THE EXPRESSION OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 AND OTHER CHEMOKINES BY OSTEOBLASTS

Dana T. Graves 1, Yanling Jiang 2, and Anthony J. Valente 3

1 Department of Periodontology and Oral Biology, 2 Department of Endodontics, Boston University School of Dental Medicine, Boston, MA 02118, 3 Department of Medicine, University of Texas Health Science Center, San Antonio, Texas

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Expression of chemokines by osteoblastic cells
4. Monocyte chemoattractant protein-1
5. MCP-1 expression by osteoblastic cells in vivo
6. Regulation of MCP-1 expression
7. Summary
8. References

1. ABSTRACT

Chemokines are low molecular weight secretory proteins that function principally as stimulators of leukocyte recruitment. There are four defined chemokine subfamilies based on their primary structure, CXC, CC, C and CX3C. Members of the CC chemokine subfamily, such as monocyte chemoattractant protein 1 (MCP-1) are chemotactic for monocytes and other leukocyte subsets. Because monocytes produce factors that regulate bone formation or resorption, such as PDGF, IL-1 or TNF, chemokines that initiate their recruitment are likely to be important in regulating osseous metabolism. In the studies below, data is presented demonstrating mechanisms of MCP-1 expression in osteoblastic cells. These studies establish that MCP-1 is induced during osseous inflammation and in developmentally regulated bone remodelling, and is associated with enhanced monocyte recruitment when applied to osseous lesions.

2. INTRODUCTION

Chemokines are low molecular weight secretory proteins that function principally to stimulate leukocyte recruitment, and in some cases, leukocyte activation (1,2). They are secondary inflammatory mediators that are induced by primary mediators such as interleukin-1 and tumor necrosis factor. There are four defined chemokine subfamilies based on their primary structure, CXC, CC, C and CX3C. The best characterized are the CC and CXC chemokines. Members of the CC chemokine subfamily, such as monocyte chemoattractant protein 1 (MCP-1), RANTES, MIP-beta and MIP-1alpha are chemotactic for monocytes, subsets of lymphocytes and natural killer cells, whereas CXC chemokines generally, but not exclusively, induce neutrophil chemotaxis.

3. EXPRESSION OF CHEMOKINES BY OSTEOBLASTIC CELLS

Bone remodeling involves the action of osteoclasts and osteoblasts. In a healthy young adults, bone resorption is linked to bone formation, so that the amount of bone loss is coupled with the formation of an equivalent amount of new bone (3). Since leukocytes produce factors capable of modulating both osteoclast and osteoblast activity, their recruitment is likely to represent a significant event in regulating osseous metabolism. Monocytes in particular have been identified as important regulators of bone activity since they produce bone resorptive factors such arachidonic acid metabolites, IL-1, TNF and others. They also produce growth factors which may contribute to the stimulation of bone formation. Thus, by inducing monocyte recruitment, chemokines could indirectly affect bone resorption or formation.

A number of different chemokines are expressed by either osteoblastic cells or osteoclasts. For example, the CC chemokine, MIP-1alpha is expressed by osteoblasts at bone remodeling sites (4). This expression is associated with bone remodelling as osteoclasts are frequently observed in the vicinity of these osteoblasts, suggesting that either osteoclast precursors are recruited by MIP-1alpha or osteoclastogenesis is stimulated by it. The latter is supported by recent findings that MIP-1alpha and MIP-1beta (and IL-8) stimulates motility of osteoclastic cells (6). This does not appear to be a general effect of chemokines, since MIP-1beta, and MCP-1 do not have this effect on osteoclasts (6).

