Inflammation, Endoplasmic Reticulum Stress, Autophagy, and the Monocyte Chemoattractant Protein-1/CCR2 Pathway

Pappachan E. Kolattukudy, Jianli Niu

Abstract: Numerous inflammatory cytokines have been implicated in the pathogenesis of cardiovascular diseases. Monocyte chemoattractant protein (MCP)-1/CCL2 is expressed by mainly inflammatory cells and stromal cells such as endothelial cells, and its expression is upregulated after proinflammatory stimuli and tissue injury. MCP-1 can function as a traditional chemotactic cytokine and also regulates gene transcription. The recently discovered novel zinc-finger protein, called MCPIP (MCP-1-induced protein), initiates a series of signaling events that causes oxidative and endoplasmic reticulum (ER) stress, leading to autophagy that can result in cell death or differentiation, depending on the cellular context. After a brief review of the basic processes involved in inflammation, ER stress, and autophagy, the recently elucidated role of MCP-1 and MCPIP in inflammatory diseases is reviewed. MCPIP was found to be able to control inflammatory response by inhibition of nuclear factor-κB activation through its deubiquitinase activity or by degradation of mRNA encoding a set of inflammatory cytokines through its RNase activity. The potential inclusion of such a novel deubiquitinase in the emerging anti-inflammatory strategies for the treatment of inflammation-related diseases such as cardiovascular diseases and type 2 diabetes is briefly discussed. (Circ Res. 2012;110:174-189.)

Key Words: cardiovascular disease ■ type 2 diabetes ■ angiogenesis ■ adipogenesis ■ osteoclastogenesis

Inflammation is widely recognized to be at the root of a host of serious human diseases from heart disease to diabetes and cancer. Inflammation involves permeability changes in vascular walls leading to enhanced movement of immune cells from the vascular lumen into the tissue. Acute inflammation can occur in response to pathogen attack (infection) or tissue injury. This process involves activation of leukocytes and production of reactive oxygen that kills invading pathogens. The pathogen and cell debris resulting from tissue injury are phagocytosed into the leukocytes, where they undergo degradation. This acute inflammatory process is short-lived because it clears up when the injury or infection is resolved. Persistent inflammatory stimuli or dysfunction of the resolution phase results in chronic inflammation, which has been recognized as the underlying factor in the development of various diseases including cardiovascular diseases, obesity-induced type 2 diabetes, rheumatoid arthritis, and cancer.1-3 Consequently, anti-inflammatory therapies are being explored for prevention and treatment of these diseases.4-6 As heart disease progresses, the inflammatory...
response is activated, contributing to the deleterious effects on the heart and vasculature, progression of ventricular dysfunction, and heart failure.7,8 Key players that contribute to the inflammatory response include agents that mediate blood leukocyte recruitment into tissues and cause local activation and interaction with resident tissue cells. Monocyte chemoattractant protein, MCP-1/CCCL2, is one of the best-studied chemokines that is expressed by mainly inflammatory cells and stromal cells such as endothelial cells, and its expression is upregulated after proinflammatory stimuli and tissue injury.9,10 MCP-1 can function as a traditional chemotactic cytokine and also regulates gene transcription.9,10 Many reports indicate that endoplasmic reticulum (ER) stress and autophagy are involved in the induction of inflammatory response and contributes to the pathogenesis of chronic inflammatory diseases.11,12 Understanding the mechanisms involved in the regulation of inflammation may help to identify novel therapeutic targets with important clinical applications. This review will discuss the pathogenesis of cardiovascular and associated diseases involving MCP-1-mediated processes. We focus on the key inflammatory signaling pathways related to the development of cardiovascular diseases. We present experimental evidence that suggest that MCP-1 triggers the expression of a recently identified novel zinc-finger protein that induces ER stress, which leads to autophagy and controls inflammatory response by negatively regulating nuclear factor-κB (NFκB), a master controller of inflammation, by catalyzing deubiquitination. Potential novel strategies to treat cardiovascular disease through the use of agents that target MCP-1/CCR2 signaling are also briefly reviewed.

Inflammatory Signaling Pathways in Cardiovascular Disease

There is abundant evidence that inflammatory processes are involved in cardiovascular injury resulting from ischemia and/or reperfusion, thrombosis, and infection.7,8,13 For example, myocardial inflammation has been implicated as a secondary injury mechanism after ischemia and reperfusion.13 Inflammation is also a major component of the damage caused by infectious diseases such as myocarditis and rheumatic heart disease and is also a fundamental contributor to atherosclerosis, ischemic heart disease, and heart failure, as well as transplant vasculopathy and stroke.7,8 The inflammatory response is a complex process consisting of many components and their interactions, and these inflammatory molecules and pathways are tightly interrelated and alter cellular physiology, leading to various pathologies in the cardiovascular system.7,8 The major cellular players involved in the inflammatory reaction are monocytes/macrophages, neutrophils, mast cells, blood platelets, and T cells. They are activated rapidly in response to tissue injury or infection and exert diverse actions. In the cardiovascular system, endothelial and smooth muscle cells, cardiomyocytes, and fibroblasts participate in the inflammatory reaction.8 Tissue macrophages are the primary cells implicated in cardiovascular inflammation. The presence of damaged cells and cell debris can cause the resident macrophages to be activated, leading to release of proinflammatory cytokines and other molecules, such as reactive oxygen species (ROS) and proteases, which can cause damaging or protective effects on neighboring cells (eg, cardiomyocytes). In response to injury, both endothelial cells and cardiomyocytes can change into a proinflammatory phenotype and produce some inflammatory mediators, such as MCP-1.11–15 These molecules are key contributors to tissue injury by their ability to attract circulating blood cells (eg, monocytes, neutrophils, and lymphocytes) and facilitate the
transmigration of these cells into the site of damage. These infiltrating cells can also produce inflammatory mediators that can locally activate and interact with resident tissue cells, resulting in the initiation and progression of cardiovascular disease (Figure 1).

