

**MODERN APPROACH TO PATHOGENETIC TRAITS OF THE
OSTEOARTHRITIS**

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Abstract. Osteoarthritis is a pathological lesion of the joints, characterized by structural changes in the articular cartilage and subchondral bone, as well as clearly or imperceptibly moderate synovitis. Osteoarthritis is an urgent medical and social problem for most countries of the world due to its high prevalence, which reaches about 25% of the population. This disease is most common among women and is one of the causes of reduced work capacity and increased disability. The article discusses the relationship between cytokines and markers of endothelial dysfunction and nonspecific immune reactivity, the main mechanisms of the development of degenerative-dystrophic and inflammatory processes at the microcirculatory level, since microcirculatory imbalance is one of the main mechanisms in joint diseases. The study of cytokine networks and changes in their structure, analysis of correlations between changes in cytokine concentrations relative to each other, as well as in combination with other factors directly actualized in the process of diagnosing and treating a patient,

is a promising area of modern medicine. In osteoarthritis, endothelial dysfunction is a component of microcirculatory disorders. Desquamated endothelial cells and vasculoendothelial growth factor are the main indicators of damage to the microvasculature. Under the influence of pro-inflammatory cytokines, homeostasis in the microcirculatory link is destabilized. A necessary element in the diagnosis of osteoarthritis is the detection of an early marker - monocytic chemoattractant protein-1 (MCP-1).

Keywords: Osteoarthritis, cytokines, monocytic chemoattractant protein, desquamated endothelial cells, vasculoendothelial growth factor

Cytokines.

Today, joint diseases of various localization are one of the most common reasons for seeking medical attention. This category of diseases can rightfully be considered socially significant, since it affects a significant part of the population and leads to the development of persistent pain syndrome, most often due to synovitis. The most common pathology accompanied by joint damage is osteoarthritis (OA); in the elderly, its frequency is 97%. OA is characterized by pain and synovitis. So, speaking of OA, we cannot fail to highlight the commonality of the course, which is manifested, first of all, by inflammation, pain (one of the leading mechanisms of pain syndrome formation is the inflammatory process) and a significant decrease in the quality of life (QOL) [1]. In this disease, cytokines (CK) play an important role in pathogenesis, in particular in the determination of the inflammatory process [2].

According to modern sources, OA is a chronic degenerative disease of the joints, accompanied by progressive destruction of cartilage, thickening of the subchondral bone, the formation of osteophytes, degenerative changes in the structure of the ligaments and menisci, and hypertrophy of the articular capsule. OA is a multifactorial disease and among the possible risk factors for OA are old age, obesity, heredity, and joint injuries. A separate position in the structure of the causes of OA development is occupied by cytokine damage [3].

The pathogenesis of OA is as follows:

1. Decrease in the resistance of the matrix to the load due to the loss of proteoglycans;
2. Thinning of the surface layers of the cartilage;
3. Razvlechenie plate;
4. Cracking up to the complete disappearance of the cartilage.

During OA, such representatives of the CK family as IL-1 β , -4, -6, 17, -18 and TNF- α are important. In chondrocytes, the action of these CKs leads to an increase in the synthesis of proteases, a decrease in the synthesis of proteoglycans, a tissue inhibitor of metalloproteases, the progression of catabolic processes in cartilage, and an increase in the destruction of cartilage matrix components. To date, the opinions of researchers increasingly agree that the development of OA begins with chronic synovitis, subchondral bone and ligamentous apparatus. Inflammation in these structures leads to synovitis, osteitis, enthesitis. The outcome of inflammation is the formation of osteophytes and destruction of the articular surface. Based on this, it is possible to draw conclusions about the importance of inflammatory mediators, whose influence extends to all joint tissues [4]. Despite the fact that OA cannot be called arthropathy in the usual sense of this term, due to the absence of neutrophils and systemic inflammation in the synovial fluid, the role of CK in cartilage resorption in this disease is undeniable [5]. IL-1 β can be called the main pro-inflammatory CK, which is characterized by a variety of biological functions, in particular, a vasodilating effect due to the activation of prostaglandins and nitric oxide, stimulation of the synthesis of procoagulants and activation of adhesion molecules, suppression of albumin synthesis, stimulation of the secretion of "acute phase" proteins, stimulation of collagenase and induction of hypercalcemia, blocking the function of smooth muscle cells and cardiomyocytes, as well as IL-1, which leads to a pronounced induction of the synthesis of all pro-inflammatory CK - TNF- α , IL-6, IL-2, colony-stimulating factor - granulocyte-monocytes, IL-4, which is manifested by the activation of both cellular and humoral immune responses [6]. IL-4 is an anti-inflammatory CK that plays a key role in the regulation of cellular activity. It also activates proliferation and