When osteoblastic cells are stimulated with inflammatory mediators in vitro, they produce CC chemokines such as MCP-1, MCP-2, and MCP-3 (7), which can recruit and activate monocytes, and selectively stimulate subpopulations of lymphocytes, NK cells, basophils and eosinophils (1,2). Osteoblastic cells also produce CXC chemokines (GCP-2, IL-8, GRO-alpha, GRO-gamma, and IP-10) that share in common the capacity to specifically recruit and/or activate neutrophils (8). Osteoblasts and osteoclasts produce IL-8 constitutively.
Expression of MCP-1 and other chemokines by osteoblasts

This production is enhanced by IL-1beta or TNF-alpha stimulation (9,10). IL-8 production has been found in Pagetic osteoblast-like cells and osteosarcoma cells (11). Human osteoclastic cells grown in culture produce high levels of IL-8 when stimulated by the pro-inflammatory factors, IL-1 and TNF (10). The expression of IL-8 may be important in bacterial infection of bone since IL-8 is a potent neutrophil activator and chemotactic.

Chemokine expression may play an important role in inflammatory cell recruitment in cartilage and bone destruction that occurs in rheumatic disease. Interleukin-8 (IL-8), MIP-1alpha and MCP-1 have been implicated in rheumatoid arthritis (RA), while MIP-1alpha has been detected in synovial arthritis (12,13). Osteoblastic cells cultured from patients with rheumatoid arthritis or osteoarthritis exhibit strong induction of MCP-1, MIP-1alpha and IL-8 (14). Chemokine production has also been implicated in failures of total hip replacement implants. When histologic sections of failed hip replacements are analyzed, polyethylene debris is surrounded by macrophages at the bone-implant interface (15). Expression of chemokine mRNAs is also observed at these sites. Thus, particles released from wear of hip prostheses may induce the production of cytokines and chemokines leading to the recruitment of inflammatory cells. The production of bone-resorptive cytokines by these cells could then lead to loosening of the implant.

4. MONOCYTE CHEMOTACTIC PROTEIN-1

MCP-1, a monomeric polypeptide, is typically secreted in two predominant forms with molecular weights of 9 and 13 kD. Lectin blots indicate that disaccharide galactose-b-3D-N-acetyl galactosamine is present on the 13 kD MCP-1 isoform but not the 9 kD isoform. MCP-1 induces recruitment of monocytes, a subset of T lymphocytes, eosinophils, and basophils (1,2). Leukocyte recruitment requires coordinated activity involving the expression of adhesion molecules on endothelial cells and leukocytes. Chemotactic signals induce the activation of integrins on monocytes, particularly integrins of the β2 family, which interact with counter-receptors on vascular endothelial cells. MCP-1 has been shown to both up-regulate the expression of β2 integrin on monocyte cell surfaces (figure 1) and the activation of these molecules. In addition, β2 integrins are involved in the adhesion of monocytes to extracellular matrix proteins in response to chemokines such as MCP-1 (figure 2). After diapedesis, monocytes migrate through connective tissue by binding to extracellular matrix proteins such as collagen, fibronectin and laminin. Monocyte migration across these proteins in response to MCP-1 also is dependent upon β2 integrins (figure 3).

Significant expression of MCP-1 in normal cells usually requires stimulation by pro-inflammatory agents, while it appears to be constitutively produced by different human tumors (16-19). MCP-1 expression may account for much of the monocyte chemotactic activity produced by tumor cells and contribute to the presence of tumor-
Expression of MCP-1 and other chemokines by osteoblasts

Figure 3. Differential Effect of mAb TO beta-1 and beta-2 Integrins On Monocyte Migration On Laminin or Fibronectin. The role of integrins in monocyte migration was measured using the sub-agarose migration assays as we described in (70). Blockage of monocyte migration with mAb's directed to beta-1 and beta-2 integrins (mAb13 & L130 respectively) on (A) laminin and (B) fibronectin. Each data point represents the mean ± S.E.M. of quadruplicate samples. Reproduced with permission from: Amer. Journal of Physiology 267: C1112-C1118, 1994 (The American Physiological Society).