The inflammatory reaction is generally triggered by the activation of pattern recognition receptors (PRRs), for example, toll-like receptors (TLRs) and nucleotide oligomerization domain-like receptors (NLRs), which recognize pathogens or pathogen-associated molecular patterns (PAMPs).16,17 Endogenous products derived from damaged cells and tissues, termed damage-associated molecular pattern (DAMP), can initiate an intense inflammatory response in the same way as pathogens.18,19 We demonstrated that Fas ligands released by dying infiltrating inflammatory cells can initiate an intense inflammatory response and contribute to the development of ischemic cardiomyopathy and heart failure.20–22 PRRs are present not only in immune cells such as macrophages and dendritic cells but also in the “nonprofessional” immune cells such as endothelial cells, vascular smooth muscle cells, and cardiomyocytes.16 Currently, very little is known about the effects of NLRs in the cardiovascular system. TLRs are implicated in a range of cardiovascular diseases and syndromes including atherosclerosis, sepsis, ischemia, cardiac remodeling, and heart failure.23 The TLR signaling network has already been reviewed.14,24 Briefly, the binding of PAMP/DAMP to a PRR leads to a sequential cascade of different transcriptional regulatory events, resulting in inflammatory responses. NFκB, which is activated by many stimuli, is a central player involved in the inflammatory processes associated with the development of cardiovascular diseases.25,26 NFκB activation triggers the production of molecules such as adhesion molecules, chemokines, and proinflammatory cytokines, all of which are involved in inflammatory responses. In turn, these responses lead to infiltration of neutrophils, monocytes, and lymphocytes into the sites of tissue damage, where activation and interaction with resident tissue cells result in sustained inflammation. Other distinctive signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway, the phosphoinositide 3-kinase (PI3K)-related signaling pathway, and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway, interacting with the TLR4 signaling pathway, were also implicated in the pathogenesis of cardiovascular diseases.27,28 These different pathways activate a series of downstream transcriptional factors and produce a great quantity of inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α and initiate inflammatory responses.

**ER Stress in Inflammation**

In recent years, considerable evidence has demonstrated that inflammation within the cardiovascular system is linked to endoplasmic reticulum (ER) stress and unfolded protein response (UPR), which alters gene expression and translational programs to overcome stressful conditions and to restore ER homeostasis.11,12,29,30 Recent evidence suggests that ER stress is a key factor in the inflammatory response and a potential mediator of inflammation in cardiovascular disease.31–33

**ER Stress and UPR Signaling**

The intracellular pathways that mediate UPR have been reviewed.34,35 These UPR signaling cascades are initiated through 3 ER-localized transmembrane proteins, namely, inositol-requiring enzyme 1 (IRE1), pancreatic ER kinase (PERK), and activating transcription factor 6 (ATF6). In the absence of stress, these 3 proteins associate with the 78-kDa glucose-regulated protein (GRP78) and remain in an inactive state. Under ER stress, GRP78 preferentially binds to unfolded or misfolded proteins, resulting in the release and activation of these transmembrane proteins. In response to ER...
stress, IRE1α autophosphorylation can elicit endoribonuclease activity that cleaves off a 26-base intron from the mRNA encoding X-box binding protein-1 (XBP1), resulting in expression of active XBPI with potent transcriptional activity that activates expression of UPR target genes.36

PERK is one of the stress kinases responsible for phosphorylation of eIF2 protein and consequent inhibition of protein synthesis.37 Phosphorylated eIF2α can also increase the expression of ATF4 by alternate translation.38 This translational induction of ATF4 can induce expression of UPR target genes involved in oxidative stress. PERK phosphorylation can also activate nuclear erythroid 2-related factor 2–driven expression of genes that encode antioxidant enzymes that are thought to counteract oxidative stress initiated by ER stress.39

On release of ATF6 from GRP78, it translocates from ER to the Golgi, where site-1 protease (S1P) and S2P cleaves off the cytosolic domain of ATF6 from the membrane, resulting in the translocation of its functional fragment containing a basic leucine zipper transcription factor to the nucleus, leading to the activation of transcription of a set of UPR genes encoding chaperones and protein-modifying enzymes related to protein folding and ER-associated degradation (ERAD).40,41 If these signaling mechanisms fail to resolve the mismatch between protein load and handling capacity over a sustained period of time, the UPR induces autophagy as an alternative coping mechanism to remove unfolded or misfolded proteins in the ER lumen that cannot be degraded by ERAD. Although the proteins targeted by autophagy and the ubiquitin proteasome system are different, the 2 systems serve a similar purpose contributing to maintenance of cellular homeostasis.

Integration of ER Stress and Inflammation

On tissue injury, inflammatory cells (eg, neutrophils and macrophages) are recruited to the site of damage, leading to the production of inflammatory cytokines and generation of ROS. Such factors could trigger ER stress. It has been shown that activation of TLR signal can activate IRE1 and its downstream target XBPI, which is required for the production of proinflammatory cytokines such as TNF-α, MCP-1, IL-6, IL-8, and CXCL3 in macrophages and endothelial cells, resulting in enhanced TLR responses contributing to inflammation.42–44 XBPI is also required for the differentiation of B lymphocytes and dendritic cells, both of which are critical in mediating inflammatory response and production of cytokines.45

The 3 UPR signal pathways are tightly interrelated with inflammatory pathways (Figure 2). An increase in the protein load in the ER has been shown to induce NFκB activation.29,32 In response to ER stress, PERK-eIF2α–mediated translation suppression of IκB can directly induce NFκB activation by increasing the ratio of NF-κB to IκB, thereby allowing the excess NFκB to enter the nucleus to trigger expression of inflammatory cytokines.46 IRE1α has been increasingly recognized as a key molecule integrating ER stress signaling to inflammatory signaling pathway by interaction with TRAF2 (TNF receptor-associated factor 2), which activates protein kinases, resulting in the activation of several inflammatory signals.57–59 On ER stress, the autophosphorylation of IRE1α leads to its binding to TRAF2 leading to recruitment of IκB kinase (IKK) and promotes NFκB-mediated inflammation. The IRE1α-TRAF2 complex can also interact with JNK (Jun N-terminal Kinase), which activates transcription factor activator protein 1 (AP-1), resulting in expression of inflammatory genes.50 ATF6 has been linked to the acute-phase-response, a broad immunologic process associated with infiltration and activation of inflammatory cells and production of inflammatory mediators.51 Cleavage of ATF6 leads to transcriptional activation of a set of acute-phase proteins such as C-reactive protein (CRP), which is a well-known marker of systemic inflammatory response. CRP can cause elevated expression of MCP-1 receptor and thus contribute to inflammation.52,53 In addition, ATF6 itself can trigger NFκB-mediated inflammation through Akt kinase phosphorylation.54

Inflammatory pathways and ER stress are integrated also through the intracellular calcium and the generation of ROS.12,32 Nitric oxide (NO) synthesized by inducible NO synthase (iNOS) in inflammatory cells can activate ER stress signaling pathways through disturbance of ER Ca2+ homeostasis, or enhancing ROS generation, or inhibiting protein disulfide isomerase (PDI) by NO-induced S-nitrosylation.33 Under ER stress conditions, the increased protein folding triggered by UPR can result in the generation of ROS due to Ca2+ release from ER through inositol-trisphosphate receptors.35 ROS generated by inflammation can also induce ER stress by inhibition of protein disulfide isomerase (PDI) and subsequent accumulation of polyubiquitinated proteins, which can trigger NFκB signaling activation and the inflammatory response.55 Thus, ER stress is an important component of chronic inflammation, which plays a critical role in the development of cardiovascular diseases.

