Macrophage Inflammatory Protein-1 Alpha (MIP-1 alpha)/CCL3: As a Biomarker

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Abstract

Macrophage inflammatory protein-1 alpha (MIP-1α/CCL3) is a chemotactic chemokine secreted by macrophages. It performs various biological functions, such as recruiting inflammatory cells, wound healing, inhibition of stem cells, and maintaining effector immune response. It activates bone resorption cells and directly induces bone destruction. Cells that secrete MIP-1α/CCL3 are increased at sites of inflammation and bone resorption. MIP-1α/CCL3 plays an important role in the pathogenesis of various inflammatory diseases and conditions that exhibit bone resorption, such as periodontitis, multiple myeloma, Sjögren syndrome, and rheumatoid arthritis. Biological fluids from patients with these diseases exhibit elevated levels of MIP-1α/CCL3. This finding indicates that MIP-1α/CCL3 protein may have diagnostic potential for the detection of several inflammatory diseases and conditions. This chapter discusses the biological functions of MIP-1α/CCL3; describes several diseases associated with MIP-1α/CCL3, particularly periodontitis; and delineates the potential application of MIP-1α/CCL3 as a biomarker.

List of Abbreviations

Aa	Aggregatibacter actinomycetemcomitans
ARDS	Acute Respiratory Distress Syndrome
BALF	Bronchoalveolar Lavage Fluid
CCR1	Chemokine (C-C motif) Receptor 1
CCR5	Chemokine (C-C motif) Receptor 5
CFU-A	Colony-Forming Unit-Adipocyte
CFU-S	Colony-Forming Unit-Spleen
CX3CR1	CX3C Chemokine Receptor 1
CXCR4	CXC Chemokine Receptor 4
GCF	Gingival Crevicular Fluid
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICAM-1	Intracellular Adhesion Molecule
Ig	Immunoglobulin
IL-1	Interleukin-1
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
IP-10/CXCL10	Interferon-Gamma-Inducible Protein-10/Chemokine (CXC) Ligand 10

J774.2	Cell Line
LAgP	Localized Aggressive Periodontitis
LPS	Lipopolysaccharide
MCP-1/CCL2	Monocyte Chemoattractant Protein-1
MIP-1α/CCL3	Macrophage Inflammatory Protein-1 Alpha/Chemokine (C-C Motif) Ligand 3
MIP-1β/CCL4	Macrophage Inflammatory Protein-1 Beta/Chemokine (C-C Motif) Ligand 4
MM	Multiple Myeloma
MNP	MIP-1 Alpha Nuclear Protein
NK	Natural Killer
OHI	Oral Hygiene Instruction
OSCC	Oral Squamous Cell Carcinoma
PDL	Periodontal Ligament
PGE2	Prostaglandin E_2
PICF	Peri-implant Crevicular Fluid
PMN	Polymorphonuclear
RA	Rheumatoid Arthritis
RANTES/CCL5	Regulated on Activation Normal T Cell Expressed and Secreted/Chemokine (C-C motif) Ligand 5
RSV	Respiratory Syncytial Virus
RT-PCR	REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION
S-BALP	Serum Bone-Specific Alkaline Phosphatase
SCI	Stem Cell Inhibitor
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SRP	Scaling and Root Planing
SS	Sjögren Syndrome
TIMP	Tissue Inhibitors of Metalloproteinases
TNF-α	Tumor Necrosis Factor Alpha
UDPD	Urine Deoxypyridinoline

Key Facts of MIP-1α/CCL3

- MIP-1α/CCL3 is a type of chemotactic cytokine.
- MIP-1α/CCL3 belongs to the CC subfamily of chemokines.
- MIP-1α/CCL3 was first discovered by Stephen D. Wolpe in 1988.
- MIP-1α/CCL3 is a multifunction peptide that is secreted by a variety of hematopoietic and non-hematopoietic cells upon stimulation.
- MIP-1α/CCL3-expressing cells are usually found in areas of inflammation and bone resorption.

- MIP-1α/CCL3 recruits macrophages, lymphocytes, and eosinophils via the CCR1 or CCR5 receptor.
- MIP-1α/CCL3 preferentially attracts activated CD8$^+$ T cells.
- MIP-1α/CCL3 exerts strong HIV-suppressive activity.
- MIP-1α/CCL3 plays an important role in the bone remodeling process by inducing osteoclastogenesis.
- MIP-1α/CCL3 is associated with periodontitis and multiple myeloma because of MIP-1α/CCL3's biological functions of recruiting inflammatory cells and osteoclastogenesis activity.
- Increased concentrations of MIP-1α/CCL3 can be detected in biological fluids of persons suffering from inflammatory and osteoclastogenic diseases.

Definitions of Words and Terms

Chemokine Chemokines are type of cytokines that play an important role in recruiting various cells.

Biomarker A protein that can be detected in tissue or bodily fluids and that helps differentiate a disease state from a normal biological state.

Bone Remodeling The process of bone resorption and bone formation.

Sequence Homology Identical gene sequences.

Tumor Metastasis Migration of tumor cells from the original site to a distant site (other tissue).

Transcriptional Factors Proteins that help regulate gene expression (increase or decrease).

Introduction

The mediators responsible for recruitment, activation, and migration of immune and inflammatory cells to the site of inflammation have been studied for years. Many of these mediators are cytokines and chemokines. Cytokines belong to a group of signaling proteins that are secreted by various cells after cellular activation (Foster 2001). Growth factors, inflammatory cytokines, and chemokines are types of cytokines. Chemokines play an important role in recruiting inflammatory and immune cells to the site of infection or inflammation. They are also known as chemoattractant cytokines (Foster 2001). The typical structure of a chemokine consists of a configuration of four cysteine residues. Depending on the presence or absence of amino acid between the first two cysteine residues, chemokines are classified into two main subfamilies, CXC (alpha) and CC (beta), with the X denoting one amino acid that
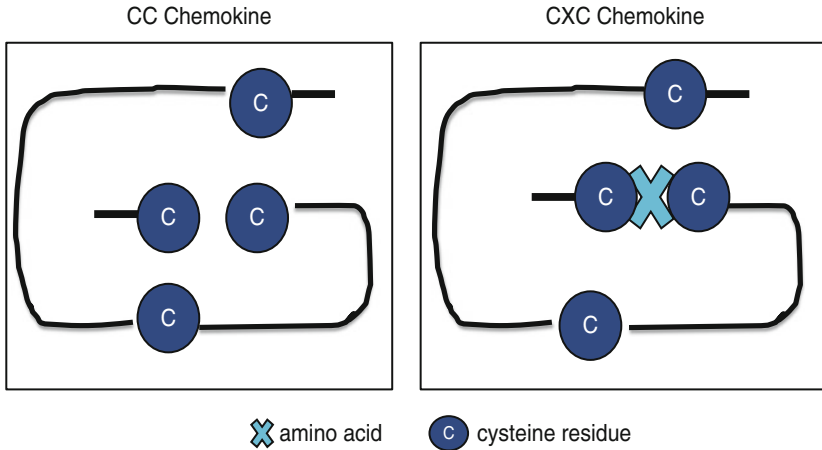
Fig. 1 **Structure of chemokine**. Schematic representation of two main families of chemokines. In CC chemokine, the first and second conservation cysteine residues are adjacent to each other. In CXC chemokine, the first two cysteine residues are separated by an amino acid

separates the two terminal cysteines (Fig. 1). The configuration of chemokines in the CC subfamily consists of two adjacent cysteines (Fernandez and Lolis 2002).