associated macrophages. MCP-1 was first purified by Valente and co-workers (20) and subsequently sequenced independently by two different groups (21,22). The murine form of MCP-1, JE, was initially identified as an immediate early gene induced in response to platelet-derived growth factor (23). The structure and properties of murine and human MCP-1 have recently been reviewed by Rollins (24). It is striking that MCP-1 is expressed in a number of inflammatory conditions associated with monocyte recruitment, including delayed hypersensitivity reactions, atherosclerosis, pulmonary fibrosis, bacterial infection, arthritis, and renal disease (1,2,24). MCP-1 stimulates monocyte chemotaxis, as well as many of the cellular events associated with chemotaxis (i.e., Ca^{2+} flux and integrin expression). In monocytes, MCP-1 weakly induces cytokine expression (25). In addition, this chemokine elicits a respiratory burst at high concentrations, which leads to the generation of oxygen radicals (26). MCP-1 is also a potent inducer of histamine release from basophils and has been implicated as an important mediator in allergic inflammation (27). The principal effect of injecting MCP-1 in vivo is the recruitment of monocytes (21).

In early studies, MCP-1 was shown to bind to freshly isolated monocytes through high affinity cell surface binding sites (28,29). Subsequently, a MCP-1 receptor (CCR2) was cloned (30) and identified as a member of the large family of G-protein-coupled protein receptors that contain 7 transmembrane-spanning domains. Two forms of the receptor were identified (CCR2-A and CCR2-B) that differ only in the C-terminal cytoplasmic domain of the protein and apparently arise by alternative splicing. Both forms of the receptor mediate ligand-dependent Ca^{2+}-mobilization, cellular migration and inhibition of adenyl cyclase. However, differences in the C-terminal appear to result in specificity for Gα-protein subtypes, suggesting that there are functional differences in the CCR2 subtypes (31). The CC chemokine receptors identified so far all demonstrate multiple ligand specificities. In addition to MCP-1, CCR2 binds the related chemokines, MCP-2, MCP-3 and MCP-4 (2,24). Similarly, MCP-1 has been shown to bind the receptor CCR4, which also binds RANTES and MIP-1alpha. This cross-reactivity between ligands and receptors suggests a measure of redundancy in the chemokine system for the trafficking and activation of leukocytic cells. However in CCR2-knockout mice, the recruitment of monocyte/macrophages in response to some inflammatory stimuli is markedly impaired as is the ability of macrophages to clear infection by the intracellular bacterium Listeria monocytogenes (32,33). Furthermore, disruption of the MCP-1 gene also leads to impairment in the monocyte recruitment process and shares features with the CCR2 knockout model (34). Thus despite multiple ligand specificity, some functions of the chemokines and chemokine receptors may remain unique and essential.

Although chemokines were first described based upon their capacity to stimulate recruitment or activation of leukocytes, it has become evident that they possess other functions, including regulating events in bone marrow. For example, MCP-1 in conjunction with TGF-beta, inhibits cycling of early progenitor cells in the bone marrow (35). In support of this, antibody to MCP-1 antagonizes the inhibitory effect that long-term cultures of stromal cells have on progenitor cell proliferation.

5. MCP-1 EXPRESSION BY OSTEOBLASTIC CELLS IN VIVO

Our laboratories have recently focused on the expression of MCP-1 and bone cells. These studies have elucidated mechanisms for the regulated expression of MCP-1, which may be generally applicable to other cell types. The significance of these studies stems from the likelihood that MCP-1 expression (as well as that of other
remodelling occurs postnatally in erupting teeth. Bone
observation that developmentally regulated bone
remodeling bone (38). These studies took advantage of the
developmentally regulate d expression of MCP-1 in
response to bacterial stimulation (37).

bone.

the recruitment of monocyt es/macrophages to inflamed
suggest that MCP-1 is an important mediator involved in
present (r = 0.69, p < 0.01).

osteolytic lesions (figure 4) . Correlation analysis revealed
mononuclear phagocytes and MCP-1 production in these
and a temporal association with the recruitment of
principal cells expressing MCP-1 in inflamed bone, while
murine mandible, and ce lls expressing MCP-1 were
experiments, inflammatory lesions were created in the
osseous inflammation is induced (36). In these