increases the functional activity of B-lymphocytes, and also induces the production of IgE and IgG by activated B-lymphocytes, stimulating the humoral link of immunity [7]. Based on the research data of Kopylova D.A. (2012), according to the level of IL-4 in patients with OA, she noted a negative correlation between the concentration of IL-4 and the severity of pain syndrome (WOMAC index, Leken's algofunctional index, VAS index), as well as the prospects of using IL-4 as an indicator of the severity of OA manifestations. [eight]. IL-6 is also a pro-inflammatory CK (one of the most active), and an increase in its concentration always accompanies an active course of the inflammatory process [9]. IL-17 can be called a kind of "mediator" between adaptive and innate immunity, and it is also an inducer of such pro-inflammatory factors as TNF- α , IL-6 and IL-1 β . IL-18 is a regulatory CK and is a co-stimulator of IFN- γ production by T cells, and also stimulates the release of IL-2, proliferation and perforin-mediated activity of natural killer cells and, according to Anisimova N.Yu. et al. (2011), IL-18 is very significant during the inflammatory process [10]. TNF- α plays an important role in the process of immunoregulation, the development of inflammation and hemodynamic disorders in various diseases (infectious and non-infectious), is an inducer of apoptosis (by activating caspase-3 or caspase-8) and can be used in complex analysis as a nonspecific marker for assessing the degree of the inflammatory process in dynamics [11].

Understanding the mechanism of cytokine-induced inflammation can be helpful in preventing complications. Chepeleva M.V. et al. (2014) investigated the incidence of aseptic instability in patients undergoing knee arthroplasty and found that the highest correlation coefficient was observed in such indicators as IL-6 and TNF- α [12]. Larsson S. et al. note the relationship between the levels of IL-6 and TNF- α with the progression of osteoarthritis in patients with a history of meniscectomy [13]. Based on these data, it is possible to assert the diagnostic value of the above CK in the diagnosis and treatment of patients with OA.

The concomitant pathology in OA is interesting primarily for its influence on the cytokine profile. Zhuravleva L.V. and Oleinik M.A., having data on the level of CK in

patients with type 2 diabetes mellitus, OA and obesity, note that the highest values of proinflammatory cytokines (IL-1 β and TNF- α) were recorded in the group of patients with a diagnosis of OA and type 2 diabetes mellitus and obesity. High concentrations of CK in this case correlated with more severe X-ray changes, greater functional impairment and more pronounced pain syndrome, which obviously indicates their effect on the degradation of articular cartilage, the development of inflammation in the joint, and the severity of pain in OA [14]. Speaking of comorbid obesity, one cannot fail to mention the ability of subcutaneous fat for secretory activity. Leptin is a kind of marker for obesity, as its content increases in parallel with the body mass index. With the development of leptin desensibilization, the concentration of leptin in the blood is quite high, but the main effect of leptin - a decrease in appetite is not realized [15]. There was also a place in the structure of comorbid states of hypertension. Hypertension in patients with OA can be a very unfavorable prognostic factor. Analyzing the results of their research work, scientists say that in patients with essential hypertension, when it is combined with OA, there are changes in the immune system, manifested by immune-inflammatory and autoimmune reactions. And even despite the complex treatment carried out, the level of CK in patients with concomitant pathology of OA and AH, compared with the group of healthy individuals, decreased insignificantly. In addition, the study materials reported that patients had a high number of activated lymphocytes with phenotypes CD25 +, HLA-DR +, CD54 +, CD95 +. The appearance of these in the peripheral blood may indicate the development of an immune response caused by the action of the IL-1 cytokine. There is no doubt that a significant role in the increase in the content of activated subpopulations in patients was played by morphological changes in organs - targets of hypertension - kidneys, heart, blood vessels, the damage of which is accompanied by autoimmunization to their own tissues.

An increase in the level of CK in the synovial fluid is the main cause of the progression of joint inflammation in OA. The most important link in the development of the inflammatory process in this case is the expression of CK, metalloproteinases,

and synoviocytes against the background of low SOCS activity, whose function is the negative regulation of JAK / STAT [16]. The study of the effects of CK and their interaction directly in the joint cavity in various arthropathies is of great interest to scientists. An in vitro study of the synovial fluid (punctate) of patients with OA was carried out. In the course of the work, the concentrations of IL-17, IL-6, IgA, BAFF (B-cell activation factor), transforming growth factor- β 1 (TGF- β 1) and anti-endotoxin antibodies (anti-LPS-IgA) were determined, an external influence on the experimental environment by introducing bacterial antigens. Based on the results of this study, a positive relationship was established between the level of IL-17 and the production of anti-LPS-IgA in the joint, independent of the level of IL-6, the concentration in the synovial fluid of TGF- β 1 and BAFF correlated with the level of anti-LPS-IgA and total IgA, with the blockade of IL-17 production, the content of TGF- β 1 and antiLPS-IgA significantly decreased. Summing up, it can be noted that TGF- β 1, BAFF and bacterial antigens are in this case a kind of determinants of the effect of IL-17 on IgA production. This work can be safely called the next step in understanding the pathogenesis of inflammatory diseases of the joints [17]. In addition to cytokines, during OA, chemokines are produced by synovial cells, such as chemoattractable CKs, which are small cationic protein molecules synthesized in cells and tissues during the body's immune response to the appearance of a pathogen, allergen, damage and control the nature and magnitude of the infiltration of immune cells. All chemokines can be differentiated into 4 groups:

1. CCL (CCL1-CCL28);
2. CXC (CXCR or CXCL);
3. C (XCL1 bXCL2); 4. CX3C (CX3CL1) [15].

The uniqueness of the spectrum of CK and chemokines in the synovial fluid for the differential diagnosis of diseases such as RA and OA complicated by secondary arthritis, in particular, B-cell chemoattractant and CXCR5, as well as the possible significance of the B-cell link (increased expression of m-RNA factor produced by stromal cells in the pathogenesis of OA [18]).