Chemokines are associated with various biological functions such as homeostasis, leukocyte recruitment, wound healing, tumor metastasis, angiogenesis, and activation of the innate and adaptive immune responses (Murphy et al. 2000; Rossi and Zlotnik 2000; Fernandez and Lolis 2002). The biological functions of chemokines occur after activating specific G protein-coupled protein receptors found on the surface of a variety of cells including monocytes/macrophages, neutrophils, basophils, eosinophils, dendritic cells, lymphocytes, and platelets (Menten et al. 2002b). Upon activation, chemokines induce migration and accumulation of inflammatory cells at the source of chemokine production. It is interesting to note that chemokines can bind to more than one chemokine receptor (Fig. 2). For example, chemokine macrophage inflammatory protein-1 alpha (MIP-1α/CCL3), a chemoattractant cytokine associated with the recruitment of leukocytes in response to tissue injury or inflammation, can bind to chemokine receptors CCR1 and CCR5 (Maurer and von Stebut 2004). Owing to these dual receptors, multiple cells can be recruited by this protein.

This chapter focuses on the chemokine MIP-1α/CCL3, its biological functions, its association with several inflammatory diseases, and its potential use as a biomarker for the diagnostic assessment of various diseases, particularly periodontal disease.

Background

Macrophage inflammatory protein (MIP)-1α/CCL3 is an inflammatory chemokine produced by cells during infection or inflammation. It belongs to the CC chemokine family, which displays potent chemotactic properties. The MIP-1 protein was first
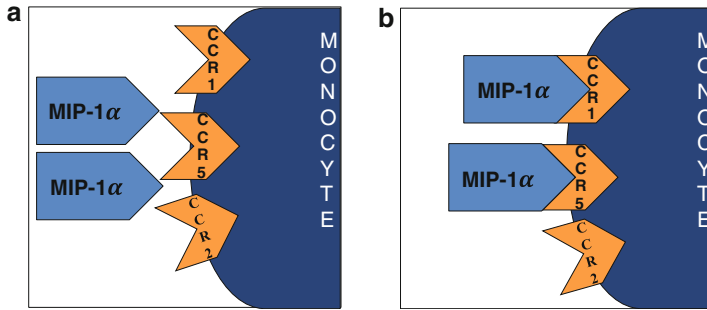
Fig. 2 **A chemokine can bind to more than one chemokine receptor**. (**a**) Schematic representation of MIP-1α/CCL3 (chemokine) approaching toward the cell expressing the chemokine receptors CCR1, CCR2, and CCR5. (**b**) MIP-1α/CCL3 binds to both CCR1 and CCR5 (chemokine receptors)

identified by Stephen D. Wolpe in 1988. His research group reported the appearance of a new heparin-binding protein from the murine macrophage cell line in response to endotoxin stimulation. This protein was called macrophage inflammatory protein (MIP) because of its biological function of inducing an inflammatory response characterized by neutrophil infiltration (Wolpe et al. 1988). The initial report of the separation of MIP-1 on SDS-PAGE gel was described by Sherry et al. (1988). Their findings suggested that the MIP-1 protein was composed of two peptides. Partial sequencing revealed two proteins: MIP-1α and MIP-1β. Although these proteins exhibited slight variation in the sequence of amino acid residue at their NH_2 terminals, they share approximately 56.7 % sequence homology (Sherry et al. 1988). The genomic nucleotide sequence of murine MIP1α is highly homologous to human counterparts LD78alpha/GOS19-1/SCYA3 (Widmer et al. 1991). Many researchers have independently isolated human and murine MIP-1α and MIP-1β in their laboratories. For this reason, MIP-1α has more than one name in published reports. However, with the introduction of the new nomenclature, human MIP-1α is currently called chemokine (C-C motif) ligand 3 (CCL3), and MIP-1β is called chemokine (C-C motif) ligand 4 (CCL4) (Zlotnik and Yoshie 2000).

MIP-1α/CCL3 is synthetized as a precursor protein with 92 amino acids. The premature protein splits at several sites, resulting in the formation of biologically active mature protein. Chemokines exhibit a high affinity toward proteoglycans. An increased tendency of MIP-1α/CCL3 to bind with heparin (a common proteoglycan) has been demonstrated experimentally by Wolpe et al. (1988), and this binding can enhance their activity (Ali et al. 2000).

Cells Secreting MIP-1α/CCL3

Most mature hematopoietic cells can induce the synthesis of MIP-1α/CCL3 and MIP-1β/CCL4. Monocytes, T lymphocytes, B lymphocytes, neutrophils, dendritic cells, and natural killer cells are known to secrete MIP-1α/CCL3 (Fig. 3)
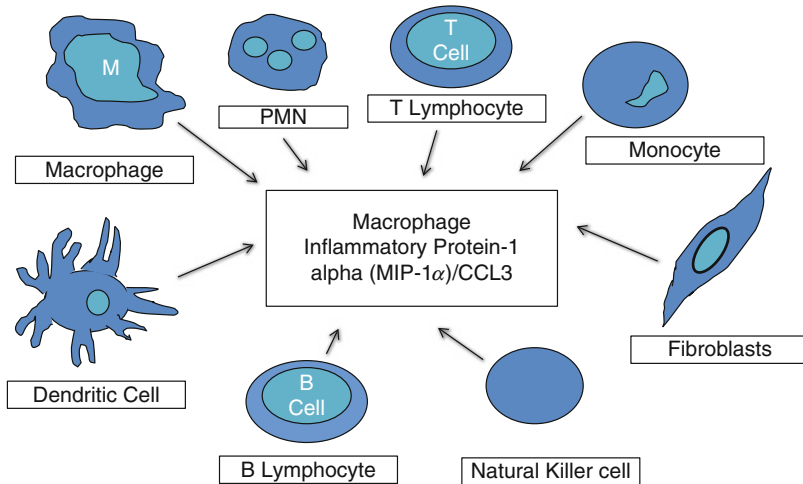
Fig. 3 Cells that secrete MIP-1α/CCL3

(Menten et al. 2002a, b). Under normal conditions, synthesis of MIP-1α/CCL3 occurs at very low levels. However, upon stimulation of receptive cells with endotoxins such as lipopolysaccharide (LPS) (Suzuki et al. 2000), virus proteins (Melchjorsen et al. 2003), or pro-inflammatory cytokines (IL-1β) (Standiford et al. 1993a), cellular signaling events are activated, and this activation induces increased production of MIP-1α/CCL3. MIP-1α levels are regulated by certain transcriptional factors. MIP-1 alpha nuclear protein (MNP) is one such transcriptional factor that induces the transcription of the MIP-1α/CCL3 gene (Ritter et al. 1995). On the other hand, prostaglandin E_2 (PGE2) has been shown to inhibit the expression of MIP-1α/CCL3 from cells (dendritic cells) previously exposed to LPS (Jing et al. 2003). Non-hematopoietic cells such as osteoblasts (Kukita et al. 1997) and epithelial cells (Ryu et al. 2007) can secrete MIP-1α/CCL3 upon stimulation. MIP-1α/CCL3 is found to be one of the chemokines copiously expressed in inflamed gingival tissue and by oral epithelial keratinocytes (Gemmell et al. 2001; Kabashima et al. 2002; Ryu et al. 2007).