ER Stress-Mediated Autophagy in Cardiovascular Disease

Multiple components of the UPR signaling link ER stress to cell death, including activation of transcription factors, proteinases, kinases, and Bcl-2 family proteins.35 ER stress-mediated signaling can often induce apoptotic and nonapoptotic cell death.55,56 Several UPR signaling pathways have been linked to the induction of autophagy.57 For example, activation of IRE1 can induce autophagy, probably through interaction with TRAF2 and activation of JNK, which are known to control the key autophagy regulator Beclin-1 expression.58 PERK-dependent eIF2α phosphorylation also plays an important role in the induction of autophagy (Atg) through upregulation of Atg12.57 It was recently reported that XBPI ablation induces autophagy and protects against amyotrophic lateral sclerosis.59 A basal-level autophagy plays an important role in the normal healthy functioning of cardiomyocytes. Thus, cardiac-specific deletion of the autophagy gene, atg5, in adult mice induced rapid development of cardiac hypertrophy and contractile dysfunction.60 The human disease resulting from the impairment of autophagy manifests severe cardiomyopathy with left ventricular dysfunction.61,62 Autophagy is also thought to play a key role in hypoxic adaptation that can be critical for survival of the heart.
through mild ischemia conditions.61,63 Autophagy helps generate ATP from the digestion products, and this helps cells to survive through the hypoxic period, thus allowing full recovery when hypoxia is eased. Autophagy can protect against cell death by removing damaged mitochondria and consequently reducing proapoptotic factors such as ROS and cytochrome C, as well as by removing toxic protein aggregates.63,64 Thus, autophagy contributes to the survival of cardiac myocytes during transient mild hypoxia or chronic mild hypoxia.

On the other hand, under the nearly complete ischemia that occurs in the risk area in myocardial infarction, autophagy is unable to prevent cardiomyocyte death except in the border zone, which may get some oxygen from adjacent areas through diffusion.61 At the reperfusion phase, autophagy occurs, but the cells die because autophagy is no longer capable of protecting against death caused by the extreme stress. Thus, ischemia/reperfusion-induced infarct size and the number of autophagosomes were smaller in beclin-1−/− deficient mice.65 In vitro, inhibition of autophagy in cardiomyocytes with chemical inhibitors or gene knockdown protected against ischemia reperfusion.66 There is good evidence for the role of autophagy in cell death caused by ischemia/reperfusion in the brain. Focal cerebral ischemia induces delayed cell death of neurons in the peri-infarct zone.67 In such cells, elevated expression of beclin-1 and accumulation of autophagosomes/autolysosomes were found. Furthermore, brain-specific knockout of atg7 in mice caused inhibition of ischemia-induced death of neurons and autophagy. Thus, autophagy is involved in cell death in both the heart and the brain subjected to the extreme stress of ischemia/reperfusion. It appears that autophagy can be protective under mild stress, whereas it contributes to damage by causing cell death under very severe stress.

There is increasing evidence that autophagy plays a role in differentiation, development, and cell death.68 Studies have shown that autophagy is important for cell differentiation during erythropoiesis,69,70 lymphopoiesis,71,72 and adipogenesis.73,74 This aspect is discussed in a later section. When autophagy cannot control protein and organelle quality, the result is a form of nonapoptotic cell death known as “autophagy-dependent necroptosis” or “necroptosis,” which involves Fas/TNF-α death domain receptor activation and inhibition of receptor-interacting protein (RIP) 1 kinase.75,76 Such a death process contributes to the pathogenesis of cardiovascular diseases, including ischemic injury and car-

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**Figure 2. Signal transduction events that link ER stress to inflammation.** Unfolded proteins in the ER cause release of IRE1, PERK, and ATF6. Once released, IRE1α binds TRAF2, activating signaling downstream kinases that activate NF-κB and AP-1, causing expression of genes associated with inflammatory response. The intrinsic ribonuclease activity of IRE1α also results in production of XBP1, inducing production of inflammatory cytokines by enhancing TLR signaling and differentiation of B lymphocytes and dendritic cells. PERK activates its intrinsic kinase activity, causing phosphorylation of eIF2 and attenuation of translation of IκB, resulting in excess of NFκB moving to the nucleus and induces expression of genes involved in inflammatory pathways. Selective activation ATF4 by PERK induces production of inflammatory cytokines and regulates redox homeostasis. Activation of ATF6 also induces activation of NFκB. ATF6 can also induce production of XBP1 (not shown).
diomyopathy. It has been also shown that necroptosis is a driver of injury-induced inflammation by death receptors. Thus, prolonged ER stress can lead to autophagy and apoptotic or necroptotic cell death or differentiation, depending on cellular context.

**ER Stress in Cardiovascular Disease**

There is compelling evidence that ER stress plays fundamental roles in the development and progression of cardiovascular diseases such as ischemic heart diseases and atherosclerosis. In the heart, hypoxia, ischemia/reperfusion (I/R), pressure overload, and inflammation can result in activation of ER stress. Several studies have demonstrated that ER stress is activated in rodent models of myocardial ischemia/reperfusion, pressure overload, and myocardial infarction. In humans with heart failure, cardiac ER stress has been evidenced by the existence of spliced XBP1 and the finding that the development of atherosclerosis was associated with cardiac ER stress as indicated by induction of GRP78, ATF4, and CHOP gene expression. We have reported that ER stress-associated genes, including CHOP, are induced in the heart of a mouse model with ischemic heart disease. It was demonstrated that myocardial I/R induce ER stress response and CHOP-mediated signaling that subsequently causes cardiomyocyte apoptosis and enhances myocardial inflammation, possibly by the transcriptional induction of proinflammatory cytokine genes such as IL-1β and IL6. However, CHOP deficiency almost completely inhibited cardiomyocyte apoptosis in the reperfused myocardium and neuronal cell death in the reperfused mouse brain. CHOP deficiency also suppressed cardiac hypertrophy and heart failure induced by pressure overload. Overexpression of the tamoxifen-activated form of ATF6, one of the ER stress sensors on the ER membrane, induced expression of ER chaperones such as GRP78 and GRP94, resulting in protection against myocardial reperfusion injury. Treatment of mice with the ATF6-specific inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride led to myocardial dysfunction and an increased mortality rate after myocardial infarction. In addition, general inhibition of ER stress response by a protein kinase C inhibitor led to suppressed cardiomyocyte apoptosis and limited infarct size. Experimental autoimmune cardiomyopathy induced by injection of the β1 adrenergic receptor peptide has been also shown to be associated with cardiac ER stress as indicated by induction of Grp78 and CHOP gene expression. Furthermore, transgenic mice expressing a mutant of KDEL receptor (a retrieval signal in the early secretory pathway) or expressing dominant negative mutant of ATF6 showed dilated cardiomyopathy, enhanced expression of CHOP, and apoptotic cardiomyocyte death and compromised cardiac function.