So, based on the opinion of various authors, both Russian and foreign, we can confidently note the importance of the cytokine link in the pathogenesis of OA. The study of cytokine networks and changes in their structure, analysis of correlations between changes in CK concentrations relative to each other, as well as in combination with various other factors directly actualized in the process of diagnosing and treating a patient, is a promising area of modern medicine. The development and improvement of medications capable of a targeted effect on certain representatives of the cytokine spectrum will radically change the approach to the treatment of OA, since by effectively affecting the main link of pathogenesis, it is possible to change the course of the disease and thereby increase the patient's chances of recovery.

Endothelial dysfunction (ED) is one of the main mechanisms in joint diseases. Vascular endothelium - a monolayer of specialized cells of mesenchymal origin, which lines blood and lymphatic vessels, heart cavities

[nineteen]. Endothelial cell sensitivity and blood flow velocity are directly related to each other. This connection is observed in most of the main arteries, which manifests itself in the form of the ability of endotheliocytes to synthesize and release factors that promote relaxation or contraction of vascular smooth muscles. Endotheliocytes have a high secretory capacity; Based on this, it can be assumed that “endothelial tissue” is a unique endocrine organ that provides homeostasis of the vascular walls [9-13]. In OA, ED is a component of microcirculatory disorders, but its role has not been sufficiently considered [1; 2]. ED is accompanied by the activation of vasoconstrictors, which contribute to vasoconstriction, which provide microcirculatory disorders [20].

Desquamated endothelial cells (DEC), vasculoendothelial growth factor (VEGF), a signaling protein produced by cells to stimulate vasculogenesis, are the most important indicators of damage to the endothelial lining of blood vessels [20].

Vascular endothelial growth factor (VEGF) is a family of closely related growth factors having a conserved pattern of eight cysteine residues and sharing common VEGF receptors. Originally known simply as VEGF, vasculotropin (VAS) or vascular permeability factor (VPF), this factor is now sometimes called VEGF-A. Four

additional family members (placental growth factor, PIGF; VEGF-B; VEGF-C; and VEGF-D) have been identified to date.

VEGF-A (VEGF) is a potent growth factor for blood vessel endothelial cells, showing pleiotropic responses that facilitate cell migration, proliferation, tube formation, and survival. It is also one of the most potent permeability factors, so that VEGF-A is a common link of inflammation, permeability and angiogenesis. VEGF-A mRNA expression patterns are closely related to proliferation of blood vessels during the developing embryo and wound healing or in the ovary. Local hypoxia is a potent inducer of VEGF-A expression from adjacent cells but it is not synthesized in endothelial cells, indicating a paracrine regulation of vessel formation. In the developing embryo VEGF-A mRNA is expressed by cells within tissues undergoing capillarization. In most adult tissues the level of VEGF-A expression is low except in the kidney (Bowman's capsule podocytes). Expression of VEGF-A can be induced in macrophages, T cells, astrocytes, osteoblasts, smooth muscle cells, fibroblasts, endothelial cells, cardiomyocytes, skeletal muscle cells and keratinocytes. It is also expressed in a variety of human tumors. Due to alternative splicing of a single gene, VEGF-A may exist in four isoforms, designated by their expected final amino acid length (VEGF121, VEGF165, VEGF189 and VEGF206). These isoforms show similar biological activities but bind with different affinities to the heparin and result in different secretion patterns. The smallest isoform (VEGF121) is secreted and completely diffusible, the largest (VEGF206) is almost completely attached to the extracellular matrix, and the other two show intermediate heparin binding affinities. VEGF-A exerts its actions through two receptors (VEGFR-1 and VEGFR-2) [21]. PIGF is expressed in the placenta and somewhat less in the heart, lung and thyroid gland. Placentally expressed PIGF may act as an autocrine on trophoblasts, which express both PIGF and its receptor (VEGFR-1). Since these cells also make VEGF-A, natural heterodimers (PIGF/VEGF-A) have also been detected. Two alternatively spliced isoforms of PIGF have been identified. Hypoxia does not induce PIGF synthesis, but the formation of heterodimers would be affected due to hypoxic control

over VEGF-A expression. VEGF-B is largely cell-associated and expressed mostly in the heart, skeletal muscle, brain and kidney. It is often co-expressed with VEGF-A and heterodimers of A/B have been detected. VEGF-B expression is not regulated by hypoxia. The long half-life of its mRNA (>8 hours) suggests a chronic rather than acute regulation. VEGF-B exerts its actions through one receptor (VEGFR-1). VEGF-C, also called VEGF-related factor (VRP) or VEGF-2, in the adult is expressed primarily in the heart, placenta, lung, kidney, muscle, ovary and small intestine. During embryo development it is expressed in the cephalic mesenchyme, tail region and allantois and along the somites. VEGF-C may play roles in the development of the venous and lymphatic vasculature systems. VEGF-C exerts its actions through two receptors (VEGFR-2 and VEGFR-3). VEGF-D, also called c-fos induced growth factor (FIGF), is a VEGF homologue induced by c-fos. It is expressed in adult lung, heart and small intestine and in fetal lung. It is reported mildly mitogenic for endothelial cells. VEGF-D and VEGF-C share 23% amino acid sequence homology. VEGF-D exerts its actions through two receptors (VEGFR-2 and VEGFR-3) [22].