Biological Function of MIP-1α/CCL3

The biological function of MIP-1α/CCL3 are broadly illustrataed in Fig. 4 and discussed below.

Inflammation

The inflammatory process consists of sequential cellular events that are associated with the expression of several mediators (cytokines and chemokines, including MIP-1 α /CCL3) that induce the recruitment of leukocytes to the site of

Biological Functions of MIP-1α/CCL3

Inflammation
(chemo-attractant: macrophages,
PMN,eosinophils)

Wound Healing
(recruiting macrophages)

Inhibition of Hematopoietic cell
proliferation

Osteoclast activation
(promoting bone resorption)

Selective CD8 T-lymphocyte
recruitment

Tumor migration and metastasis

HIV suppression factors
(prevents T cells from HIV infection)

inflammation (Fig. 5). After injury, vascular endothelial cells are activated (Fig. 5, step 1). The interaction between activated endothelial cells and leukocytes migrating in the circulatory system into the site of inflammation depends on cytokine production and endothelial adhesion molecules (Luscinskas et al. 1991). Unrestricted production of cytokines and chemokines can lead to tissue destruction (Fig. 5, step 3). Activated adhesion molecules help to retain leukocytes and transmigrate them into the inflamed tissues after injury (Fig. 5, step 4). It has been reported that intracellular adhesion molecules (ICAM-1) play an important role in the interaction between monocytes and endothelial cells to produce MIP-1α/CCL3 (Lukacs et al. 1994). In vitro studies have shown a strong inflammatory reaction characterized by neutrophil accumulation after subcutaneous injection of MIP-1α/CCL3 into mice footpads (Wolpe et al. 1988). Thus, MIP-1α/CCL3 is suggested to play an important role in the inflammatory process by promoting the recruitment of leukocytes (PMNs, macrophages, and lymphocytes) to the site of inflammation.

Role of MIP-1α/CCL3 in Viral Infections

MIP-1α/CCL3 is important for inducing the inflammatory response against viruses. For example, upon exposure of lung tissue to respiratory syncytial virus (RSV), a strong inflammation response is induced by MIP-1α/CCL3. MIP-1α/CCL3 induces recruitment and migration of eosinophils and basophils. Eosinophil
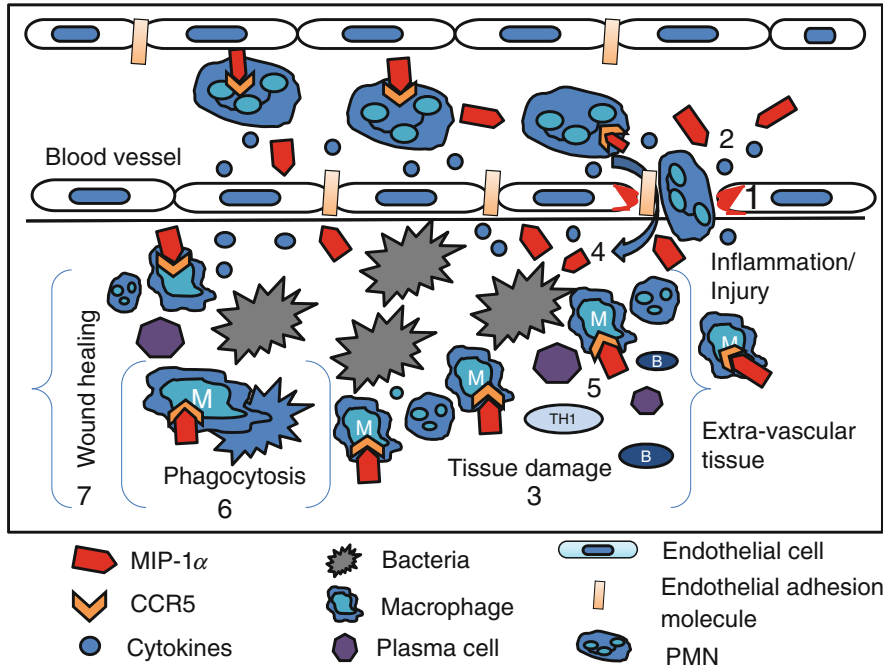
Fig. 5 Role of MIP-1α/CCL3 in inflammation and wound healing. Schematic representation of tissue injury followed by (1) activation of endothelial cell. (2) Numerous cytokines and chemokines are released in this process. (3) Unrestricted production of cytokines and chemokines causes tissue destruction. (4) Endothelial cell adhesion molecules produce MIP-1α/CCL3, and they recruit and retain polymorphonuclear (PMN) cells into the area of injury. (5) MIP-1α/CCL3 promotes the recruitment of macrophages and lymphocytes to the site of bacterial infection. (6) Macrophages induce phagocytosis to eliminate the bacteria and to produce growth factors that can induce wound healing. (7) Macrophages secrete various growth factors that help promote wound healing

degranulation results in severe tissue damage and is suggested to contribute to the pathological process triggered by viral infections (Garofalo et al. 1992). MIP-1α/CCL3 is the most abundantly produced chemokine in mice infected with RSV, and respiratory epithelial cells and adjacent endothelial cells have been shown to be the major source of MIP-1α/CCL3. Furthermore, transgenic mice lacking the MIP-1α/CCL3 gene showed a marked decreased inflammatory response when infected with RSV (Haeberle et al. 2001). An increased level of MIP-1α/CCL3 has been detected in bronchoalveolar lavage fluid (BALF) from patients with different lung diseases such as allergen-mediated asthma (Holgate et al. 1997), acute respiratory distress syndrome (ARDS) (Goodman et al. 1996), and pulmonary fibrosis (Ziegenhagen et al. 1998). Genetically altered MIP-1α/CCL3 null mice infected with Coxsackie virus fail to develop myocarditis, and when inoculated with influenza virus, the degree of pulmonary inflammation was reduced (Cook 1996). These findings suggest that MIP-1α/CCL3 plays a significant role in promoting inflammation in response to different viral infections.