Expression of MCP-1 and other chemokines by osteoblasts

Figure 4. MCP-1 Expression Is Temporally Correlated
With The Recruitment Of Mononuclear Phagocytes. To
statistically correlate the expression of MCP-1 and the
recruitment of monocytes/macrophages in bone, osseous
lesions were induced in BALB/c mice. Experimental
animals were sacrificed at 0 (no exposure), 7, 14, 21, or
42 days after induction of inflammation. Immunohistochemistry was carried out using a polyclonal
antiserum to murine MCP-1 (closed triangles) and adjacent
sections were incubated with F4/80 monoclonal antibody to
detect mononuclear phagocytes (open circles). The graph
shows the percentage of MCP-1 positive cells lining bone
and the number of F4/80 positive cells in the same field.
The X-axis represents the number of days following
osseous induction with 0 (zero) representing control
animals. Bars represent the SEM. Statistical analysis of
MCP-1 positive cells lining bone and F4/80 positive cells
showed them to be significantly correlated (N = 15, r =
0.69, p <= 0.01). Reproduced with permission from: Endocrinology 136: 2752-2759, 1995.

chemokines) affects osseous metabolism. Because of its
role in stimulating monocyte recruitment, we investigated
MCP-1 expression by osteoblasts under conditions where
osseous inflammation is induced (36). In these
experiments, inflammatory lesions were created in the
murine mandible, and cells expressing MCP-1 were
identified by immunohistochemistry. Osteoblasts were the
principal cells expressing MCP-1 in inflamed bone, while
MCP-1 expression was virtually non-existent in non-
inflamed normal bone. Moreover, there was both a spatial
and a temporal association with the recruitment of
mononuclear phagocytes and MCP-1 production in these
osteolytic lesions (figure 4). Correlation analysis revealed
that the number of MCP-1 positive cells was significantly
associated with the number of monocytes/macrophages
present (r = 0.69, p < 0.01). These in vivo results strongly
suggest that MCP-1 is an important mediator involved in
the recruitment of monocytes/macrophages to inflamed
bone. In vitro studies support the notion that MCP-1 is one
of the principal CC chemokines induced in osteoblasts in
response to bacterial stimulation (37).

The above approach was expanded to investigate
developmentally regulated expression of MCP-1 in
remodeling bone (38). These studies took advantage of the
observation that developmentally regulated bone
remodelling occurs postnatally in erupting teeth. Bone
resorption occurs above the tooth, along the path of
resorption, while bone formation occurs at the opposite end
(39). In a murine model, on days 5 and 8 postpartum, the
greatest number of MCP-1 positive cells was observed in the
occlusal area of the erupting first molar (figure 5). Bone
lining cells consistent with osteoblasts were the principal
cell type expressing MCP-1. There was significant
correlation between the number of MCP-1 positive cells
and monocyte recruitment. An association between the
formation of osteoclasts and MCP-1 expression was also
noted, suggesting that the recruited monocytes may act to
enhance osteoclastogenesis. In the area of bone formation,
MCP-1 production coincided with monocyte recruitment.
This was unexpected since monocyte recruitment had
previously been correlated with resorption but not bone
formation. It is possible, though not proven, that monocytes
recruited to the erupting side of the tooth participate in
bone resorption by elaborating bone resorbing cytokines,
whereas those recruited to sites of bone formation would be
functionally distinct. The latter could promote bone
formation through the production of growth factors. Que
and Wise have similarly described a relationship between
tooth eruption and MCP-1 expression (40). They reported
that mediators which promote bone-remodelling, such as
IL-1, TGF-beta and CSF-1, enhance the expression of the
MCP-1 in areas of tooth eruption in vivo and in cultured
cells associated with tooth eruption in vitro (41). Thus,
expression of MCP-1 may be critical for recruiting
monocytes to initiate the cellular events of developmentally
regulated bone remodelling which occurs during tooth
eruption.