Monocyte chemoattractant protein-1 (MCP-1).

Chemokines are small molecules that play a crucial role as chemoattractants for several cell types, and their components are associated with host immune responses and repair mechanisms. Chemokines selectively recruit monocytes, neutrophils, and lymphocytes and induce chemotaxis through the activation of G protein-coupled receptors. Two well-described chemokine families (CXC and CC) are known to regulate the localization and trafficking of immune cells in cases of injury, infection, and tumors. Monocyte chemoattractant protein 1 (MCP-1/CCL2) is one of the important chemokines from the CC family that controls migration and infiltration of monocytes/macrophages during inflammation [23]. CCL2 is profoundly expressed in osteoporotic bone and prostate cancer-induced bone resorption. CCL2 also regulates physiological bone remodeling in response to hormonal and mechanical stimuli. Parathyroid hormone (PTH) has multifaceted effects on bone, depending on the mode of administration. Intermittent PTH increases bone in vivo by increasing the number

and activity of osteoblasts, whereas a continuous infusion of PTH decreases bone mass by stimulating a net increase in bone resorption. CCL2 is essential for both anabolic and catabolic effects of PTH. In this review, we will discuss the pharmacological role of PTH and involvement of CCL2 in the processes of PTH-mediated bone remodeling [24].

CC Chemokines and Their Receptors

The primary functions of chemokines are to recruit monocytes, neutrophils, and lymphocytes, inducing chemotaxis by activating G-protein-coupled receptors. The chemokine family is mainly composed of four groups (CC, CXC, C, and CX3C) based on the relative position of cysteine residues. The C chemokine family has one cysteine, whereas the CC chemokine family has two adjacent cysteines near the amino terminus of the protein. The CXC and CX3C chemokine families have either one or three amino acids separating the two cysteines.

In particular, CC and CXC chemokines have a defined role in bone remodeling [25]. The monocyte chemoattractant subfamily is a member of the CC chemokine family, which includes CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4), CCL12 (MCP-5), CCL5 (RANTES), CCL3 (MIP-1 α), CCL20 (MIP-3 α), and CCL4 (MIP-1 β) [26]. Among these chemokines, CCL2 is one of the most highly studied. Various cell types produce CCL2, including vascular endothelial, fibroblasts, epithelial, smooth muscle cells, astrocytic, monocytic, and microglial cells. However, principal sources of CCL2 are mononuclear leukocytes (27).

CCL2, originally known as JE, is a low-molecular-weight polypeptide whose primary function is to promote monocyte and macrophage migration to sites of inflammation. For example, CCL2 is associated with monocyte infiltration in inflammatory diseases such as rheumatoid arthritis and different tumors associated with inflammatory responses. The high-affinity CCL2 receptor, CCR2, is a member of the group of G protein-coupled receptors that contain seven transmembrane spanning domains. The genomic sequence of CCR2 is highly homologous and conserved through different species. Two alternatively spliced forms of the receptor have been identified,

CCR2A and CCR2B, varying only in the C-terminal domain of the protein. Although CCR2B is the predominant form, both forms of the receptor bind with high affinity to CCL2, but induce different biological responses. The CCR2 expression has been reported in numerous tissues, including bone, blood, brain, heart, kidney, liver, lung, ovary, pancreas, spinal cord, spleen, and thymus. It has been found that most chemokine receptors have the ability to bind several chemokines. Several reports revealed that the chemokine receptor, CCR2, could bind to five different CCL members such as CCL2, CCL7, CCL8, CCL12, and CCL13. However, CCL2 is the most potent inducer of the signal transduction pathways leading to monocyte transmigration (3). In addition, CCL2 also binds to the CCR4 receptor, which also has CCL5 and CCL20 as ligands (4).

CCL2/MCP-1 Chemokine and Bone Remodeling

Bone remodeling is imperative for physiological bone homeostasis. It comprises two phases: bone resorption by osteoclasts and bone formation by matrix-producing osteoblasts. The osteoblasts originate from mesenchymal stem cells in the bone marrow stroma. Osteoclasts are large, multinucleated cells formed from the fusion of mononuclear progenitors of the monocyte/macrophage in the process of osteoclastogenesis. The precise balance between bone resorption and formation is critical for the maintenance of bone mass and systemic mineral homeostasis. Any disturbance of this balance causes various bone diseases, including osteoporosis, which is characteristically defined as low bone mass and microarchitectural deterioration and extremely susceptible to fracture risk. The physiological bone remodeling process is controlled by various local and systemic factors and their expression and release in a well-organized manner. These include calcitonin, PTH, vitamin D₃ [1,25(OH)₂ vitamin D₃], and estrogen. In addition to systemic hormonal regulation, other growth factors such as IGFs, TGF-β, FGFs, EGF, BMPs, Wnt family proteins, and chemokines also play a significant role in the regulation of physiological bone remodeling [28].

It has been reported that CXC and CC chemokines promote the migration of osteoclast precursor cells and facilitate the process of osteoclastogenesis and bone resorption.