The ER stress-mediated inflammatory pathway has been suggested to play a role in the development of atherosclerosis. Activation of ER stress is implicated in atherosclerotic lesions at all stages of atherosclerosis. Increased expression of ER stress-associated genes, including CHOP, was detected in endothelial cells subjected to atherosclerosis-prone shear stress and macrophages and smooth muscle cells within ruptured plaques. The levels of expression of ER stress-associated genes in atherectomy specimens from patients with unstable angina pectoris were higher than those from patients with stable angina pectoris. A causal link between ER stress and progression of atherosclerosis was indicated by the finding that the development of atherosclerosis was suppressed in the CHOP-deficient mice mated with 2 distinct models of atherosclerotic mice. Accumulation of free cholesterol in the ER is considered as one of the main causes of ER stress leading to activation of UPR and CHOP-induced apoptosis in atherosclerotic lesions, and ER stress induced apoptosis in macrophages is thought to be a major contributor to the instability of atherosclerotic plaques.

**MCP-1 Links ER Stress and Cardiovascular Inflammation**

Several studies have linked ER stress with production of various proinflammatory molecules such as IL-8, IL-6, MCP-1, and TNF-α. Of these, MCP-1 is widely recognized to be a major component of chronic inflammation associated with a variety of major diseases including cancer, obesity-associated type 2 diabetes, and cardiovascular diseases. We review the key role of MCP-1 in inflammation, ER stress, and the development of cardiovascular disease, with brief reference to a major risk factor, obesity, and diabetes.

**Role of MCP-1 in ER Stress Involved in Atherosclerosis**

The pathogenic role of MCP-1 in atherosclerosis is well established. MCP-1 is produced by many cell types, including fibroblasts, cardiomyocytes, endothelial and smooth muscle cells, and monocytes. Many stimuli such as oxidative stress, cytokines, metabolic factors, and shear stress can cause the production of MCP-1. Hyperlipidemia and oxidized lipids can induce MCP-1 production in vascular cells and stimulate the local accumulation of monocytes in the vascular wall, resulting in increased foam cell formation, inflammation, and progression of atherosclerosis. MCP-1, and TNF-α, various proinflammatory molecules such as IL-8, IL-6, MCP-1, and TNF-α. Several studies have linked ER stress with production of MCP-1/MCPIP in Inflammatory Diseases.
phages. These findings suggest that MCP-1 may link ER stress to inflammation, which leads to atherosclerosis.

Role of MCP-1 in ER Stress Involved in Heart Disease
MCP-1 induction is a prominent feature of ischemic myocardium, and increased expression of MCP-1 contributes to the pathogenesis of cardiac hypertrophy, inflammation, and heart failure induced by chronic mechanical overload, myocardial infarction, and diabetes. Disruption of the MCP-1/CCR2 axis decreases and delays macrophage infiltration, reduces production of cytokines, TNF-α, IL-1β, and transforming growth factor (TGF)-β, as well as attenuates cardiac remodeling. MCP-1 gene knockout or inhibition of MCP-1 function, by overexpression of N-terminal deleted MCP-1 gene, and genetic disruption of CCR2 also demonstrate the central role of MCP-1/CCR2 in cardiovascular diseases. To investigate the role of chronic inflammation in the development of cardiovascular disease, we generated mice with cardiac-specific expression of MCP-1. These mice manifested most of the pathological and molecular alterations of human ischemic cardiomyopathy. This transgenic mice model manifested features found in human diseases such as intra-arterial thrombosis, which are said to be not usually manifested in animal models. These transgenic mice manifested activation of ER stress, inflammation, and apoptotic cell death in the myocardium, resulting in the development of ischemic cardiomyopathy and heart failure at 6 to 7 months of age, suggesting that ER stress probably is involved in the development of myocardial inflammation and dysfunction. In support of this conclusion was the finding that cerulium oxide nanoparticles, free radical scavengers, attenuated oxidative and ER stress and ischemic cardiomyopathy. Hyperglycemia-induced cardiomyocyte death is also mediated through ER stress.

The young transgenic animals expressing MCP-1 in the heart manifested a preconditioning-like cardioprotection against I/R injury and postinfarction cardiac dysfunction. It appears that MCP-1–induced ER chaperones may contribute to “preconditioning” of the heart against subsequent myocardial oxidative and ER stress as well as inflammation caused by ischemia. This conclusion is supported by the recent findings that ischemic preconditioning or postconditioning reduced cardiac damage associated with activation of UPR. Overexpression of GRP94 attenuates myocyte necrosis induced by ischemia in cultured H9c2 cardiac myoblasts. Cardioprotection by ischemic preconditioning is found to be closely related to induction of GRP78. MCP-1–induced protein, called MCPIP, turned out to be the first member of a novel family of CCCH zinc-finger proteins containing 4 members that we designate MCPIP 1, 2, 3, and 4, encoded by zc3h12a, zc3h12b, zc3h12c, and z2ch12d, respectively. The best-studied member MCPIP1 is often simply called MCPIP. A genome-wide analysis of the CCCH zinc-finger gene family revealed 58 such genes in mice and 55 in humans. At least 7 of them were found to be expressed in macrophage-related organs such as thymus, spleen, lung, intestine, and adipose tissues.