The transmission and progression of human immunodeficiency virus (HIV) infection is greatly controlled by chemokines and chemokine receptors. While $CD4^+$ T lymphocytes are the primary target cells, chemokine receptors CCR5 and CXCR4 act as primary coreceptors for entry of the virus (Alfano and Poli 2005). Because MIP-1α/CCL3 binds to the chemokine receptor CCR5, it can play an important role in the etiopathogenesis of HIV infection. Some $CD4^+$ T cells secrete HIV-suppressive factors; hence, they are not preferentially infected with HIV (Abdelwahab et al. 2003). Apart from T lymphocytes, macrophages are well-known target cells that are infected with HIV. Macrophages infected with HIV exhibit increased levels of MIP-1α/CCL3 expression, and this expression correlates with viral replication (Canque et al. 1996). Various chemokines such as regulated on activation normal T cell expressed and secreted (RANTES/CCL5), MIP-1α/CCL3, and MIP-1β/CCL4 demonstrate HIV-suppressive activity (Cocchi et al. 1995). Furthermore, experimental studies have shown antibodies against RANTES/CCL5, MIP-1α/CCL3, and MIP-1β/CCL4 can entirely block HIV-suppressive activity (Cocchi et al. 1995). These findings suggest that chemokines have a potential role in the prevention and treatment of HIV infection.

Wound Healing

At the site of injury, macrophages perform multifunctional roles to promote wound healing. They secrete various growth factors that encourage the proliferation of cells such as fibroblasts, endothelial cells, and keratinocytes. They also produce proteins that promote extracellular matrix remodeling (collagenase) and proteases. Macrophages can produce several enzymes that degrade infectious agents and induce phagocytosis (Fig. 5, step 6). Delayed wound healing and decreased phagocytic activities are found when the numbers of macrophages and monocytes are reduced (Leibovich and Ross 1975). Mediators such as MIP-1α/CCL3 may help promote wound healing activity by recruiting macrophages to the site of tissue injury. An in vitro study by DiPietro et al. reported an increase in MIP-1α/CCL3 mRNA expression and protein levels after excisional wound injury. Furthermore, they also reported that neutralization of MIP-1α/CCL3 by anti-MIP-1a antiserum decreases collagen synthesis activity, angiogenic activity, and the number of infiltrating macrophages (DiPietro et al. 1998). These findings reflect the important role of MIP-1α/CCL3 in mediating macrophage migration into wounds to mediate tissue repair.

Inhibition of Hemopoietic Stem Cell Proliferation

MIP-1α/CCL3 is implicated in the inhibition of hematopoietic stem cell proliferation. This function was not revealed until a molecule derived from macrophage (J774.2 cell culture) designated as a stem cell inhibitor (SCI) was detected by Graham et al. It was shown that SCI inhibits the proliferation of both primitive cells (CFU-A assay) and hematopoietic stem cells (CFU-S assay). Further studies

showed that SCI is identical to MIP-1α/CCL3, as demonstrated by its amino acid sequence (Graham et al. 1990). Furthermore, MIP-1α/CCL3 may play a pivotal role in protecting the hematopoietic stem cell from negative effects triggered by cytotoxic drugs during cancer treatment.

Osteoclast Activation and Multiple Myeloma

MIP-1α/CCL3 promotes the orientation and migration of osteoclasts (Fuller et al. 1995). It stimulates the process of osteoclastogenesis by directly activating osteoclast cells (Kukita et al. 1992; Watanabe et al. 2004). Increased MIP-1α/CCL3 mRNA expression has been detected in human bone marrow myelocytes and human bone tissues (Kukita et al. 1997). Moreover, CCR1, CCR3, and CX3CR1 are some of the chemokine receptors expressed by osteoclast cells (Lean et al. 2002). It is suggested that MIP-1α/CCL3 plays a key role in the bone remodeling process because it binds with the CCR1 receptor, which is predominantly expressed by osteoclasts.

Multiple myeloma (MM) is characterized by the accumulation of malignant plasma cells in the bone marrow and is associated with increased osteoclast activity. Consistent with the role of MIP-1α/CCL3, the expression of MIP-1α/CCL3 mRNA is higher in bone marrow samples from patients with MM than healthy control subjects (Choi et al. 2000), and levels of MIP-1α/CCL3 from isolated bone marrow plasma of patients with active MM are increased compared with bone marrow plasma from patients with stable disease (Choi et al. 2000). MIP-1α/CCL3 binds to CCR1 or CCR5 to induce osteoclast formation, and neutralizing antibodies of CCR1 and CCR5 inhibit the osteoclast-induced bone resorption activity of MIP-1α/CCL3 (Oba et al. 2005). A strong positive correlation exists between bone resorption markers such as urine deoxypyridinoline (UDPD), serum bone-specific alkaline phosphatase (S-BALP), and serum MIP-1 levels (MIP-1α/CCL3 and MIP-1β/CCL4) in patients with multiple lytic bone lesions (Hashimoto et al. 2004). Also, serum MIP-1α/CCL3 levels correlate with the severity and prognosis of MM (Terpos et al. 2003, 2005). Therefore, it can be concluded that bone destruction in MM is a process highly associated with MIP-1α/CCL3. On the other hand, IL-1β, a potent osteoclast-activating factor, has been implicated in the etiopathogenesis of MM. However, MM patients have shown either low to no detectable levels of IL-1β protein (Choi et al. 2000). Thus, it is suggested that MIP-1α/CCL3 is more potent than IL-1β in inducing bone resorption in MM patients. Since the concentrations of the mediators causing bone destruction are elevated, evaluating their levels can serve as a diagnostic tool for the detection of disease activity.

Malignancy, Tumor Migration, and Tumor Metastasis

MIP-1α/CCL3 has been recently implicated in the regulation of tumor metastasis. Oral squamous cell carcinoma (OSCC) patients with metastatic lymph nodes have

increased mRNA expression of MIP-1α/CCL3 in comparison with same patients with non-metastatic lymph nodes, thereby suggesting a possible role of MIP-1α/CCL3 in tumor migration (Silva et al. 2007). MIP-1α/CCL3 is also produced by stimulated leukocytic cell lines or lymphoblasts from pediatric patients with acute lymphoblastic leukemia or acute myeloid leukemia (Struyf et al. 2003). In a study involving a microchemotaxis chamber, MCF-7 breast cancer cell line responded to MIP-1α/CCL3 and MIP-1β/CCL4 suggesting their potential role in the migration of breast cancer cells (Youngs et al. 1997). Wu et al. reported that CCL3-CCR5 axis may play an important role in enhancing various tumor cell functions including tumor metastasis (Wu et al. 2008). They found increased expression of the CCR5 receptor and not the CCR1 receptor in mice injected with renal cell carcinoma, a cancer known to metastasize to the bone. Their reports suggest that CCL3-CCR5 axis contributes to neovascularization by enhancing MMP-9 expression which can help degrade extracellular matrix. Furthermore, the CCL3-CCR5 axis may help in tumor progression by increasing HGF-expressing fibroblasts (angiogenic factor). The concept of tumor migration is based on the ability of chemokines to attract tumor cells to secondary sites. Thus, the quantity of chemokines produced by lymphoma or leukemia cells and the types of chemokines present at distant sites of metastasis may determine the migration of tumor cells (Menten et al. 2002a).