Reports of several authors indicated that the expression of CCL2 is developmentally regulated, and the recruitment of mononuclear cells in the occlusal area and basal area of the tooth is associated with bone resorption and bone formation, respectively, suggesting a differential role of monocytes in bone formation and bone resorption [29]. Mechanical stresses including pressure induce chemokine (CXCL2 and CCL2) expression in osteoblasts resulting in inflammatory reactions and bone remodeling [30].

Several chemokines have been involved in different stages of osteoclastogenesis. The roles of CCL2 and its receptor CCR2 have been characterized in bone cells. The work of Rahimi et al. showed that mice with an inflammatory lesion in the mandible had elevated staining for CCL2, mainly by osteoblasts RANKL stimulates the formation of osteoclasts in human peripheral blood monocyte cultures, in part, due to an increase in CCL2 production, which was shown by using blocking antibodies to CCL2. CCL2-deficient mice have reduced osteoclast-specific genes (DC-STAMP, NFATc1, and cathepsin K), suggesting impaired osteoclast differentiation. Further, CCL2 deficiency resulted in increased bone mass and decreased bone resorption markers (CTX-1 and TRACP 5b), however, no changes in bone formation markers, suggesting that impaired osteoclastogenesis is responsible for the bone phenotype observed in CCL2 null mice. Studies have shown that both CCL2 and CCR2 knockout mice exhibit inadequate monocyte recruitment in response to various inflammatory conditions. It has been reported that CCR2 null mice have high bone mass and decreased osteoclast number, size, and activity. In osteoclast progenitor cells, CCR2 activates downstream signaling through NF- κ B and ERK1/2. This publication also reported that CCR2 knockout mice develop resistance to ovariectomy-induced bone loss, suggesting the involvement of the chemokine receptor CCR2 in estrogen's effects on bone. However, recent work by Mader et al. showed that although CCR2 null mice

have larger and stronger tibiae compared to wild-type mice, they concluded that this was due to greater body mass rather than reduced bone resorption. In addition, they did not observe protection against ovariectomy-induced bone loss. Recently, it has been shown that topical treatment with the CCR2 antagonist (JNJ17166864) reduced alveolar bone loss from bacterial infection in mice supporting a role for CCR2 in bone loss.

Role of CCL2 in PTH Action on Bone

Parathyroid hormone is synthesized and secreted by parathyroid glands and exerts its functional role in bone mass regulation by an endocrine mode [31]. The PTH/parathyroid hormone-related peptide (PTHrP) receptor, also known as PTH1R, is the common receptor for both PTH and PTHrP. PTH1R is mostly expressed in bone, cartilage, and kidney cells [32].

Parathyroid hormone can exert both catabolic and anabolic effects on bone. It is well established that daily injections of low doses of PTH increase bone mass in animals and humans [33]. Continuous administration of PTH or PTHrP induces bone resorption by activating osteoclasts indirectly through their actions on osteoblastic cells [34]. Several effects of PTH on osteoclast formation are mediated by stimulation of RANKL and inhibition of OPG mRNA expression [35]. PTH's anabolic effect can now be explained by evidence that PTH increases the proliferation and differentiation of osteoblasts in vitro and in vivo [36], decreases osteoblast apoptosis and activation of bone lining cells [37]. PTH-mediated cAMP/protein kinase A signaling is required for Runx2 transactivation, which in turn upregulates the expression of osteoblast genes. In addition, intermittent PTH also activates ERK1/2-mitogen-activated protein kinase and phosphatidylinositol phosphate signaling pathways, resulting in increased osteoblast proliferation.

The PTH1R exists predominantly on osteoblasts, osteocytes, and preosteoblast-like cells. It has been well established that osteoblast-secreted factors play an essential role in PTH-mediated osteoclastic bone resorption. M-CSF and RANKL are two well-known factors necessary for proliferation of osteoclast progenitors and their

differentiation into mature osteoclasts [38]. Intermittent PTH increases bone formation and promotes bone remodeling [39]. We have shown that CCL2 is the most highly upregulated gene in rat femurs 1 h after the 14th daily hPTH [1–34] injection [40], with nearly 200-fold stimulation of its mRNA expression. Osteoclasts and monocytes are likely to be the central targets for CCL2 in bone. PTH-induced osteoblastic expression of CCL2 facilitates osteoclast recruitment, differentiation, and fusion of osteoclast precursors and finally provides a rationale for increased osteoclast activity in the anabolic effect of PTH. We reported a significant increase in serum CCL2 levels 2 h after PTH injection compared with basal levels in rats treated daily with hPTH. We also found a profound increase in CCL2 expression in osteoblasts *in vivo* by immunohistochemistry and *in vitro* in UMR 106-01 and rat primary calvarial osteoblastic cells after PTH treatment. As well, CCL2 null mice were completely unable to increase trabecular BMD and bone volume compared to wild-type mice after daily injections of PTH. In addition, these mice did not show the increase in macrophage numbers, osteoclast surface, and osteoclast number observed in wild-type mice after PTH injections. We concluded that the reduction in PTH-mediated bone formation in CCL2 null mice was due to the lack of osteoclast and macrophage activity and that osteoblast CCL2 expression is a key mediator for the anabolic effects of PTH on bone [41].