MCPIP Mediates Inflammation-Associated Differentiation Through Oxidative and ER Stress and Autophagy
Differentiation associated with inflammation is involved in at least 3 pathophysiological processes: (1) angiogenesis associated with cardiovascular diseases, obesity/type 2 diabetes, and tumor growth; (2) adipogenesis associated with obesity; and (3) osteoclastogenesis associated with inflammatory bone erosion. Recent evidence shows that the MCP-1/MCPIP system plays a critical role in all of these differentiation processes. Angiogenesis plays a major role in the reparative and remodeling processes after myocardial injury as well as in the pathogenesis of atherosclerosis and in providing blood to the growing adipose tissue and tumors. MCP-1 is an angiogenic factor associated with the recruitment of monocytic cells. MCP-1 mobilizes and transdifferentiates bone marrow macrophage lineage cells into endothelial-like cells. Recent reports strongly suggest that increased expression of MCP-1 in adipose tissue causes macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis associated with obesity in mice. It was shown that an acute increase in circulating concentration of MCP-1 elicited systemic insulin resistance irrespective of adipose tissue inflammation in mice. That the role of MCP-1 in type 2 diabetes is mediated through its binding to the receptor CCR2 was demonstrated by results obtained with CCR2−/− mice. CCR2 deficiency attenuated the development of obesity in high fat–fed mice.
mice. CCR2 deficiency resulted in reduced macrophage content, increased adiponectin expression, amelioration of hepatic steatosis, and insulin resistance. Treatment with a pharmacological antagonist of CCR2 lowered macrophage content of adipose tissue and improved insulin sensitivity in obese mice. Thus, the MCP-1/CCR2 system plays a critical role in obesity-induced diabetes in mice. Many recent observations suggest that in humans, MCP-1 plays a similar role in obesity-induced diabetes. The increased level of serum MCP-1 levels found in humans correlated with markers of the metabolic syndrome, including obesity, insulin resistance, type 2 diabetes, hypertension, and increased serum concentration of triacylglycerol. In diabetic patients, CCR2 expression in monocytes is elevated.

MCP-1/MCPIP plays a major role in angiogenesis, as indicated by the finding that tube formation in human umbilical vein endothelial cells (HUVEC) was induced by forced expression of MCPIP. MCP-1–induced tube formation was inhibited by knockdown of MCPIP, indicating that MCP-1–induced angiogenesis is mediated through MCPIP. In this case, the transcription factor activity of MCPIP probably plays an important role. Chromatin immunoprecipitation analysis indicated that cadherins (cdh)12 and cdh19 are targets of MCPIP. Knockdown of cdh12 and cdh19 caused marked inhibition of MCPIP-induced tube formation by HUVEC. Because cadherins are likely to be involved in cell-cell adhesion of endothelial cells required for tube formation, it is reasonable to conclude that MCPIP-induced expression of these cadherins is important for angiogenesis. Angiogenesis also involves MCPIP-mediated induction of hypoxia inducible factor (HIF)-1α, which induces vascular endothelial growth factor (VEGF), which causes induction of oxidative stress leading to ER stress and autophagy required for the differentiation. Selective inhibition of each of these postulated steps with chemicals or gene knockdown inhibited subsequent steps in the sequence and tube formation (A. Roy and P.E. Kolattukudy, unpublished). Additional interplay between the intermediary processes is probably involved in this differentiation process. For example, the ER stress-induced UPR can contribute to VEGF expression. Three UPR signaling pathways can independently regulate VEGF expression under ER stress through different regulatory regions of the VEGF gene.

MCP-1/MCPIP is involved in adipogenesis. MCP-1 treatment or forced expression of MCPIP caused induction of CCAAT/enhancer-binding protein (C/EBP) family of transcription factors and peroxisome proliferator-activated receptor (PPAR)-γ as well as adipocyte markers and a robust accumulation of lipid droplets in 3T3-L1 cells in the absence of the classic adipogenesis-inducing ingredients. Surprisingly, MCPIP expression was found to induce robust adipogenesis in the absence of PPARγ, which had been thought to be indispensable for adipogenesis. MCPIP expression in 3T3-L1 cells caused inducible NO synthase (iNOS) induction and elevated reactive oxygen/nitrogen species production that caused ER stress that led to autophagy required for differentiation. Inhibition of any of these steps with selective chemical inhibitors or gene knockdown inhibited the postulated subsequent steps and adipogenesis (C Younce and P.E. Kolattukudy, unpublished). Involvement of autophagy in adipogenesis was demonstrated by the inhibition of adipogenesis by knockdown of Atg7 or Atg5 in 3T3-L1 cells. The role of autophagy in adipogenesis in vivo was demonstrated by the finding that Atg-deficient mice had fewer adipocytes, and adipocyte-specific knockout of Atg7 resulted in a decrease in white adipose tissue.

MCP-1–induced osteoclast differentiation is involved in inflammatory bone erosion involved in diseases such as rheumatoid arthritis, multiple myeloma, and bone metastasis. MCP-1 was reported to cause differentiation of monocyctic cells into osteoclast precursors; MCP-1 deficiency caused a reduction in osteoclasts, and such mice showed osteopetrosis. The MCP-1–mediated differentiation of bone marrow–derived monocyctic cells to osteoclast precursors was recently found to be mediated through MCPIP-induced oxidative and ER stress that resulted in autophagy required for the differentiation. Involvement of autophagy in hypoxia-induced osteoclastogenesis was recently reported.

MCPIP-Induced Death Mediated Through Oxidative and ER Stress and Autophagy

MCPIP was found to induce death in HEK293 cells. Microarray analysis showed that MCPIP expression caused elevation of expression of death-associated genes such as components of caspase activation, cytochrome C release, unique subsets of the Bcl-2 family of genes, and the TNF receptor family members. In transgenic animals with cardiomyocyte-targeted MCP-1 expression, MCPIP was found in dying cardiomyocytes, suggesting that cardiomyocyte death might have been mediated through MCPIP. Forced expression of MCPIP in the H9c2 cardiomyoblasts caused cell death, as indicated by caspase 3 activation and TUNEL assay. Hyperglycemia-induced cardiomyocyte death, which probably plays a critical role in diabetic cardiomyopathy, was found to be mediated through production of MCP-1 and induction of MCPIP.

In terminally differentiated cardiomyocytes, the MCP-1–initiated processes, mediated through MCPIP, leads to death. The intermediate processes initiated by MCPIP that leads to cardiomyocyte death were recently elucidated. MCPIP expression causes induction of reactive oxygen/nitrogen species, which caused ER stress, which led to autophagy. In cardiomyocytes, autophagy is not able to cause cell survival but causes cell death. This postulate regarding the sequence of events is supported by the finding that inhibition of oxidative stress, ER stress, or autophagy by chemical inhibitors or by gene knockdown prevented each subsequent step and cell death. Activation of JNK and p38 and induction of p53 and PUMA (p53 upregulated modulator of apoptosis) by MCPIP appears to be involved in the MCPIP induction of apoptosis. A similar chain of events is involved in hyperglycemia-induced, MCP-1/MCPIP-mediated cell death in H9c2 cells and in neonatal rat cardiomyocytes. Many forms of stress on the myocardium, such as pressure overload, chronic ischemia, and I/R, induce autophagy and cell death and thus contribute to the cardiovascular pathol-
Histone deacetylase inhibitors were shown to attenuate cardiac hypertrophy by suppressing autophagy.

