Targeting the CCL2–CCR2 signaling pathway: potential implications of statins beyond cardiovascular diseases

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Abstract

Background: The chemokine ligand CCL2 and its cognate receptor CCR2 have been implicated in the pathogenesis of a wide variety of diseases. Hence, the inhibition of the CCL2/CCR2 signaling pathway has been of great attention in recent studies. Among suggested medications, statins known as HMG-COA reductase inhibitors with their pleiotropic effects are widely under investigation.

Method: A comprehensive literature search on Scopus and PubMed databases was conducted using the keywords ‘CCL2’, ‘CCR2’, ‘monocyte chemoattractant protein-1’, ‘HMG-COA reductase inhibitor’, and ‘statin’. Both experimental and clinical studies measuring CCL2/CCR2 expressions following statin therapy were identified excluding the ones focused on cardiovascular diseases.

Results: Herein, we summarized the effects of statins on CCL2 and CCR2 expression in various pathologic conditions including immune-mediated diseases, nephropathies, diabetes, rheumatic diseases, neuroinflammation, inflammatory bowel diseases, gynecologic diseases, and cancers.

Conclusion: For the most part, statins play an inhibitory role on the CCL2–CCR2 axis which implies their potential to be further developed as therapeutic options in non-cardiovascular diseases either alone or in combination with other conventional treatments. However, the existing literature mostly focused on experimental models and is therefore inadequate to reach a conclusion.

Keywords: CCL2; CCR2; MCP-1; statins; HMG-COA reductase inhibitor; non-cardiovascular diseases

Introduction

The chemokines comprise a large family of small heparin-binding cytokines with molecular weights ranging from 8 to 14 kDa and a strikingly conserved tertiary structure [1]. Chemokines mediate their physiological functions through G protein-coupled chemokine receptors (GPCRs) which are embedded in the cell membrane. Conformation of GPCRs by binding to their corresponding ligands induces a series of downstream signals leading to leukocyte motility, adhesion, degranulation, mitogenesis, and apoptosis [2–5]. From a functional standpoint, chemokines are classified into hematostatic and inducible subtypes. Hemostatic chemokines carry out the housekeeping function and form the architecture of lymphoid organs, whereas inducible chemokines participate in the recruitment of immune cells into inflammation sites during tissue injury [6]. Currently, up to 50 chemokines and 20 chemokine receptors have been identified in human beings [7]. According to their genetic organization and configuration of the first two cysteine residues, chemokines are categorized into four major subfamilies: CC, CXC, C, and CX3C; with their corresponding receptors named CCR, CXCR, CR, and CX3CR. CC chemokines, the most diverse and abundant subtype, have adjacent cysteines near the N-terminus. Five members of monocyte chemoattractant proteins (MCPs) including CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4), and CCL12 (MCP-5) are distinguished within the CC-chemokine subfamily and share about 60% sequence similarities [1, 8–10]. As their names suggest, these chemokines predominantly affect mononuclear leukocytes (e.g. monocytes, macrophages, and T cells) [11].

CCL2, the first purified and well-investigated human CC chemokine, is a 13 kDa protein consisting of 76 amino acids [12]. The CCL2 coding gene is mapped at chromosome 17 (chr. 17, q11.2), and its expression could be induced by oxidative stress and different cytokines such as interleukins (ILs), tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and bacterial lipopolysaccharide (LPS) [13, 14]. CCL2 is produced by a wide range of cells and could regulate the recruitment of various cells such as monocytes, dendritic cells, natural killer (NK) cells, and memory T cells into the site of inflammation [15–19]. The significance of CCL2/CCR2 axis in human health and disease is mirrored by its contribution to the progression of different pathological conditions such as cardiovascular and non-cardiovascular diseases. Although the role of this signaling pathway in a

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wide range of disorders is quite well described, the potential therapeutic strategies that hinder the progression of such conditions by targeting the CCL2/CCR2 axis have not been adequately distinguished [20–22].

Competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase also known as statins, are the most efficient medications to lower serum concentrations of low-density lipoprotein cholesterol (LDLc) [23]. In both primary and secondary intervention trials, statins have dramatically reduced cardiovascular morbidity and mortality [24, 25]. The pleiotropic effects of statins are mainly independent of lipid-lowering properties and mostly beneficial [26–40], though adverse effects also exist [33, 41, 42]. The ability of statins to interfere with the expression, secretion, and function of immune mediators makes them a potential treatment for inflammatory diseases [43–49]. The scope of this review is to introduce the pathophysiologic roles of the CCL2–CCR2 signaling pathway in different non-cardiovascular diseases. We also summarize the CCL2–CCR2 targeting agents, with a particular emphasis on statins.

**Literature search**

The literature search was performed on original articles published up to June 2022 in English using PubMed and Scopus databases with the string: CCL2 and statin. For each term, all synonyms and related keywords were also searched. The specific examples of the search terms used in this review were: ‘Hydroxymethylglutaryl CoA Reductase Inhibitor’, ‘HMG CoA Reductase Inhibitor’, ‘Atorvastatin’, ‘Fluvastatin’, ‘Lovastatin’, ‘Pitavastatin’, ‘Pravastatin’, ‘Rosuvastatin’, ‘Simvastatin’ AND ‘CCL2 Chemokine’, ‘Monocyte Chemotactic and Activating Factor’, ‘Monocyte Chemoattractant Protein 1’, ‘Chemokine (C-C Motif) Ligand 2’, and ‘Monocyte Chemotactic Protein 1’. All articles were screened and those related to cardiovascular diseases were removed. This review includes both preclinical and clinical studies focused on pathological conditions where CCL2 expression plays a more predominant role. The relevant data from the included articles were extracted and summarized in Table 1. Also, the data presented in Table 2 describes the current clinical knowledge about the role of statin therapy on CCL2 levels. Figure 1 provides an overall illustration of the principal cytokines and immune cells involved in different diseases that are particularly affected by statins.

**The effect of statin therapy on CCL2 in the immune system**

**Infection and sepsis**

During microbial invasion, damaged tissues express CCL2 which in turn initiates a cascade of signals resulting in leukocyte infiltration, monocyte activation, and T-cell proliferation [12, 103, 104]. The trafficking of pluripotent cells into the sites of tissue repair is a well-orchestrated process. Precise regulation of the immunological responses optimizes the host defense and suppresses uncontrolled microbial growth [105]. Lack of CCL2 was found to be associated with the impaired clearance of bacteria and enhanced susceptibility to infection [106]. The antibacterial effects of CCL2 were revealed to be mediated through modulation of IFN-γ production [107] On the other hand, elevated levels of CCL2 during cytokine release syndrome (CRS) induced by pathogens such as COVID-19 increased the risk of serious complications [108]. Therefore, modulation of CCL2–CCR2 axis arises as an interesting target for the treatment of sepsis and infectious diseases. Several in vivo studies have reported that blocking CCL2 by anti-CCL2 antibodies could diminish leukocyte penetration into the infection site [109, 110]. Also, a recent review discussed the available therapeutic agents aimed at inhibiting CCL2–CCR2 in patients with severe COVID-19 infection [111]. In vitro studies performed on monocytes, respiratory syncytial virus (RSV)-infected macrophages, and peripheral blood lymphocytes (PBL) showed suppression of CCL2 in the presence of statins as well as other chemokines and cytokines including CXCL1, CCL4, CCL5, CCL7, CCL13, CCL18, TNF-α, IL-1, IL-6, IL-8, and TGF-β1 [50–52]. That being said, prophylactic treatment with simvastatin improved survival in mice models of lethal sepsis but increased the CCL2 and IL-6 production [53].