Some recent clinical studies have shown a positive relationship between PTH and MCP-1 levels. Sukumar et al. found a positive association between serum levels of CCL2 and PTH in women with primary hyperparathyroidism (PHPT) [42]. Elevated CCL2 levels increase the risks of hypertension, hyperlipidemia, type 2 diabetes mellitus, and coronary artery disease. A small clinical study by Patel et al. also showed that an immediate decline in high serum CCL2 concentrations after parathyroidectomy of PHPT patients further prove the positive association between MCP-1 and PTH levels in patients with PHPT [43].

Parathyroid hormone-related peptide is a genetically related peptide that shares homology with PTH within its amino-terminal domain, which is considered to contain

the essential bioactivity of PTH and thus could mimic several functions of PTH. PTHrP is synthesized in bone and cartilage and exerts its functions in autocrine and paracrine modes. It has been shown that PTHrP augments bone metastasis in animal models of both prostate cancer and breast cancer. Regulation of CCL2 has investigated its promoter region, which consists of two C/EBP binding sites, two NF- κ B binding sites, and a GC box. The C/EBP binding sites, NF- κ B binding sites, and GC box are important for the response to insulin activation, IL-1 and TNF- α activation, and SP1 binding, respectively. Very little is known of how PTH regulates the CCL2 promoter in osteoblastic cells, and it remains a mystery since this is a primary response gene regulated by the PKA pathway, yet there is no obvious CRE in the upstream region. It has been stated that PTHrP provokes CCL2 promoter activity in hFOB cells through NF- κ B and C/EBP activation [44]. but none has established how the PKA pathway can regulate these factors to stimulate CCL2 transcription.

Conclusion

Bone remodeling is a complex process under the control of several factors, including hormones, growth factors, and other inflammatory mediators. Among them, chemokines and their particular receptors play a vital role as chemoattractant and growth factors for bone cell recruitment and regulation of osteoclastogenesis, respectively.

Chemokines recruit and activate leukocytes at the site of inflammation in response to infection or tissue damage. These leukocytes are primary cells responsible for inflammatory responses and bone metabolism, since they are capable of acting as growth regulators of both osteoblast and osteoclast activity. Growing evidence suggests that CCL2 and its receptor CCR2 are involved in the physiological bone remodeling process. In vitro and in vivo models, together with both ligand and receptor transgenic animals, have made a significant contribution to understanding the molecular mechanisms behind the role of CCL2 in PTH-mediated bone effects.

A more comprehensive understanding of the chemokines that regulate bone remodeling could reveal new possibilities for the development of novel and more

successful drug therapies for the treatment of severe bone loss diseases, including osteoporosis, rheumatoid arthritis, or cancer-mediated bone metastasis. In conclusion, modulation of the CCL2/CCR2 axis may provide the potential mechanism to therapeutically limit the bone resorption and blunt bone loss.

References

1. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* (2006) 354(6):610–21. doi:10.1056/NEJMra052723

[CrossRef Full Text](#) | [Google Scholar](#)

2. Li X, Liu H, Qin L, Tamasi J, Bergenstock M, Shapses S, et al. Determination of dual effects of parathyroid hormone on skeletal gene expression in vivo by microarray and network analysis. *J Biol Chem* (2007) 282(45):33086–97. doi:10.1074/jbc.M705194200

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

3. Hayashida K, Nanki T, Girschick H, Yavuz S, Ochi T, Lipsky PE. Synovial stromal cells from rheumatoid arthritis patients attract monocytes by producing MCP-1 and IL-8. *Arthritis Res* (2001) 3(2):118–26. doi:10.1186/ar149

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

4. Kusano KF, Nakamura K, Kusano H, Nishii N, Banba K, Ikeda T, et al. Significance of the level of monocyte chemoattractant protein-1 in human atherosclerosis. *Circ J* (2004) 68(7):671–6. doi:10.1253/circj.68.671

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

5. Sorensen TL, Ransohoff RM, Strieter RM, Sellebjerg F. Chemokine CCL2 and chemokine receptor CCR2 in early active multiple sclerosis. *Eur J Neurol* (2004) 11(7):445–9. doi:10.1111/j.1468-1331.2004.00796.x

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

6. Li X, Loberg R, Liao J, Ying C, Snyder LA, Pienta KJ, et al. A destructive cascade mediated by CCL2 facilitates prostate cancer growth in bone. *Cancer Res* (2009) 69(4):1685–92. doi:10.1158/0008-5472.CAN-08-2164

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

7. Wise GE, Frazier-Bowers S, D'Souza RN. Cellular, molecular, and genetic determinants of tooth eruption. *Crit Rev Oral Biol Med* (2002) 13(4):323–34. doi:10.1177/154411130201300403

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

8. Binder NB, Niederreiter B, Hoffmann O, Stange R, Pap T, Stulnig TM, et al. Estrogen-dependent and C-C chemokine receptor-2-dependent pathways determine osteoclast behavior in osteoporosis. *Nat Med* (2009) 15(4):417–24. doi:10.1038/nm.1945

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

9. Graves DT, Jiang Y, Valente AJ. Regulated expression of MCP-1 by osteoblastic cells in vitro and in vivo. *Histol Histopathol* (1999) 14(4):1347–54.