To investigate the potential effect of MCP-1 on
the regulation of bone in vivo, experiments were performed
in which exogenous MCP-1 was applied, and the impact on
monocyte recruitment and osteoblast number was assessed
(42). At day 5 following MCP-1 application, a 3-fold
increase in the number of mononuclear phagocytes was
observed (figure 6). By day 28, the number of monocytes
had returned to the baseline level, indicating that MCP-1
caused a transient increase in recruitment. Furthermore,
MCP-1 application resulted in a significant 2-fold increase
in the number of osteoblasts compared with controls treated
with vehicle alone. To rule out the possibility that MCP-1
might directly stimulate proliferation of osteoblastic cells,
in vitro experiments were undertaken and demonstrated
that it was not mitogenic for osteoblastic cells (42). It is
possible that MCP-1 induces recruitment of mononuclear
phagocytes, which in turn produce mitogenic factors that
stimulate osteoblast proliferation. These results are
consistent with reports that monocyte/macrophage products
are mitogenic for osteoblastic cells in vitro (43). Thus,
MCP-1 expression may be indirectly associated with bone
formation as suggested by the tooth eruption studies.

6. REGULATION OF MCP-1 EXPRESSION

It is thought that monocytes play an important
role in regulating osseous metabolism. Significant
monocyte recruitment occurs at sites of bone injury and
remodeling. As mentioned above, monocytes affect bone
through the production of factors that can stimulate bone
Expression of MCP-1 and other chemokines by osteoblasts

Figure 5: Quantitation of MCP-1 Positive Cells, Monocytes And Osteoclasts During Tooth Eruption. Mice were sacrificed from birth to day 14 postpartum. Adjacent serial sections of the mandibular first molars were examined to assess changes in MCP-1 expression., Monocyte recruitment and osteoclastogenesis during developmentally regulated tooth eruption. MCP-1 immunopositive osteoblastic cells, F4/80 positive mononuclear phagocytes, and bone-lining TRAP positive osteoclasts were counted using an image analysis system as described in (38). A,B,C: occlusal area (bone resorption); D,E,F: basal area (bone formation). Reproduced with permission from (American Journal of Pathology) 150 #5 May (1997).

resorption or formation. Our laboratory and that of Van Damme and colleagues were the first to demonstrate that MCP-1 could be expressed by osteoblastic cells in vitro (17,18). The studies described below have addressed the regulation of MCP-1 expression in osteoblastic cell lines and normal osteoblastic cells.

The cell line, MG-63, has been used as an osteoblastic cell “model” for chemokine studies. In addition to MCP-1, MCP-2 and MCP-3 were isolated as novel monocyte chemoattractants from conditioned medium of MG-63 cells (7). MCP-2 and MCP-3 share with MCP-1 a target specificity for monocytes, activated T lymphocytes and basophils, but unlike MCP-1, they are also active for eosinophils (44,45). In most non-transformed cells, chemokines are induced by inflammatory mediators. For example, TNF-alpha induces murine MCP-1 expression via activation of fos and jun in an osteoblastic cell line (46,47). The CXC chemokine, IP-10, is also expressed by osteoblastic cells and its expression is regulated by cytokines such as IFN-gamma, IL-1-alpha, and TNF-alpha (48). A number of other CXC chemokines (GCP-2, IL-8, GRO-alpha, and GRO-gamma) have also been isolated from the conditioned medium of MG-63 cells (8). GCP-2, IL-8, GRO-alpha and GRO-gamma contain the characteristic ELR amino acid motif found in CXC chemokines between the N-terminus and the first cysteine residue and are potent chemoattractants for neutrophils (49).

Williams and colleagues tested normal human osteoblastic cells to identify monocyte chemoattractants produced by stimulated osteoblasts (50). Results showed that IL-1 stimulation induced normal human osteoblastic cells to produce monocyte chemotactic activity. Without IL-1 stimulation, osteoblast-conditioned medium had no effect on monocyte chemotaxis. The addition of IL-1 induced the expression of monocyte chemotactic activity in the medium within 6 hours. Since the increase in chemotactic activity was time-dependent, this indicated that it was not simply caused by the presence of IL-1 in conditioned medium. In fact these studies confirmed reports that IL-1 alone has little or no chemotactic activity for monocytes. Evidence that MCP-1 antiserum inhibits virtually all IL-1 stimulated monocyte chemotactic activity produced by normal human osteoblastic cells suggested that MCP-1 is an important osteoblast-produced chemokine (figure 7).