**Acute respiratory distress syndrome (ARDS)**

Acute lung injury (ALI) that clinically manifests as ARDS is a devastating and potentially life-threatening event caused by a variety of insults including pneumonia, sepsis, ventilator-induced trauma, and acute pancreatitis, among others [112]. ARDS is characterized by massive accumulation of protein-rich exudate, red blood cells, and neutrophils in the interstitial and alveolar spaces as a consequence of increased microvascular permeability [113]. The interrelationship of adhesion molecules, early inflammatory response, and chemotactic cytokines leads to neutrophil recruitment into the lungs [114]. It was documented that higher CCL2 levels in bronchoalveolar lavage fluid (BALF) of patients with ARDS, enhanced the propensity to fibroproliferative lung complications and mortality rate [115]. Surprisingly, in an animal model of sepsis-related ARDS, CCL2 administration decreased the mortality rate [116]. CCR2 blockade has been observed to reduce the protein concentration and leukocyte influx in BALF of mice exposed to intratracheal LPS [117]. These observations could be due to the role of the CCL2–CCR2 axis on re-epithelialization after lung injury. Whether CCL2 inhibition could exhibit beneficial effects on noninfectious causes of ARDS is unknown [118]. An experimental study by Chen et al., revealed that pretreatment with 5 µM simvastatin significantly attenuated BALF protein, cell count, and inflammatory cytokines (CCL2, CCL5, IL-6, and IL-1β) in a murine ALI model [54]. Similarly, statin administration before endotoxin-induced ALI was demonstrated to possess anti-inflammatory and antioxidant activity. Pravastatin-treated mice were also observed to have fewer leukocytes and lower levels of CCL2, IL-6, and TNF-α [55]. In a ventilator-induced lung injury (VILI) animal model, high-dose simvastatin reduced pulmonary endothelial injury, hyperpermeability, leukocyte recruitment, cytokine levels, and improved oxygenation. Although the levels of CCL3, IL-1β, and IL-12p40 significantly decreased after simvastatin treatment (20 mg/kg), CCL2 levels showed no significant difference between simvastatin and sham-treated groups [56]. ALI or even ARDS is expected to occur after procedures requiring cardiopulmonary bypass (CPB). The findings of a preclinical study suggested that pretreatment with simvastatin inhibited CPB-induced nuclear factor kappa B (NF-κB) activation and reduced the levels of CCL2, IL-6, and TNF-α in serum, BAL fluid, and lung tissue in a dose-dependent manner. Lung injury score was lower in the simvastatin-treated group [57].
Targeting the CCL2–CCR2 signaling pathway

Allergic airway disease (AAD)

Allergic asthma is a chronic inflammatory condition characterized by an overabundance of eosinophils, mast cells, basophils, and T-helper 2 (Th2) lymphocytes in the lower respiratory tract [119]. CCL2 may elicit effects on the development of AADs by promoting Th2 polarization, inducing leukotriene and histamine release, and mediating the homing of regulatory and effector cells within the pulmonary system.

Table 1. The effects of statin therapy alone or in combination with other drugs on the levels of CCL2 and other cytokines in different pathologic conditions.