T Lymphocyte Recruitment

MIP-1α/CCL3 acts as a potent chemoattractant for T lymphocytes and therefore plays an important role in recruiting T cells during the cell-mediated immune response (Taub et al. 1993; Cook et al. 1999). The two main subsets of T lymphocytes are CD4 (helper T cells) and CD8 (cytotoxic T cells). These cells are known to produce generous amount of cytokines and are broadly categorized as Th-1-type cytokines and Th-2-type cytokines. Th-1 cytokines are potent pro-inflammatory mediators and are responsible for maintaining autoimmune responses. However, a large quantity of these pro-inflammatory cytokines can induce unobstructed tissue damage. Thus, there is a need to counterbalance the mediators that promote unrestricted tissue destruction. Th-2-type cytokines induce an anti-inflammatory response, thereby counteracting the functions of Th-1 cytokines. In general, MIP-1α/CCL3 and MIP-1β/CCL4 play a key role in determining the responses of lymphocytes. For example, MIP-1β/CCL4 selectively attracts activated CD4^{+} T cells (helper), and MIP-1α/CCL3 preferentially attracts activated CD8^{+} T cells (cytotoxic) (Taub et al. 1993).

Potential Applications to Diagnosis, Risk Assessment, and Prognosis of Diseases and Conditions

MIP-1α/CCL3 is associated with the activation and migration of leukocytes, and it can induce osteoclastogenesis by activating osteoclasts. As a result, MIP-1α/CCL3 has been implicated in the pathogenesis of various inflammatory diseases and

conditions such as multiple myeloma (MM), aggressive periodontitis, sarcoidosis, Papillon-Lefèvre syndrome, rheumatoid arthritis (RA), Sjögren syndrome (SS), and cardiovascular diseases.

Periodontitis

The periodontium includes the gingiva and the attachment apparatus surrounding each erupted tooth. The gingiva is comprised of keratinized oral squamous epithelium and the underlying connective tissue, whereas the attachment apparatus consists of the periodontal ligament (connective tissue), cementum, and the supporting alveolar bone. Their function is to attach the tooth to the alveolar bone.

Periodontitis is a chronic disease that results from the interaction between oral bacteria and the host inflammatory response. It is reported to affect nearly 47 % of adults in the United States (Eke et al. 2012). Poor oral hygiene methods contribute to the accumulation of bacterial plaque formation at the interface between the tooth and the gingiva, which when chronically present causes gingivitis, a reversible inflammation of the gum tissue (i.e., gingiva). If bacterial plaque is removed daily through effective oral hygiene measures, health is quickly restored.

Periodontitis is a more serious condition than gingivitis and is characterized by the destruction of the connective tissue attachment and bone surrounding teeth. Unlike gingivitis, periodontitis requires more time and gram-negative bacteria in subgingival sites for its development. The presence of periodontal pathogens is sufficient to cause but will not predictably cause disease in all infected patients. Differential susceptibility to the bacterial by-products elicits host responses that are contributory (Seymour 1991). Although tooth-associated bacterial biofilm is the precipitating cause of periodontitis, many risk factors predispose a person to these diseases. These factors include genetic influences (IL-1β polymorphism), aging, diabetes, stress, and smoking.

MIP-1α/CCL3 Is Upregulated in Inflamed Periodontal Tissue

Periodontal pathogens have a unique advantage in that the dentogingival interface is an ideal substrate upon which a biofilm can be formed. The tooth penetrates the integument and is the only interface in the body where a calcified structure is in contact with the external environment and is subjected to colonization by bacteria. This tooth-associated biofilm confers a degree of protection and allows some degree of metabolic cooperation between the members of the community. Microbes are held in contiguity with the delicate epithelium that lines the gingival sulcus (a potential space between the tooth and gum). If allowed to remain in situ, the increasing anaerobic and gram-negative flora will elicit an inflammatory host response. Influx of polymorphonuclear (PMN) cells and macrophages takes place after activation of the immune reaction (Fig. 6). The exposure of the bacterial lipopolysaccharide (LPS) induces the initiation of various inflammatory cells to stimulate the expression of inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) in the gingival
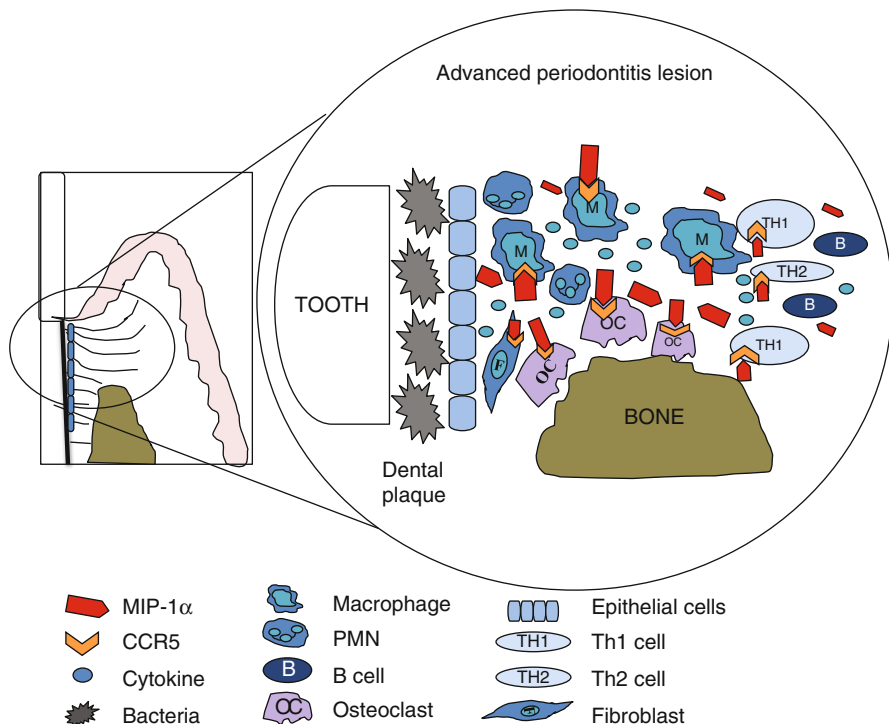
Fig. 6 Role of MIP-1α/CCL3 in periodontal disease. Schematic representation of tooth surface and accumulation of dental plaque. Advanced periodontal lesions are associated with an acute inflammation reaction characterized by PMN, macrophages, and plasma cells. Increased production of cytokines and chemokines takes place in response to bacterial LPS. MIP-1α/CCL3 binds to the cells presenting its receptors and induces recruitment of macrophages to induce inflammatory and immune response in the periodontal connective tissue. MIP-1α/CCL3 also activates osteoclasts and promotes bone destruction. The numbers of B lymphocytes and Th1 cells are increased with the progression of the advanced periodontal lesion