In vitro studies have shown that for most normal cell types there is little or no MCP-1 expression and exogenous stimulation is required for induction of MCP-1. Zhu et al studied the effects of several different classes of biologic mediators on MCP-1 expression in normal human osteoblastic cells (table 1). Cells were cultured in serum-free medium with or without factors known to regulate osseous metabolism. After 24 hours, little MCP-1 expression (< 5 ng/mL) was found in medium conditioned by unstimulated cells or in cells that were stimulated with non-inflammatory calcitropic mediators (PTH, Vit D, hydrocortisone). However, physiologic concentrations of MCP-1 were found in conditioned medium from cells treated with IL-1beta, TNF-alpha, IL-6, and TGF-beta.
Expression of MCP-1 and other chemokines by osteoblasts

Table 1. Secretion of MCP-1 by normal human osteoblasts

<table>
<thead>
<tr>
<th>Addition</th>
<th>MCP-1 (ng/ml)</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt;5</td>
<td>14 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>PTH (10 nM)</td>
<td>&lt;5</td>
<td>9 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>1,25(OH)2D3 (20 nM)</td>
<td>&lt;5</td>
<td>10 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone (140 nM)</td>
<td>&lt;5</td>
<td>11 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>PDGF-BB (15 ng/ml)</td>
<td>&lt;5</td>
<td>14 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>TGF-beta (2 ng/ml)</td>
<td>7 ± 1.1</td>
<td>11 ± 0</td>
<td></td>
</tr>
<tr>
<td>IL-6 (20 nM)</td>
<td>19 ± 0</td>
<td>23 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>TNF-alpha (20 ng/ml)</td>
<td>48 ± 2.6</td>
<td>93 ± 7.6*</td>
<td></td>
</tr>
<tr>
<td>IL-1beta (50 u/ml)</td>
<td>63 ± 1.7</td>
<td>84 ± 7.0*</td>
<td></td>
</tr>
</tbody>
</table>

Serum-free cultures of normal human osteoblastic cells were incubated with physiologic concentrations of PTH, 1,25(OH)2D3, hydrocortisone, PDGF-BB, IL-1beta, TGF-beta, or vehicle control. Conditioned media was collected after 24 or 48h and MCP-1 was assayed by RIA. Values are mean ± SEM of triplicate samples. The minimum detectable level of MCP-1 was 5ng/ml. Values that are significantly different from the unstimulated control are indicated with an * for the 48 h time point (p<0.01). Statistical significance was not determined for the 24 h period because the value of the negative control was below the detection threshold. Table 1 is reproduced from permission from: Bone, 21:321-327, 1997 (Elsevier Science Ltd).

Figure 6: MCP-1 Application In vivo Is Associated With An Increase in Osteoblast Number. Creation of osseous lesions and application of MCP-1 or the vehicle alone (control), was performed as described in (42). Animals were sacrificed five or twenty eight days following application. Monocytes/macrophages were counted as F4/80 immunopositive cells and counted with the use of an image analysis system. * indicates statistically significant compared to the negative control (p<0.01). Reproduced with permission from: Bone, 21:321-327, 1997 (Elsevier Science Ltd).

These investigators also examined the effect of osteotrophic mediators on MCP-1 mRNA levels in two osteoblastic cell lines, SaOS-2 and MG-6 (51). Constitutive expression of MCP-1 was very high in MG-63 cells and low in SaOS-2 cells. Detectable levels of MCP-1 mRNA were observed in both cell lines after stimulation with TNF-alpha (20 ng/ml). Similar to results obtained with normal cells, in the SaOS-2 cells, IL-1beta and TNF-alpha induced dose-dependent increases in the MCP-1 mRNA level of 36-fold and 28-fold, respectively, TGF-beta and IL-6 induced increases of approximately 3-fold each. In MG-63 cells, the following dose-dependent increases in the MCP-1 mRNA levels were seen: TNF-alpha, 20 fold; IL-1beta, 15 fold; TGF-beta, 9 fold; and IL-6, 2 fold.