<table>
<thead>
<tr>
<th>Pathologic condition</th>
<th>Treatment</th>
<th>Statin dosage</th>
<th>Type of study</th>
<th>Model</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune System</td>
<td>Pravastatin</td>
<td>5, 100, 500 µM</td>
<td>In vitro</td>
<td>Monocytes</td>
<td>↓</td>
<td>[50]</td>
</tr>
<tr>
<td>Infection and sepsis</td>
<td>Lovastatin</td>
<td></td>
<td>In vitro</td>
<td>RSV-infected macrophages</td>
<td>↓</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin</td>
<td>5 mg/l</td>
<td>In vitro</td>
<td>PBL</td>
<td>↓</td>
<td>[52]</td>
</tr>
<tr>
<td>ARDS</td>
<td>Simvastatin</td>
<td>40 mg/kg/day</td>
<td>In vivo</td>
<td>Lethal sepsis</td>
<td>↑</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>Simvastatin</td>
<td>5 µM</td>
<td>In vivo</td>
<td>ALI</td>
<td>↓</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>Pravastatin</td>
<td>5 mg/kg/day</td>
<td>In vivo</td>
<td>VILI</td>
<td>→ CCL3, IL-1β → CXCL2, IL-6</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td>Simvastatin</td>
<td>20 mg/kg</td>
<td>In vivo</td>
<td>CPB</td>
<td>↓</td>
<td>[57]</td>
</tr>
<tr>
<td>AAD</td>
<td>Simvastatin</td>
<td>10 µM</td>
<td>In vitro</td>
<td>MTE cells</td>
<td>↓</td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin + inhaled beclomethasone</td>
<td>40 mg/day</td>
<td>RCT</td>
<td>Asthma</td>
<td>↓ CCL4, CCL7, IL-8, TGF-α</td>
<td>[59]</td>
</tr>
<tr>
<td>Transplant rejection</td>
<td>Pravastatin</td>
<td>30 mg/kg/day</td>
<td>In vivo</td>
<td>CGVHD</td>
<td>↓</td>
<td>[60]</td>
</tr>
<tr>
<td>Cancer</td>
<td>Simvastatin</td>
<td>20 mg</td>
<td>RCT</td>
<td>Renal transplant</td>
<td>↓</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>Pitavastatin</td>
<td></td>
<td>In vitro</td>
<td>Monocytic tumor cells</td>
<td>↓</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td>Simvastatin + paclitaxel</td>
<td>5, 10 µM</td>
<td>In vitro</td>
<td>MSC and PCC</td>
<td>↓</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>Simvastatin + paclitaxel</td>
<td>50 µg/kg/day</td>
<td>In vitro</td>
<td>Melanoma</td>
<td>↓</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>Pitavastatin</td>
<td>20, 40 ppm</td>
<td>In vivo</td>
<td>Intestinal polyp</td>
<td>↓</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>Pravastatin</td>
<td>100 µM</td>
<td>In vitro</td>
<td>Irradiated HMVEC</td>
<td>↓</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>Pravastatin</td>
<td>40 mg/kg/day</td>
<td>In vivo</td>
<td>Dorsal skin irradiation</td>
<td>↓ CXCL1</td>
<td>[67]</td>
</tr>
<tr>
<td>Nephropathies</td>
<td>Lovastatin</td>
<td>1, 5, 20 µM</td>
<td>In vitro</td>
<td>Mesangial cells</td>
<td>↓</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>Lovastatin</td>
<td>4 mg/kg</td>
<td>In vivo</td>
<td>PA nephrosis</td>
<td>↓</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin</td>
<td>20 mg/kg/day</td>
<td>In vivo</td>
<td>Anti-GBM GN</td>
<td>↓</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin</td>
<td>20 mg/day</td>
<td>In vivo</td>
<td>IgA nephropathy</td>
<td>→</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>Pravastatin + imidapril</td>
<td>100 mg/kg</td>
<td>In vivo</td>
<td>Lupus nephritis</td>
<td>↓</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin</td>
<td>20 mg/kg/day</td>
<td>In vivo</td>
<td>CI-AKI</td>
<td>↓</td>
<td>[73]</td>
</tr>
<tr>
<td></td>
<td>Rosuvastatin</td>
<td>10 mg/kg/day</td>
<td>In vivo</td>
<td>Salt-sensitive hypertension</td>
<td>↓ TGF-β1 → IL-6, TNF-α</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>Simvastatin</td>
<td>80 mg/kg/day</td>
<td>In vivo</td>
<td>Obesity and hypertension</td>
<td>→</td>
<td>[75]</td>
</tr>
<tr>
<td>Diabetes and diabetic nephropathy</td>
<td>Pravastatin</td>
<td>0.1, 1 mM</td>
<td>In vitro</td>
<td>Podocytes</td>
<td>↓</td>
<td>[76]</td>
</tr>
<tr>
<td>Diabetes and diabetic nephropathy</td>
<td>Pitavastatin</td>
<td>10 mg/kg/day</td>
<td>In vivo</td>
<td>Diabetes</td>
<td>↓</td>
<td>[77]</td>
</tr>
<tr>
<td>Diabetes and diabetic nephropathy</td>
<td>Pravastatin</td>
<td>10 mg/day</td>
<td>Clinical trial</td>
<td>Type 2 DM</td>
<td>↓</td>
<td>[78]</td>
</tr>
</tbody>
</table>
High concentrations of CCL2 have been detected in BALF of allergic asthmatic subjects; similarly, allergen challenge elevated CCL2 production in BALF [120–122]. The severity of asthma is attributable to the intensity of leukocyte influx into the airways. Thus, interfering with the chemokine system that involves leukocyte recruitment may represent an emerging therapeutic approach for asthma [123]. Anti-CCR2 and anti-CCL2 antibodies known as Cortistatin and Chemerin, showed suppressive effects on asthma by inhibiting the recruitment of inflammatory cells [124–126]. Simvastatin inhibited CCL2, CCL7, and CCL8 expression in mouse tracheal epithelial (MTE) cells, while inducing CCL20 and did not change CCL5, CCL12, IL-4, IL-10, and TNF-α expression levels [58]. A randomized controlled trial (RCT) by Thomson et al., compared sputum mediator concentrations between atorvastatin (40 mg/day), an inhaled corticosteroid (ICS), and their combination in smokers with asthma. Atorvastatin significantly reduced the levels of CCL4, CCL7, IL-12p70, and TGF-α in sputum. Reductions in CCL2, IL-7, and IL-8 were found to be associated with improvements in asthma quality of life questionnaire (AQLQ) and/or asthma control questionnaire (ACQ) scores [59].

