent study localized targets of inhibition to discrete sites within the IL-2 gene enhancer (41). Inhibition may result from interactions between glucocorticoid receptors and transcription factors that bind to these sites.

Notably, the differential glucocorticoid sensitivity of I-309 expression in mast cells and in T-cells argues that this gene is likely to be induced by different pathways in these two cell types. A detailed analysis of cis-acting regulatory elements, and the definition of the transcription factors that bind to and transactivate gene expression through these elements, will be required to fully understand the differences between these pathways.

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