The above studies indicate that MCP-1 is regulated by inflammatory cytokines. Several cis regulatory elements and trans-acting factors involved in this regulation have been identified in a variety of cell types. NF-kappaB binding sites appear to be involved in response to IL-1beta and TNF-alpha, (52-54). Additional cis elements close to these kappaB sites (55,56) and a 7 bp response element in the 3' untranslated region of the MCP-1 promoter have also been shown to mediate MCP-1 transcription in 3T3 cells in response to PDGF (55-57). Interestingly, the response elements to IL-1 and TNF appear to lie at some distance from the transcription initiation site. There is also a cluster of regulatory elements in the proximal promoter region, including response elements for the AP-1 trans-acting factors, which appear to mediate TPA-induced MCP-1 transcription (58,59).

Valente et al investigated the regulation of MCP-1 expression by interferon-gamma (60). IFN-gamma is a leukocyte-derived cytokine that has been shown to inhibit osteoclast formation (61) and yet paradoxically, it has been shown to be an effective treatment for osteopetrotic patients who suffer from an inadequate amount of osteoclasts (62). A number of studies have shown that IFN-gamma rapidly induces MCP-1 mRNA accumulation and MCP-1 in a variety of cell types (63-66). In some cell types MCP-1 is regulated at the post-transcriptional level (67). To assess the level at which MCP-1 mRNA levels were enhanced by IFN-γ, nuclear run on experiments were carried out in MG-63 osteoblastic cells (figure 8). Although the basal levels of MCP-1 mRNA are high in MG-63 cells, IFN-gamma stimulation resulted in a marked increase in MCP-1 transcription. In the same report, it was also shown that IFN-γ did not enhance MCP-1 mRNA stability. Thus, MCP-1 expression in IFN-gamma-stimulated osteoblastic cells is regulated at the transcriptional level.

IFN-gamma has been shown to induce transcription in several genes through a transcriptional element termed the GAS (gamma interferon activated site). The consensus sequence (TTNCNNNAA) has been defined for GAS. A GAS element exists within the proximal promoter region of MCP-1 at -214 relative to the ATG start codon (figure 9A.). The core sequence of this element (TTCTTGGAA) resembles closely the symmetrical dyad sequence defined as the binding site for the Stat family of transcription factors (with the exception of Stat2).
Expression of MCP-1 and other chemokines by osteoblasts

Figure 7. Antibody to MCP-1 Inhibits Monocyte Chemotactic Activity Produced by Stimulated Osteoblasts. Normal human osteoblasts were stimulated with IL-1β and the conditioned media was collected and diluted with plain media as indicated. Each dilution was preincubated with MCP-1 antiserum (closed circles) or control IgG (open circles). Samples were then tested for recruitment of peripheral blood monocytes using a modified Boyden chamber as indicated in (50). Reproduced with permission from: Amer. Journal of Physiology, 263:C194-C199, 1992 (The American Physiological Society).

Figure 8. Nuclear Run-on Analysis of MCP-1 Transcription. MG-63 cells were incubated with or without (100 U/ml) IFN-gamma for 6 h. Nuclei from control or IFN-gamma-stimulated cells were isolated, and 32P-labeled nuclear run-on products were hybridized to denatured plasmid DNAs slot-blotted on nylon membrane. Bands represent hybridization with MCP-1 (row 1), GAPDH (row 2), and pBluescript vector without insert (row 3). Reproduced with permission from: Amer. Journal of Physiology, 263:C194-C199, 1992 (The American Physiological Society).

Adjacent and 5' to this site in the MCP-1 promoter is a CT-rich element (GCTT CCCT TTCC TAC) which shows some homology to the consensus sequence for the interferon-stimulated response element (ISRE) (68), that has been shown to mediate transcriptional responses to both IFN-γ and IFN-α/β. The importance of this site is suggested by the high degree of conservation between species (figure 9B).