connective tissue. Chemokines are upregulated during the course of periodontal disease as a result of the interaction between the bacterial proteins and cellular surface receptors. Experiments have shown that MIP-1α/CCL3 expression is induced by polymorphonuclear (PMN) cells and gingival epithelial cells and not by gingival fibroblasts and human MG63 osteosarcoma cells (osteoblastic cells) when subjected to bacterial lipopolysaccharide (LPS) and interleukin (IL-1β) (Ryu et al. 2007). Epithelial keratinocytes in the oral cavity can produce various chemokines such as MCP-1/CCL2, RANTES/CCL5, MIP-1α/CCL3, and interferon-γ-inducible protein-10 (IP-10/CXCL10) (Gemmell et al. 2001). An immunohistochemistry study showed that the number of cells secreting MIP-1α/CCL3 and chemokine receptor CCR5 and CXCR3 is higher in inflamed gingival tissue than in normal healthy gingival tissue (Kabashima et al. 2002). Examination of gingival tissue biopsy samples from patients with various degrees of inflammation

(gingivitis and periodontitis) showed that cells from the periodontitis samples contained more MIP-1α/CCL3 than MCP-1/CCL2. Moreover, MIP-1 α /CCL3 exhibited higher levels with increase in inflammation (Gemmell et al. 2001).

MIP-1α/CCL3 plays a significant role in recruiting inflammatory cells that are associated with later stages of inflammation. A correlation has been reported between MIP-1α/CCL3-positive cells and an increased proportion of CD8^{+} T cells (cytotoxic cells) and B cells in association with increased inflammation (Gemmell et al. 2001). Correspondingly, microchemotaxis experiments have shown that MIP-1α/CCL3 has an increased tendency to attract cytotoxic T cells and B cells (Schall et al. 1993). MIP-1α/CCL3 functions by recruiting inflammatory cells to the inflamed tissue, upregulating the expression of MIP-1 α /CCL3 by gingival epithelial cells to promote an acute inflammation response, and recruiting B lymphocytes in the later stages of periodontal disease. On the other hand, relative amounts of RANTES/CCL5 and IP-10/CXCL10 (specific for activated T cell) from the gingival tissues of periodontitis and gingivitis samples remained stable or reduced suggesting lack of recruitments of T-cell subsets by these chemokines. These findings indicate that MIP-1 α /CCL3 plays a crucial role in recruiting leukocytes at early and later stages of periodontitis (Gemmell et al. 2001).

Previous reports have suggested that inflammatory mediators can temporarily alter the gene expression of a subset of cells. Interestingly, fibroblasts from gingival tissue express higher levels of chemokine (MIP-1α/CCL3) and cytokine (IL-6) than periodontal ligament (PDL) fibroblasts when they are subjected to LPS stimulation (Morandini et al. 2010). Thus, the differential response between gingival and PDL fibroblasts appears due to different surface receptors that bind to chemokines.

Role of MIP-1α/CCL3 in Aggressive Periodontitis

Aggressive periodontitis is a specific type of periodontitis that is characterized by a genetic component and rapid attachment loss and severe bone destruction. It predominantly affects children and usually involves molars and incisors. Patients often exhibit levels of bacterial plaque that are inconsistent with the amount of periodontal tissue destruction. An increased incidence of Aggregatibacter actinomycetemcomitans (Aa), a gram-negative bacteria, is a predominant feature of this disease. The local host inflammatory response is characterized by intense infiltration of PMNs, macrophages, and lymphocytes, predominantly plasma cells (about 70 %) (Liljenberg and Lindhe 1980). Activated cells produce unrestricted expression of cytokines and chemokines that can further stimulate cells to produce prostaglandin E2 and tissue-degrading enzymes (Masada et al. 1990; Offenbacher et al. 1993; Okada and Murakami 1998).

MIP-1α/CCL3 has been suggested to play an important role in aggressive periodontitis by inducing the activation of osteoclasts and mediating Th-1 cytokines that promote tissue destruction (Fig. 6). MIP-1α/CCL3 can also induce osteoclast formation and stimulate osteoclastogenesis (Kukita et al. 1992; Kukita et al. 1997). MIP-1α/CCL3 elicits bone resorption primarily through its receptor CCR1, which is predominantly expressed by osteoclast cells. As a result, increased level of MIP-1α/CCL3 is one of the diagnostic features of this disease. Fine et al. performed

a longitudinal study to evaluate salivary levels of MIP-1α/CCL3 from periodontally healthy Aa$^+$ and Aa$^-$ subjects. Three subject groups were studied: seven subjects who developed localized aggressive periodontitis (LAgP) with clinical signs of bone loss, seven healthy subjects with no bone loss (Aa$^+$), and seven healthy subjects with no bone loss (Aa$^-$). Fine et al. found significantly higher levels (about 50 fold) of MIP-1α/CCL3 from subjects in the LAgP Aa$^+$ group than the healthy control groups. Furthermore, levels of MIP-1α/CCL3 were found to be higher 6–9 months before the detection of bone loss. Although the number of study subjects was small, this study demonstrates the potential role of MIP-1α/CCL3 in promoting macrophage recruitment and osteoclast activation. The fact that MIP-1α/CCL3 levels were higher before the detection of bone loss suggests that this protein may play a role as a biomarker for detecting patients at risk of bone loss during the course of periodontitis.

MIP-1α/CCL3 RNA and its receptor CCR5 have also been detected at higher levels in gingival biopsy samples from patients with aggressive periodontitis than in samples from patients with chronic periodontitis (Garlet et al. 2003). Consistent with these results was the finding that the chemokine receptor CCR5 is preferentially expressed on Th1 cells rather than on Th2 cells (Loetscher et al. 1998). Thus, the expression of MIP-1α/CCL3 and its receptor CCR5 in aggressive periodontitis suggests an inclination toward the production of the Th1 cell response. One study showed that a higher level of IFN-γ (Th-1-type cytokine) is found in aggressive periodontitis, a finding suggesting the possibility of differential patterns of cytokine expression in periodontal tissue (Garlet et al. 2003). Thus, depending on the recruitment of lymphocyte subsets, differential cytokine expression is expected in areas of aggressive periodontitis.