It has been recognized that death and differentiation share some common components such as caspases. However, the biological processes shared between death and differentiation pathways remain obscure. Although a role for individual processes such as oxidative stress, ER stress, and autophagy in cell death and differentiation has been recognized in scattered reports, how these processes are interrelated in bringing about death or differentiation has not been clear. Inflammatory processes lead to death of cells in cardiomyopathy, whereas inflammation can also lead to differentiation processes such as angiogenesis, adipogenesis, and osteoclastogenesis. We have found that in all of these processes, oxidative stress resulting from inflammation causes ER stress, which leads to autophagy, which can lead to cell death in the case of terminally differentiated cells such as cardiomyocytes or differentiation in preadipocytes and monocytic cells (Figure 3).

**MCPIP Controls Inflammation by Inhibition of NFκB Activation**

Assessment of changes in the expression of all of the CCCH zinc-finger gene family during lipopolysaccharide (LPS) activation of mouse macrophages showed that most of this family of genes is associated with macrophage activation. Induction of MCPIP by inflammatory agents such as LPS, TNF-α, and IL-1 appears to be a mechanism by which inflammation can be controlled and thus can be viewed as an autoregulatory feedback mechanism. For example, macrophage activation induced by LPS and cytokines was inhibited by MCPIP expression. Thus, forced expression of MCPIP inhibited LPS-induced production of inflammatory cytokines and oxidative stress. Knockdown of MCPIP expression with siRNA enhanced LPS-induced inflammatory gene expression. Reporter gene expression controlled by TNF-α and iNOS promoters was severely inhibited by MCPIP. NFκB is the well-known integrator of inflammation caused by a variety of inflammatory agents including LPS, IL-1, and TNF-α and thus could be a target of MCPIP action. In fact, forced expression of MCPIP inhibited LPS-induced NFκB activation.

Expression of MCPIP inhibited p65-induced activation of TNF-α, iNOS, and NFκB promoters, both in vitro and in vivo.

How MCPIP regulates NFκB activation is only beginning to be explored. Mice lacking MCPIP (MCPIP−/−) showed systemic inflammation with elevated serum levels of TNF-α and MCP-1. Bone marrow–derived macrophages secreted greater amounts of proinflammatory cytokines. Elevated levels of TNF-α, IL-β, IL-6, and MCP-1 were found in MCPIP−/− macrophages, and the LPS-induced levels of such inflammatory cytokines were much higher in MCPIP−/− macrophages. Expression of such cytokines and iNOS in intestine, lung, and thymus was much higher in

**Figure 3. Schematic representation of the MCP-1/MCPIP-induced processes in inflammatory diseases.** MCPIP promotes oxidative stress that leads to autophagy that can cause different forms of cell death or differentiation that leads to angiogenesis, adipogenesis, and osteoclastogenesis.
MCPIP\(^{-/-}\) mice that showed stunted growth and shortened life span of less than 4 months. Lungs of the MCPIP\(^{-/-}\) mice showed elevated phosphorylation of JNK and IKK\(\beta\), suggesting that the absence of MCPIP caused constitutive activation of JNK and NF\(\kappa\)B signaling. LPS-induced phosphorylation of JNK, ERK \(\frac{1}{2}\), and IKK\(\beta\) was higher in MCPIP\(^{-/-}\), indicating that MCPIP is essential for downregulation of LPS-induced JNK and NF\(\kappa\)B activation. Forced expression of MCPIP in the Raw 264.7 macrophage cell line blocked LPS-induced phosphorylation of JNK and IKK\(\beta\) and nuclear translocation of p65. Gene array analysis of LPS-induced gene expression changes in Raw 264.7 cells showed that expression of genes encoding inflammatory agents TNF, IL-1\(\beta\), IL-6 and MCP-1, whose transcription is known to be controlled by NF\(\kappa\)B and JNK signaling, were repressed by MCPIP.\(^{151}\) Thus, NF\(\kappa\)B, the master controller of inflammation is probably the target of MCPIP.

The in vivo role of MCPIP as an inhibitor of NF\(\kappa\)B has been recently demonstrated.\(^{154}\) Cardiac specific expression of MCPIP in mice resulted in marked attenuation of LPS-induced deterioration of myocardial contractile function.\(^{154}\) MCPIP expression caused marked reduction in the LPS-induced production of inflammatory cytokines, iNOS expression, and peroxynitrite formation as well as reduced caspase activation and apoptosis in the heart. That these protective effects were mediated through inhibition of IKK activation was suggested by the decreased phosphorylation of IKK\(\alpha/\beta\), reduced degradation of I\(\kappa\)B\(\alpha\), and decreased nuclear translocation of the p65 subunit of NF\(\kappa\)B.\(^{154}\)

**MCPIP Inhibits NF\(\kappa\)B Activation Through Its Deubiquitinase Activity**

Ubiquitination plays critical roles in JNK and NF\(\kappa\)B signaling.\(^{155}\) Expression of MCPIP in HEK293 cells caused a decrease in ubiquitinated protein levels, suggesting that MCPIP might either inhibit ubiquitination or deubiquitinate proteins.\(^{152}\) A direct test for deubiquitination activity showed that purified MCPIP cleaved both K48- and K63-linked polyubiquitin, with a preference for higher molecular weight polyubiquitin chains such as Ub\(_6\) and Ub\(_8\). N-ethyl maleimide inhibited polyubiquitin hydrolyase activity of MCPIP, suggesting that it is a Cys proteinase-type enzyme.

In vivo substrates of MCPIP have not been clearly identified. TRAFs are known to be ubiquitinated.\(^{155}\) Stimulus-dependent autoubiquitination of TRAF2 and TRAF6 activates TAK1, which plays a key role in NF\(\kappa\)B activation (Figure 4). Ubiquitinated TRAF2 and TRAF6 levels were lowered by expression of MCPIP in HEK293 cells.\(^{152}\) MCPIP also affected RIP, which modulates NF\(\kappa\)B activity. MCPIP expression caused a major decrease in I\(\kappa\)B\(\alpha\) ubiquitination. MCPIP\(^{-/-}\) caused a drastic increase in basal levels of ubiquitinated TRAF2, TRAF3, and TRAF6 in splenocytes, and LPS treatment caused a further increase in these polyubiquitinated proteins. Isolated polyubiquitinated TRAF2 and TRAF3 were deubiquitinated by incubation with MCPIP.\(^{152}\) Thus, TRAF family members involved in NF\(\kappa\)B signaling are deubiquitinated by MCPIP.