[PubMed Abstract](#) | [Google Scholar](#)

10. Hoshino A, Ueha S, Hanada S, Imai T, Ito M, Yamamoto K, et al. Roles of chemokine receptor CX3CR1 in maintaining murine bone homeostasis through the regulation of both osteoblasts and osteoclasts. *J Cell Sci* (2013) 126(Pt 4):1032–45. doi:10.1242/jcs.113910

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

11. Li X, Qin L, Bergenstock M, Bevelock LM, Novack DV, Partridge NC. Parathyroid hormone stimulates osteoblastic expression of MCP-1 to recruit and increase the fusion of pre/osteoclasts. *J Biol Chem* (2007) 282(45):33098–106. doi:10.1074/jbc.M611781200

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

12. Rahimi P, Wang CY, Stashenko P, Lee SK, Lorenzo JA, Graves DT. Monocyte chemoattractant protein-1 expression and monocyte recruitment in osseous inflammation in the mouse. *Endocrinology* (1995) 136(6):2752–9. doi:10.1210/endo.136.6.7750500

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

13. Tamasi JA, Vasilov A, Shimizu E, Benton N, Johnson J, Bitel CL, et al. Monocyte chemoattractant protein-1 is a mediator of the anabolic action of parathyroid hormone on bone. *J Bone Miner Res* (2013) 28(9):1975–86. doi:10.1002/jbmr.1933

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

14. Zheng MH, Fan Y, Smith A, Wysocki S, Papadimitriou JM, Wood DJ. Gene expression of monocyte chemoattractant protein-1 in giant cell tumors of bone osteoclastoma: possible involvement in CD68+ macrophage-like cell migration. *J Cell Biochem* (1998) 70(1):121–9. doi:10.1002/(SICI)1097-4644(19980701)70:1<121::AID-JCB12>3.3.CO;2-D

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

15. Ugucioni M, Loetscher P, Forssmann U, Dewald B, Li H, Lima SH, et al. Monocyte chemotactic protein 4 (MCP-4), a novel structural and functional analogue of MCP-3 and eotaxin. *J Exp Med* (1996) 183(5):2379–84. doi:10.1084/jem.183.5.2379

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

16. Schall TJ. Biology of the RANTES/SIS cytokine family. *Cytokine* (1991) 3(3):165–83. doi:10.1016/1043-4666(91)90013-4

[CrossRef Full Text](#) | [Google Scholar](#)

17. Sarafi MN, Garcia-Zepeda EA, MacLean JA, Charo IF, Luster AD. Murine monocyte chemoattractant protein (MCP)-5: a novel CC chemokine that is a structural and functional homologue of human MCP-1. *J Exp Med* (1997) 185(1):99–109. doi:10.1084/jem.185.1.99

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

18. Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F, et al. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci U S A* (1990) 87(13):5134–8. doi:10.1073/pnas.87.13.5134

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

19. Standiford TJ, Kunkel SL, Phan SH, Rollins BJ, Strieter RM. Alveolar macrophage-derived cytokines induce monocyte chemoattractant protein-1 expression from human pulmonary type II-like epithelial cells. *J Biol Chem* (1991) 266(15):9912–8.

[PubMed Abstract](#) | [Google Scholar](#)

20. Barna BP, Pettay J, Barnett GH, Zhou P, Iwasaki K, Estes ML. Regulation of monocyte chemoattractant protein-1 expression in adult human non-neoplastic astrocytes is sensitive to tumor necrosis factor (TNF) or antibody to the 55-kDa TNF receptor. *J Neuroimmunol* (1994) 50(1):101–7. doi:10.1016/0165-5728(94)90220-8

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

21. Brown Z, Strieter RM, Neild GH, Thompson RC, Kunkel SL, Westwick J. IL-1 receptor antagonist inhibits monocyte chemotactic peptide 1 generation by human mesangial cells. *Kidney Int* (1992) 42(1):95–101. doi:10.1038/ki.1992.266

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

22. Yoshimura T, Robinson EA, Tanaka S, Appella E, Leonard EJ. Purification and amino acid analysis of two human monocyte chemoattractants produced by phytohemagglutinin-stimulated human blood mononuclear leukocytes. *J Immunol* (1989) 142(6):1956–62.

[PubMed Abstract](#) | [Google Scholar](#)

23. Yoshimura T, Yuhki N, Moore SK, Appella E, Lerman MI, Leonard EJ. Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. *FEBS Lett* (1989) 244(2):487–93. doi:10.1016/0014-5793(89)80590-3

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

24. Taub DD, Proost P, Murphy WJ, Anver M, Longo DL, van Damme J, et al. Monocyte chemotactic protein-1 (MCP-1), -2, and -3 are chemotactic for human T lymphocytes. *J Clin Invest* (1995) 95(3):1370–6. doi:10.1172/JCI117788

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

25. Ajuebor MN, Flower RJ, Hannon R, Christie M, Bowers K, Verity A, et al. Endogenous monocyte chemoattractant protein-1 recruits monocytes in the zymosan peritonitis model. *J Leukoc Biol* (1998) 63(1):108–16.