Association of MIP-1α/CCL3 with Periodontitis as It Can Be Measured in Bodily Fluids

Visual evaluation, periodontal pocket depth, clinical attachment level, plaque index, bleeding upon probing, and demonstration of alveolar bone loss by radiographic images are some of the traditional parameters for diagnosing periodontal disease. However, these conventional diagnostic measures fail to recognize highly susceptible subjects who are at risk of future tissue breakdown. Also, a substantial amount of damage must take place before these diagnostic measures can detect disease activity. Hence, scientists are attempting to develop new diagnostics for detecting ongoing periodontal disease at an early stage. New diagnostics should be able to provide information about the extent of current disease activity as well as determine which patients are highly susceptible for future disease progression. Recently, the use of serum, saliva, and gingival crevicular fluid (GCF) in clinical research has provided insight into the pathogenesis of periodontitis and potential biomarkers that may have diagnostic utility. Currently, oral fluid biomarkers such as enzymes (collagenase), host cells (PMN), bacteria (Aa, Porphyromonas gingivalis), bacterial products (LPS), and immunoglobulin (IgA) show potential for detecting periodontal disease (Khashu et al. 2012).

Periodontitis and MIP-1α/CCL3: Findings from Oral Fluids

Saliva from patients with periodontal disease contains higher concentrations of MIP-1α/CCL3 than healthy controls. Similar to the findings of Fine et al. (2009), levels of MIP-1α/CCL3 were 18-fold higher in the saliva of 40 subjects with periodontitis than in 40 healthy control subjects (Al-Sabbagh et al. 2012). Salivary concentrations of MIP-1α/CCL3 also demonstrate a strong positive correlation with clinical parameters of periodontal disease. In addition to correlating with disease severity, salivary levels of MIP-1α/CCL3 have been shown to reflect response to therapy (Sexton et al. 2011). Tymkiv et al. reported that the level of MIP-1α/CCL3 is lower in subjects with periodontitis who smoke than in similar subjects who do not smoke (Tymkiw et al. 2011). This finding could be because smoking impairs the immune response (Palmer et al. 2005). Although MIP-1α/CCL3 appears to be a good salivary biomarker of periodontitis, one study reports that concentrations of MIP-1α/CCL3 in gingival crevicular fluid were not different in subjects presenting with increasing severities of periodontal diseases (i.e., gingivitis or chronic and aggressive periodontitis) (Emingil et al. 2005). Thus, this finding indicates more research is needed and could reflect the episodic nature of periodontal destruction activity (Socransky et al. 1984; Table 1).

Peri-implantitis and MIP-1α/CCL3: Findings from Oral Fluids

In dentistry, the concept of osseointegration is revolutionizing the success of dental implants as replacements for missing teeth. The demand for dental implants has grown considerably. However, implants can fail because of peri-implant disease. Peri-implant mucositis is the inflammation of the soft tissue surrounding the implant, whereas peri-implantitis is a irreversible inflammation surrounding an osseointegrated implant that leads to severe bone loss caused either by bacteria or by technical (mechanical trauma) complications (Lindhe and Meyle 2008) (Albrektsson and Isidor 1994). Several studies have shown that the progression of peri-implantitis results in a markedly higher rate of implant failure (Sakka et al. 2012). In as much as peri-implantitis is a major cause of implant failure, various salivary biomarkers have been investigated for the detection of peri-implantitis (Arakawa et al. 2012; Fonseca et al. 2012). Reports of cytokine and chemokine level in peri-implant crevicular fluid (PICF) provide valuable information about the health status of dental implants. To date, at least one study has shown that levels of PICF MIP-1α/CCL3 and IL-1β are significantly higher in patients with peri-implantitis than in patients with healthy implants (Petkovic et al. 2010).

Periodontitis, MM, and MIP-1α/CCL3: Findings from Serum and Bone Marrow

de Queiroz et al. compared the level of serum biomarkers of inflammation in 17 patients with chronic periodontitis and eight healthy control subjects. They found higher serum concentrations of MIP-1α/CCL3 in the periodontitis group

Table 1 The association of MIP-1α/CCL3 with periodontitis as measured in bodily fluids. *G* gingivitis, *CP* chronic periodontitis, *AP* aggressive periodontitis, *H* healthy periodontal tissue group, *GCF* gingival crevicular fluid, *LAgP* localized aggressive periodontitis, *OHI* oral hygiene instructions, *SRP* scaling, and root planing

Sample	Periodontal disease				Main finding	References
	G	CP	AP	H		
Serum		17		8	MIP-1α/CCL3 level was found to be elevated in the test group	de Queiroz et al. 2008
GCF	15	26	26	15	No significant difference in levels of MIP-1α/CCL3 was found between patients with gingivitis, patients with periodontitis (chronic or aggressive), and the healthy control group	Emingil et al. 2005
GCF		40 (20 smokers 20 nonsmokers)		12	MIP-1α/CCL3 levels were lower in patients with periodontitis who smoked than in those who did not smoke	Tymkiw et al. 2011
Saliva			7 Aa⁺ group who developed bone loss	7 Aa⁺, healthy group subjects with no bone loss	MIP-1α/CCL3 levels were 50-fold higher in subjects with LAgP Aa⁺ group	Fine et al. 2009
				7 Aa⁻, healthy group	The levels of MIP-1α/CCL3 were found to be increased 6–9 months before the detection of bone loss	
Saliva		40		40	MIP-1α/CCL3 levels were 18-fold higher in the periodontitis group	Al-Sabbagh et al. 2012
Saliva		33 (OHI) 35 (SRP+ OHI)			A significant reduction in MIP-1α/CCL3 levels was found in patients who responded to periodontal therapy (SRP + OHI) compared to those who did not	Sexton et al. 2011

than the healthy control group, but not statistically higher levels (de Queiroz et al. 2008). In contrast to serum studies, freshly isolated bone marrow plasma from patients with active MM exhibited higher concentrations of MIP-1α/CCL3 than plasma from patients with stable disease (Choi et al. 2000). Likewise, MM patients with more than three lytic lesions exhibit higher serum MIP-1α/CCL3 concentrations than MM patients with fewer (less than 3) lytic lesions (Terpos et al. 2005) suggesting that serum MIP-1α/CCL3 levels and markers of bone resorption correlate with disease severity of MM (Terpos et al. 2003; Hashimoto et al. 2004), but less so with periodontal disease.

Sarcoidosis and MIP-1α/CCL3

Sarcoidosis is a chronic granulomatous disease characterized by the accumulation of T lymphocytes and macrophages; this accumulation produces inflammatory reactions in various organs. Because MIP-1α/CCL3 is a potent macrophage chemotaxin, its role in the pathogenesis of sarcoidosis is significant. Patients with sarcoidosis exhibit higher concentrations of MIP-1α/CCL3 from bronchoalveolar lavage fluid (BALF) and a 2.5-fold increase in chemotactic activity when compared to control subjects. Also, reductions in chemotaxis of approximately 22 % were observed in the presence of anti-MIP-1α antibodies (Standiford et al. 1993b). These findings suggest that the plasma concentration of MIP-1α/CCL3 is related to the disease activity of sarcoidosis. Patients with active sarcoidosis, including both systemic and pulmonary involvement, showed a marked increase in plasma levels of MIP-1α/CCL3 (Hashimoto et al. 1998). However, patients with predominantly pulmonary involvement without any systemic manifestation also showed elevated concentrations of MIP-1α/CCL3. Thus, the plasma concentration of MIP-1α/CCL3 can provide valuable information about potential extrathoracic disease activity in patients with sarcoidosis and can correlate with disease activity.