Ligand-activated NF\(\kappa\)B activation is a very complex process involving a major role for phosphorylation and different types of polyubiquitination of the different components involved in NF\(\kappa\)B activation (Figure 4).\(^{155}\) TRAF6 is a ubiquitin ligase involved in NF\(\kappa\)B activation triggered by binding of the IL-1 receptor or TLRs. TRAF6 works with a ubiquitin-conjugating enzyme complex (Ubc13) to catalyze K63 polyubiquitination, which activates the TAK1-kinase complex. This activated kinase phosphorylates IKK, which phosphorylates I\(\kappa\)B, leading to its K48 ubiquitination and degradation, which leads to translocation of the p65/p50 to the nucleus for triggering the expression of a set of NF\(\kappa\)B target genes (Figure 4). The nature of polyubiquitin chains required for activation of TAK1 or IKK activation is not fully understood. Through the use of a reconstituted system consisting of purified proteins, it was recently demonstrated that unanchored large K63 polyubiquitin chains can directly activate TAK1 by binding to the ubiquitin receptor TAB2, causing autophosphorylation and consequent activation of TAK1.\(^{156}\) Treatment of the K63...
polyubiquitin with IsoT, an enzyme that cleaves the polyubiquitin, prevented the activation, suggesting that the intact polyubiquitin was necessary for TAK1 activation. Treatment of high-molecular-weight K63-linked polyubiquitin with purified MCPIP caused hydrolysis of the polyubiquitin and inhibition of TAK1 phosphorylation (M. Zhang, C. Menaa, and P.E. Kolattukudy, unpublished). Because such unanchored polyubiquitin chains were also shown to activate IKK complex,\textsuperscript{156} hydrolytic removal of such polyubiquitin is probably one mechanism by which MCPIP inhibits NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation.

Despite the evidence for the participation of k63-linked polyubiquitin in NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation, knockout of Ubc13, encoding the E2 ligase needed to generate K63-linked chains, showed little effect on NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation, indicating that NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} can be activated independent of K63-linked polyubiquitin chains.\textsuperscript{157} LUBAC, composed of the 2 RING finger proteins HOIL-IL and HOIP, activates the NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} pathway by binding to NEMO and conjugating linear polyubiquitination on to NEMO (Figure 4). In this case, ubiquitin moieties are linked by peptide bonds between the carboxyl terminal Gly of one ubiquitin with the amino group of the N-terminal Met of another. This linear ubiquitination of NEMO is independent of Ubc13. Absence of HOIL-1, which encodes HOIL-1L and HOIL-1, caused severe impairment of TNF\textsubscript{\textit{a}}-induced and IL-1\textbeta-induced NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation in cells and in intact mice, demonstrating the involvement of linear polyubiquitination in NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation.\textsuperscript{157} MCPIP deubiquitinasates both K63- and K48-linked polyubiquitin, attached to the proteins, and unanchored large K63-linked polyubiquitin chains involved in NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation as indicated.\textsuperscript{152} Expression of MCPIP severely inhibited phosphorylation of TAK1 in cells, in which IL-1\textbeta activates NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} (C. Menaa and P.E. Kolattukudy, unpublished). Thus, it appears likely that MCPIP can inhibit NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation by hydrolyzing protein-bound and unanchored polyubiquitin chains involved in NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation (Figure 4). Our results showed that MCPIP expression caused a drastic decrease in RIP ubiquitination but showed much less effect on NEMO ubiquitination.\textsuperscript{152} Consistent with this finding, we found that purified MCPIP does not hydrolyze linear polyubiquitin (M. Zhang and P.E. Kolattukudy, unpublished). Whether deubiquitinase activity of MCPIP is critical for its inhibitory activity on NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation was examined with deletion mutants of MCPIP.\textsuperscript{152} Mutants that lost deubiquitinase activity also lost the ability to inhibit TNF-\alpha-induced NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation, suggesting that inhibition of NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation by MCPIP is through its deubiquitinase activity.\textsuperscript{152}

NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation is also regulated by other deubiquitinases.\textsuperscript{160} For example, overexpression of A20 inhibits LPS-induced activation of NF\textsubscript{\textit{F}}\textsubscript{\textit{B}}. A20 expression also inhibited NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} reporter activation caused by forced expression of Myo 88, IRAK1, IRAK2, TRAF6, and TAK1/TAB2.\textsuperscript{159} Cylindromatosis (CYLD) has been shown to inhibit NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation,\textsuperscript{158} and more recent results indicate that this inhibition might be mediated through the ability of CYLD to hydrolyze unanchored polyubiquitin chains and thus inhibit TAK1 and IKK activation.\textsuperscript{160} Both A20 and CYLD are known to play an anti-inflammatory protective role in cardiovascular diseases by inhibiting NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation.\textsuperscript{161–163} The ligand-induced NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation involves polyubiquitins held together by different linkages such as K48, K63, and linear. Deubiquitinases show different specificities for cleavage of these polyubiquitins.\textsuperscript{164} For example, CYLD cleaves linear and K63 linkages, whereas A20 cleaves K48\textsuperscript{158} and MCPIP cleaves K63 and K48. The roles of the various deubiquitinases in the regulation of NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation triggered by various inflammatory agents in different cell types involved in the development of human diseases are yet to be fully elucidated.