Transfection studies were carried out with a deletion series of MCP-1 promoter/luciferase plasmids to identify and characterize the IFN-γ-responsive elements (figure 10). When the largest construct tested was examined, construct ‘a’ (-2910/+21), IFN-γ stimulated a 1.7 fold increase in luciferase activity compared to unstimulated controls. Construct ‘a’ included the κB and other regulatory elements located in the distal enhancer region that have recently been shown to regulate MCP-1 transcription in response to IL-1β and TNF-α (52). In construct ‘b’ (-248/+21) and ‘c’ (-227/+21) the functional κB site and the majority of the 5' flanking sequence were eliminated. This IFN-gamma induced a 3 fold increase in luciferase activity in these constructs, indicating that they distal enhancer elements in the MCP-1 promoter are not required for IFN-gamma induced transcription in these cells. Since construct C contained a GAS element and an adjacent CT rich element, construct ‘d’ (-214/+21) was prepared so that it terminated at the 5' end of the GAS element and eliminated the adjacent CT element. Unexpectedly, this construct produced the greatest increase in the reporter gene activity observed in these experiments (≥10 fold). Thus the CT element appears to inhibit the response to IFN-gamma-mediated by the GAS element. When the GAS element was deleted (construct ‘e’, -198/+21), responsiveness to IFN-gamma was lost completely. Therefore these experiments indicate that in the MG-63 osteoblastic cell line, the rapid IFN-gamma-induced increase in transcription of MCP-1 appears to be mediated entirely through the GAS element located at position -214 to -198, whereas the adjacent CT element appears to negatively regulate this GAS-mediated response. We have termed this CT element the interferon response inhibitory sequence (IRIS), which describes its observed functional activity.

7. SUMMARY

The above studies demonstrate that chemokines are expressed by osteoblastic and osteoclastic cells. More specifically, the chemokine MCP-1 is produced by osteoblastic cells in vitro and in vivo, and this expression occurs in inflamed bone or is induced by inflammatory mediators. Furthermore, the application of MCP-1 in osseous lesions enhances that degree of monocyte recruitment. Because chemokines are both proinflammatory and upregulated by proinflammatory cytokines, their actions might contribute to bone resorption associated with inflammatory osteolytic lesions. However, they may also be components of the physiologic regulation of bone resorption since some chemokines are expressed at low levels constitutively by cells of osteoblastic lineage.

8. REFERENCES

Expression of MCP-1 and other chemokines by osteoblasts

Figure 9. Nucleotide sequence of the proximal 5' flanking region of the MCP-1 gene. A: Sequence from the National Center for Biotechnology (NCBI) GeneBank accession # D26087. Numbers refer to nucleotide positions relative to the translation start site. The IFN-gamma response element is underlined. B) Sequences from bovine, rat and murine MCP-1 showing homology with the human MCP-1 IFN-gamma response element. Numbers refer to position of the 5' nucleotide relative to the translation start site. Sequences taken from the GeneBank at NCBI.

Figure 10. Identification of the IFN-gamma Responsive Region In The Human MCP-1 Promoter. A schematic diagram indicating the location of the deletion constructs in the MCP-1 gene 5' flanking region and the resulting responsiveness to IFN-gamma in transfected MG-63 cells. Cells were transfected with the MCP-1/luciferase constructs and the CMV-Renilla plasmid (transfection efficiency control) as described in Materials and Methods, incubated for 3 days, then stimulated with either 1000 U/ml human recombinant IFN-γ or with medium (unstimulated control) for 4 h. Luciferase activity was determined and normalized to renilla activity. Results are expressed as fold stimulation of luciferase activity.

Expression of MCP-1 and other chemokines by osteoblasts


35. Cashman JD, C.J. Eaves, A.H. Sarris, A.C. Eaves: MCP-1, not MIP-1alpha, is the endogenous chemokine that cooperates with TGF-beta to inhibit the cycling of primitive normal but not leukemic (CML) progenitors in long-term human marrow cultures. *Blood* 92, 2338-44 (1998)


Expression of MCP-1 and other chemokines by osteoblasts