Papillon-Lefèvre Syndrome and MIP-1α/CCL3

Papillon-Lefèvre syndrome (PLS) is a rare autosomal recessive disorder initially reported by Papillon and Lefèrve in 1924. It is characterized by early loss of both deciduous and permanent dentition and by palmar-plantar keratosis. Reports have shown that a genetic mutation of the chromosome (11q14-21) that results in complete loss of cathepsin C activity is responsible for the development of PLS (Hart et al. 1999). These patients exhibit severe periodontitis, which involves uncontrolled destruction of connective tissue and alveolar bone resorption. This occurs because of the downregulation of cathepsin C that impedes the activation of neutrophil serine proteinases such as cathepsin G, proteinase 3, and elastase (de Haar et al. 2004). Neutrophil serine proteinases are important for maintaining host immune functions. Patients with PLS demonstrate impaired proteolysis of MIP-1α/CCL3 because of the inactivity of neutrophil serine proteinases resulting

in severe periodontal destruction attributed to excessive buildup of MIP-1α/CCL3 (Ryu et al. 2005).

Rheumatoid Arthritis and MIP-1α/CCL3

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease that predominantly affects synovial joints. It is genetically associated with HLA antigen DR4 (Gran et al. 1983). During the progression of this disease, inflamed synovial joints are infiltrated by various inflammatory cells, such as macrophages, lymphocytes, and plasma cells. The activated cells produce pro-inflammatory cytokines such as TNF-α, which further mediates inflammatory synovitis and induces matrix metalloproteinase to promote collagen fiber destruction. Macrophages also play an important role in secreting chemokines involved in the pathogenesis of rheumatic arthritis. MIP-1α/CCL3 expression is higher in synovial fluid and serum from RA patients than in these fluids from patients with osteoarthritis (Koch et al. 1994). Increased levels of MIP-1α/CCL3 mRNA and protein also have been found in macrophages from the synovial tissue from RA patients. The higher expression can be attributed to an increase in the recruitment of macrophages and T cells into the areas of inflamed synovium by MIP-1α/CCL3. Likewise, increased expression of the chemokine receptor for MIP-1α/CCL3, CCR5, occurs in RA patients (Patel et al. 2001).

Sjögren Syndrome and MIP-1α/CCL3

The pathogenesis of Sjögren syndrome (SS) is unclear; it is characterized by a chronic inflammation of the salivary glands, which is driven by interferon-related dysregulation resulting in damage to the glands and impaired excretion of saliva (Lessard et al. 2013). Clinically, patients exhibit dry eyes and a dry mouth; however, other organs can be involved. Biopsy samples from salivary glands of patients with SS express higher concentrations of both MIP-1α/CCL3 and MIP-1β/CCL4 than that of RANTES/CCL5 and IL-8. In these studies, the ductal epithelial cells express higher concentrations of MIP-1β/CCL4 than MIP-1α/CCL3 (Cuello et al. 1998). It has been suggested that the main causative factor in the development of SS is either the recruitment of leukocytes into the ductal epithelium by chemokines or the secretion of various pro-inflammatory cytokines by these cells.

Cardiovascular Diseases and MIP-1α/CCL3

Recent studies indicate the pathogenic role of C-C chemokines in the development of several cardiovascular diseases such as atherosclerosis (Braunersreuther et al. 2007; de Jager et al. 2013), myocardial ischemia (de Jager et al. 2008), and

congestive heart failure (Aukrust et al. 1998). This is due to their biological function of activation and migration of leukocytes into areas of inflammation. Clinical studies have recently identified elevated concentrations of circulating chemotactic chemokines, thereby suggesting their potential as diagnostic markers of cardiovascular diseases. For example, serum concentrations of MCP-1/CCL2, MIP-1α/CCL3,, and RANTES/CCL5 are elevated in patients with congestive heart failure (Aukrust et al. 1998). Patients with acute myocardial infarction associated with heart failure complication and severe left ventricular dysfunction also show a higher concentration of circulating MIP-1 α /CCL3 in comparison with healthy control patients (Parissis et al. 2002). Furthermore, a strong correlation between myocardial infarction and MIP-1α/CCL3 has been reported suggesting its strong prognostic potential to identify high-risk patients (de Jager et al. 2008, 2012). Increasing evidence suggest that C-C chemokines (MIP-1 α /CCL3) and their receptors play a significant role in the etiopathogenesis of the cardiovascular disease. Therefore, inhibition of these chemokines and their receptors could provide novel therapeutic strategies.

Conclusion

In conclusion, MIP-1α/CCL3 plays a key role in the activation and regulation of inflammatory and host defense responses. MIP-1α/CCL3 is crucial for the recruitment of macrophages and T lymphocytes from the circulation to sites of infection or injury; thus, it orchestrates acute and chronic inflammatory host responses. It also acts as a potent osteoclast activator and induces bone resorption. Hence, MIP-1α/CCL3 is a key protein that plays an important pathological role in the development of several inflammatory and autoimmune diseases such as multiple myeloma, rheumatoid arthritis, Sjögren syndrome, sarcoidosis, respiratory disease, cardiovascular disease, and periodontitis. Evaluating the levels of MIP-1α/CCL3 in biological fluids provides a great opportunity for diagnosing various inflammatory diseases and conditions at their early stages. Moreover, inhibitors of MIP-1α/CCL3 and its receptors could be potential therapeutic targets for the treatment of these diseases.

Summary Points

- MIP-1α/CCL3 is a key chemokine that plays an important role in recruiting inflammatory cells (macrophages, lymphocytes, eosinophil, and basophils) to the site of injury or inflammation.
- MIP-1α/CCL3 is expressed by several cells upon stimulation of endotoxins such as LPS, viral proteins, and pro-inflammatory cytokines.
- MIP-1α/CCL3 performs important biological functions, such as the initiation of the inflammatory response by the recruitment of macrophages, wound healing,

tumor metastasis, promoting osteoclast activation, HIV-suppressive activity, and inhibition of hematopoietic stem cells, principally by binding to their selective chemokine receptors CCR1 and CCR5.

- MIP-1α/CCL3 induces osteoclastogenesis by directly activating osteoclasts; therefore, it is suggested to be a main etiological factor in diseases such as multiple myeloma and periodontitis that are characterized by severe tissue destruction and bone destruction.
- MIP-1α/CCL3 is associated with several inflammatory diseases including Papillon-Lefèvre syndrome, peri-implantitis, sarcoidosis, Sjögren syndrome, and rheumatoid arthritis.
- Detection of increased concentrations of MIP-1α/CCL3 in bodily fluids (serum, saliva, GCR, bronchial lavage) is a promising approach for detecting disease risk, disease activity, and response to therapy regarding several inflammatory and osseous destructive diseases.

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