MCPIP may inhibit inflammation also by mechanisms other than the inhibition of NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation. Another demonstrated mechanism involves degradation of mRNA encoding inflammatory cytokines.\textsuperscript{153,165} Thus, in MCPIP-deficient mice, IL-6 mRNA decay was severely impaired in the macrophages. Overexpression of MCPIP accelerated IL6 mRNA degradation at the 3‘-untranslated region (UTR). The N-terminus of MCPIP contains a nuclease domain. Expressed MCPIP protein showed direct binding of the UTR of IL-6 mRNA and manifested RNase activity against IL-6 mRNA. Selectivity of MCPIP for degradation of mRNA was demonstrated by the finding that the expression of TNF-\alpha mRNA was not affected by the absence of MCPIP in the macrophages.\textsuperscript{153} Another zinc-finger protein, tristetrapolin, controls TNF\textsubscript{\textit{a}} mRNA stability.\textsuperscript{166} When IL-6 mRNA degradation by MCPIP was discovered, this activity was considered inadequate to account for the profound pathological changes found in MCPIP-deficient mice.\textsuperscript{153} Probably, inhibition of activation of the master controller of inflammation, which was not discovered until later, can account for the other pathological changes not explained by the IL-6 RNase activity of MCPIP. Thus, MCPIP controls inflammation not only by preventing the production of inflammatory cytokines by inhibiting NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation but also by degrading mRNA for some of the inflammatory cytokines.

How MCPIP carries out RNase and polyubiquitinase activities remains to be elucidated. Direct binding of the UTR of IL-6 mRNA was demonstrated.\textsuperscript{153} MCPIP binding to ubiquitin has been demonstrated by pull-down experiments.\textsuperscript{152} Incubation of ubiquitin vinyl sulfone with MCPIP caused covalent cross-linking of MCPIP with ubiquitin, probably through the catalytic Cys residue (M. Zhang and P.E. Kolattukudy, unpublished). The N-terminal region of MCPIP contains ubiquitin association domain, and deletion of this domain abolished ubiquitin binding.\textsuperscript{152} Several putative Cys boxes and Asp boxes, usually found in Cys proteinases, are present in MCPIP. The Cys box of MCPIP is conserved in organisms from fly to human. Mutagenesis analysis showed that D141N, C157A, D278A, D279A, and C306R had lost the inhibitory activity on NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation, and the corresponding proteins demonstrated loss of deubiquitinase activity.\textsuperscript{152} Our study confirmed the previously reported the loss of RNase activity by D141N mutation.\textsuperscript{152} However, the H88A, C157A, and C306R mutants retained RNase activity but lost deubiquitinase activity and the ability to inhibit NF\textsubscript{\textit{F}}\textsubscript{\textit{B}} activation.\textsuperscript{152} Forced expression of MCPIP in MCPIP-/- fibroblasts suppressed LPS-induced TNF-\alpha and MCP-1 release, but the C157A mutant lost this activity. All of these results suggest that deubiquitinase activity probably plays a more major role
in the anti-inflammatory function of MCPIP than the RNase activity. Because NFκB is the integrator of many inflammatory signals, inhibition of NFκB activation would be an effective means to control inflammation caused by multiple inflammatory agents.

**MCP-1/CCR2 Pathway: A Novel Therapeutic Target in Cardiovascular Disease**

With the realization that inflammation is a key factor in the initiation and progression of the cardiovascular diseases, anti-inflammatory approaches to prevent and treat such diseases have become an avenue under active investigation. The recently designed Cardiovascular Inflammation Reduction Trial, with low doses of methotrexate or placebo, is an example. Another trial, the Canakinumab Anti-inflammatory Thrombosis Outcome Study, tests whether IL-1β inhibition with a human monoclonal antibody is effective against recurrent myocardial infarction, stroke, and death from cardiovascular diseases. Statins constitute the best-characterized anti-inflammatory class of drugs for the prevention of coronary heart disease. In addition to their lipid-lowering activity, statins also exert anti-inflammatory effects. Clinical evidence has indicated that statin-induced decrease in CRP, a marker of inflammation, was significantly correlated with reduced atherosclerosis progression, independent of LDL cholesterol lowering. Further studies by the JUPITER (The Justification for Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial demonstrated that the decrease in the levels of CRP was in parallel with the clinical benefit, suggesting a protective effect of targeting inflammation in the prevention of cardiovascular events.

The MCP-1/CCR2 pathway was recently recognized as a new therapeutic target for treatment of cardiovascular diseases. Interference with MCP-1 interaction with CCR2 has shown beneficial effects against the development of cardiovascular disease. In recent years, small molecules that interfere with MCP-1/CCR2 interaction have been developed and were shown to suppress inflammation in the mouse model of multiple sclerosis, renal ischemia-reperfusion injury, and diabetic nephropathy. A recent clinical study also demonstrated that MLN1202, a humanized monoclonal antibody that blocks MCP-1/CCR2 interaction, can reduce the levels of inflammation in patients with high risk for major adverse cardiovascular events. The synthetic peptide fragment from the C-terminal domain of MCP-1 was recently reported to inhibit monocyte migration and has anti-inflammatory activity in patients with stable angina after coronary stenting. Recent findings that a novel CCR2 antagonist ameliorated insulin resistance in high fat–fed mice suggests that the MCP-1/CCR2 pathway could be a target for drugs against type 2 diabetes. Future clinical trials are needed to test the MCP-1/CCR2 pathway as a potential therapeutic target to reduce cardiovascular events and risk factors such as obesity/diabetes.

**Conclusion and Future Direction**

The realization that chronic inflammation is a critical factor in the development of many of the major human diseases such as cardiovascular diseases, type 2 diabetes, rheumatoid arthritis, and cancer opens up new avenues for therapeutic intervention. Obesity, which affects a rapidly increasing segment of human population, is closely associated with some of the leading causes of death including heart disease, stroke, type 2 diabetes, and cancer. The maladies caused by obesity probably result from the fact that obesity generates a chronic inflammatory condition. It is probable that the chronic inflammation generates oxidative stress that can cause DNA damage leading to cancer. Such a mechanism might help to explain how obesity causes up to 20% of all human cancers, a major issue that attracts great attention at the National Cancer Institute. Inflammation involves oxidative stress that causes ER stress, which leads to autophagy involved in the development of many of the pathologies associated with the obesity-related major diseases. The complex sets of molecular players involved in these processes are incompletely understood, and novel players critical to inflammation-associated processes are being discovered. The involvement of deubiquitination in inflammatory processes presents many unanswered questions. Inflammation-induced oxidative stress can lead to cell death or differentiation, depending on the cellular context. What determines the cellular fate remains unknown. Whether deubiquitination or NFκB is involved in both cellular fates is not known. The biological functions of the many deubiquitinases present in different cell types in the development of human diseases remain to be elucidated. Elucidation of the critical steps involved in this complex series of events will reveal novel targets for therapeutic intervention. Thus, the anti-inflammatory approaches that are currently in clinical trials constitute only a beginning, and future elucidation of the critical steps will lead to an increasing number of opportunities to develop effective anti-inflammatory drugs to treat major human disease.

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None.

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Pappachan E. Kolattukudy and Jianli Niu

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