**Transplant rejection**

Transplantation tolerance is a critical challenge that requires the interplay of innate and adaptive immunity [127]. Sequential waves of cytokine expression induce the recruitment of phagocytes and lymphocytes into the graft [128].
Targeting the CCL2–CCR2 signaling pathway

Allo-activated T-cell response to alloantigens may exert deleterious impacts on short and long-term graft function [129]. Noninvasive investigation of CCL2 has been suggested as a predictor of graft rejection episodes [130, 131]. Furthermore, several experimental studies have explored the potential of CCL2 inhibitors to be used as an immunosuppressive therapy in transplant recipients [132, 133]. Previous research on chronic graft-versus-host disease (CGVHD) animal model suggested that the protective effects of pravastatin involved the down-regulation of chemokines such as CCL2 and CCL5. Pravastatin-treated recipients had significantly slower onset of clinical cutaneous CGVHD, less submucosal fibrosis in the lungs, and reduced numbers of inflammatory and epithelial cells in BALF [60]. These findings are consistent with the results from an RCT by Wang et al., in which 60 post-kidney transplant patients received 20 mg simvastatin for 3 months

Table 2. Clinical studies assessing the effect of statin therapy on the levels of CCL2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Statin dosage</th>
<th>Disease</th>
<th>CCL2 level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin + inhaled beclomethasone</td>
<td>40 mg</td>
<td>Asthma</td>
<td>↓</td>
<td>[59]</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>20 mg</td>
<td>Renal transplant</td>
<td>↓</td>
<td>[61]</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>10 mg</td>
<td>Type 2 DM</td>
<td>↓</td>
<td>[79]</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>40 mg</td>
<td>AD</td>
<td>↓</td>
<td>[93]</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>80 mg</td>
<td>Crohn disease</td>
<td>→</td>
<td>[97]</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>20 mg</td>
<td>Endometriosis</td>
<td>↑</td>
<td>[99]</td>
</tr>
<tr>
<td>Atorvastatin + Metformin</td>
<td>20 mg</td>
<td>PCOS</td>
<td>↓</td>
<td>[100]</td>
</tr>
</tbody>
</table>

Figure 1. Involvement of CCL2–CCR2 signaling pathway in the immunopathogenesis of rheumatic diseases, neurologic and pulmonary diseases, nephropathies, and cancers. The distinct components mostly affected by statins are indicated by arrows.
and showed significant reduction in mRNA expressions of CCL2 and CCR2 [61].

The effect of statin therapy on CCL2 in cancer

Tumorigenesis is a multistage, long-lasting, and progressive process primarily caused by the acquisition of mutations in key signaling pathways responsible for cellular growth, differentiation, and survival [134, 135]. The complex network of inflammatory cytokines expressed by cancer and stromal cells provides a highly immunosuppressive tumor microenvironment (TME) that allows the tumor to escape from tumor-reactive T cells [136, 137]. The CCL2–CCR2 axis has been observed to participate in different stages of a wide spectrum of malignancies including breast [138], prostate [139], lung [140], kidney [141], and pancreas cancer [142]. It is confirmed that CCL2–CCR2 is involved in sustaining cancer cell proliferation, stimulating cells to invade surrounding tissues, entering the circulation, and migrating into the distant metastatic foci [143]. Taken together, there is a strong correlation between CCR2 and CCL2 overexpression in tumor and immunosuppressive cells and poor prognosis in subjects suffering from cancer [144–146].