[PubMed Abstract](#) | [Google Scholar](#)

26. Paavola CD, Hemmerich S, Grunberger D, Polsky I, Bloom A, Freedman R, et al. Monomeric monocyte chemoattractant protein-1 (MCP-1) binds and activates the MCP-1 receptor CCR2B. *J Biol Chem* (1998) 273(50):33157–65. doi:10.1074/jbc.273.50.33157

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

27. Ransohoff RM. Chemokines and chemokine receptors: standing at the crossroads of immunobiology and neurobiology. *Immunity* (2009) 31(5):711–21. doi:10.1016/j.immuni.2009.09.010

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

28. Charo IF, Myers SJ, Herman A, Franci C, Connolly AJ, Coughlin SR. Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails. *Proc Natl Acad Sci U S A* (1994) 91(7):2752–6. doi:10.1073/pnas.91.7.2752

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

29. Wong LM, Myers SJ, Tsou CL, Gosling J, Arai H, Charo IF. Organization and differential expression of the human monocyte chemoattractant protein 1 receptor gene. Evidence for the role of the carboxyl-terminal tail in receptor trafficking. *J Biol Chem* (1997) 272(2):1038–45. doi:10.1074/jbc.272.2.1038

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

30. Gong X, Gong W, Kuhns DB, Ben-Baruch A, Howard OM, Wang JM. Monocyte chemotactic protein-2 (MCP-2) uses CCR1 and CCR2B as its functional receptors. *J Biol Chem* (1997) 272(18):11682–5. doi:10.1074/jbc.272.18.11682

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

31. Wain JH, Kirby JA, Ali S. Leucocyte chemotaxis: examination of mitogen-activated protein kinase and phosphoinositide 3-kinase activation by monocyte

chemoattractant proteins-1, -2, -3 and -4. Clin Exp Immunol (2002) 127(3):436–44.
doi:10.1046/j.1365-2249.2002.01764.x

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

32. Gouwy M, Struyf S, Catusse J, Proost P, Van Damme J. Synergy between proinflammatory ligands of G protein-coupled receptors in neutrophil activation and migration. J Leukoc Biol (2004) 76(1):185–94. doi:10.1189/jlb.1003479

[CrossRef Full Text](#) | [Google Scholar](#)

33. Sozzani S, Zhou D, Locati M, Rieppi M, Proost P, Magazin M, et al. Receptors and transduction pathways for monocyte chemotactic protein-2 and monocyte chemotactic protein-3. Similarities and differences with MCP-1. J Immunol (1994) 152(7):3615–22.

[PubMed Abstract](#) | [Google Scholar](#)

34. Power CA, Meyer A, Nemeth K, Bacon KB, Hoogewerf AJ, Proudfoot AE, et al. Molecular cloning and functional expression of a novel CC chemokine receptor cDNA from a human basophilic cell line. J Biol Chem (1995) 270(33):19495–500. doi:10.1074/jbc.270.33.19495

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

35. Siddiqui JA, Partridge NC. Physiological bone remodeling: systemic regulation and growth factor involvement. Physiology (Bethesda) (2016) 31(3):233–45. doi:10.1152/physiol.00061.2014

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

36. Eraltan H, Cacina C, Kahraman OT, Kurt O, Aydogan HY, Uyar M, et al. MCP-1 and CCR2 gene variants and the risk for osteoporosis and osteopenia. Genet Test Mol Biomarkers (2012) 16(4):229–33. doi:10.1089/gtmb.2011.0216

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

37. Graves DT, Jiang Y, Valente AJ. The expression of monocyte chemoattractant protein-1 and other chemokines by osteoblasts. Front Biosci (1999) 4:D571–80. doi:10.2741/A453

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

38. Volejnikova S, Laskari M, Marks SC Jr, Graves DT. Monocyte recruitment and expression of monocyte chemoattractant protein-1 are developmentally regulated in remodeling bone in the mouse. *Am J Pathol* (1997) 150(5):1711–21.

[PubMed Abstract](#) | [Google Scholar](#)

39. Maeda A, Bandow K, Kusuyama J, Kakimoto K, Ohnishi T, Miyawaki S, et al. Induction of CXCL2 and CCL2 by pressure force requires IL-1beta-MyD88 axis in osteoblasts. *Bone* (2015) 74:76–82. doi:10.1016/j.bone.2015.01.007

[CrossRef Full Text](#) | [Google Scholar](#)

40. Kim MS, Day CJ, Morrison NA. MCP-1 is induced by receptor activator of nuclear factor- κ B ligand, promotes human osteoclast fusion, and rescues granulocyte macrophage colony-stimulating factor suppression of osteoclast formation. *J Biol Chem* (2005) 280(16):16163–9. doi:10.1074/jbc.M412713200

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

41. Miyamoto K, Ninomiya K, Sonoda KH, Miyauchi Y, Hoshi H, Iwasaki R, et al. MCP-1 expressed by osteoclasts stimulates osteoclastogenesis in an autocrine/paracrine manner. *Biochem Biophys Res Commun* (2009) 383(3):373–7. doi:10.1016/j.bbrc.2009.04.020

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

42. Sul OJ, Ke K, Kim WK, Kim SH, Lee SC, Kim HJ, et al. Absence of MCP-1 leads to elevated bone mass via impaired actin ring formation. *J Cell Physiol* (2012) 227(4):1619–27. doi:10.1002/jcp.22879

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

43. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* (2009) 29(6):313–26. doi:10.1089/jir.2008.0027

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

44. Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, et al. Abnormalities in monocyte recruitment and cytokine expression in monocyte

chemoattractant protein 1-deficient mice. J Exp Med (1998) 187(4):601–8.

doi:10.1084/jem.187.4.601

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)