Before preclinical and clinical studies have introduced various CCL2 inhibitors and neutralizing antibodies such as C1142, bindarit, mNOX-36, Curcumin, and Carlumab. Moreover, CCR2 antagonists and antibodies interfering with ligand binding are found to possess antitumor activity. Several examples are RS 504393, RS 102895, CAS445479-97-0, GMMEI, Teijin Compound 1, BMS CCR2 22, PF-04136309, MLN1202, and Propagermanium. Several studies have suggested combination therapeutic strategies to avoid unexpected adverse effects [147]. Attempts have been made to determine whether statins could influence the expression of CCL2 and CCR2 in cancer. In human monocytic tumor cells, pitavastatin but not pravastatin was shown to suppress cell proliferation in a dose-dependent manner and induce S-phase arrest through down-regulation of CCR2 and CCR5. Also, low concentrations of CCL2 and CCL5 did not inhibit the antiproliferative effects of pitavastatin [62]. A recent investigation highlighted the pleiotropic effects of simvastatin on cell metabolism and suppression of CCL2, CCL3, and IL-6 in primary cancer cells (PCCs) and mesenchymal stromal cells (MSCs) from human squamous cell lung carcinoma (SCC). It was reported that simvastatin negatively impacted PCC survival by inhibiting spheroid formation by these cells [63]. The administration of 50 mg/kg/day simvastatin as an adjuvant to commercial paclitaxel was tested by Kretzer et al., in melanoma-bearing mice. Although simvastatin alone had no inhibitory effects on cancer cells, 44% tumor-growth inhibition was achieved by paclitaxel and simvastatin in combination. Interestingly, the immunohistochemical expression of CCL2 was decreased in both groups [64]. An in vivo study claimed that pitavastatin (20,40 ppm) inhibited intestinal polyp formation in a dose-dependent manner and significantly decreased serum mRNA expression levels of CCL2 and IL-6 [65]. Statins have been shown to preserve endothelial function and hence, may have beneficial effects on radiation-induced vascular injury. Pravastatin increased the adhesion of leukocytes to irradiated endothelium and inhibited the overproduction of CCL2, IL-6, and IL-8 [66]. Similarly, Holler et al., demonstrated the inhibitory effects of pravastatin on CCL2 and CXCL1 production and skin lesions following radiation [67].

The effect of statin therapy on CCL2 in nephropathies

Progressive deterioration of renal function followed by tubulointerstitial inflammation and fibrosis is a hallmark of many types of chronic nephropathies and contributes to the development of end-stage renal disease (ESRD) [148]. Increased glomerular protein filtration stimulates tubular epithelial cells to upregulate chemokines mainly via NF-κB activation [149]. Locally secreted chemokines such as CCL2 and CCL5 promote the recruitment of mononuclear leukocytes, that in turn amplify inflammation, fibroblast proliferation, collagen deposition, and tubular atrophy [150]. In lupus nephritis, urinary CCL2 levels were correlated with macrophage infiltration and renal failure severity. Thus, treatment with anti-CCL2 antibodies was found to decline monocyte influx, proteinuria, and crescent formation in animal models of lupus glomerulonephritis [151, 152]. The inhibitory effects of glucocorticoids on CCL2 production and thus disease activity are proven in these patients as well [153]. Several investigations have been directed to evaluate statins’ efficacy in nephropathies and their potential inhibitory functions against the CCL2–CCR2 pathway. Lovastatin downregulated CCL2 mRNA expression and the chemotactic activity of mesangial cells in vitro [68]. It also ameliorated the pturomycin aminonucleoside (PA) nephrosis by attenuating glomerular macrophage infiltration. Besides, glomerular expression of CCL2 mRNA was significantly lower in lovastatin-treated nephrotic rats [69]. In an experimental model of anti-glomerular basement membrane glomerulonephritis (anti-GBM GN), treatment with 20 mg/kg/day atorvastatin decreased macrophage infiltration into the glomeruli, proteinuria, formation of crescentic and neutrophilic lesions, and CCL2 levels [70]. However, low-dose atorvastatin treatment (20 mg/day) in 16 patients with IgA nephropathy did not affect CCL2 levels after 1 month [71]. It is suggested that combination therapy with Imidapril and pravastatin could improve survival and significantly reduce proteinuria and renal CCL2 levels in mice models of lupus nephritis [72]. A recent in vivo study compared the ameliorative effects of various types of statins on contrast-induced acute kidney injury (CI-AKI). Although 20 mg/kg/day atorvastatin and 10 mg/kg/day rosuvastatin remarkably reduced the mRNA levels of CCL2, IL-6, and TNF-α, 80 mg/kg/day simvastatin exerted no such alterations [73]. To explore the mechanisms behind the renoprotective functions of statins in hypertension and the possible role of CCL2, two experiments were conducted on animal models of salt-sensitive hypertension. Atorvastatin (30 mg/kg/day) significantly attenuated glomerulosclerosis, proteinuria, renal oxidative stress, CCL2 and TGF-β1 expressions. On the other hand, simvastatin-treated animals had a notable reduced interstitial fibrosis and expression of CCL2 with no change in renal mRNA expression of TGF-β [75, 74]. Although 10 mg/kg/day of simvastatin reduced renal oxidative stress, it did not affect CCL2 levels in a rat model of obesity and hypertension [76].

The effect of statin therapy on CCL2 in diabetes and diabetic nephropathy

It is widely accepted that diabetes is a perpetual state of ongoing low-grade inflammation manifested by an excess generation of reactive oxygen species (ROS), advanced glycation end products (AGEs), and proinflammatory cytokines...
Tended to be more affected by statins than renal inflammation. These findings implied that systemic vascular inflammation from a clinical trial on type 2 diabetic patients indicated that sulphasalazine [175], anti-TNF monoclonal antibodies [176], and bindarit [179] have shown inhibitory effects on CCL2 experimentally or clinically. In addition, administration of anti-CCL2 antibodies in animal models of collagen-induced arthritis (CIA) has improved joint swelling and reduced monocyte recruitment into the joint space [180]. The salutary effects of statins on monocytemediated diseases such as RA have been investigated previously. Experimental research on monocytes revealed that treatment with atorvastatin or simvastatin significantly inhibited monocyte migration, CRP-induced secretion of CCL2, CCL3, and CCL4, and up-regulation of ICAM-1 [80]. Likewise, simvastatin attenuated mRNA expression of CCL2 in FLSs derived from RA patients [81]. In an animal model of adjuvant-induced arthritis (AIA), atorvastatin markedly reduced neutrophil influx as well as the concentrations of CCL2, CCL5, TNF-α, IL-6, and IL-1β [82]. The effects of pravastatin and simvastatin on CCL2 expression in CIA were reported by two independent researchers. The pravastatin-treated mice had lower synovial expression of CCL2 mRNA, delayed onset of CIA, and decreased synovial inflammatory cells. Furthermore, CCL2, IL-6, TNF-α, and IFN-γ levels were found to be reduced in the pravastatin-cultured spleen cells [83]. Another investigation by He et al., on CIA animal models demonstrated that simvastatin inhibited NF-κB binding to MCP-1-induced protein (MCPIP) gene enhancer, alleviated aortic levels of MCPIP which in turn ameliorated endothelial dysfunction [84].

Osteoarthritis (OA)

Progressive cartilage degeneration is the most prominent pathologic feature leading to joint dysfunction in OA [181]. The CCL2→CCR2 axis plays a distinctive role in monocyte recruitment into the damaged tissues, thus contributing to the advancement of OA [182, 183]. A plethora of immune mediators, including IL-1β and TNF-α, has been found to regulate the expression of CCL2 and its receptor [184, 185]. Although targeting CCL2 produced by injured chondrocytes and FLSs could be a potential treatment for OA, there is still a lack of evidence about it. During OA development in rabbits that underwent bilateral anterior cruciate ligament transaction (ACLT), intra-articular administration of mevastatin was shown to limit cartilage degeneration. Synovial tissues treated with mevastatin showed attenuated monocyte infiltration, CCL2, and IL-1β mRNA expressions, as well as reduced synovial thickness [85]. To evaluate the role of statins in the treatment and prevention of aseptic loosening of the prosthesis, PBMCs were challenged with titanium particles and were exposed to simvastatin. Results indicated significant differences between simvastatin-treated and the control group in terms of CCL2 and TNF-α levels [86].

The effect of statin therapy on CCL2 in Rheumatic disorders

Rheumatoid arthritis (RA)

The immunopathogenesis of RA involves the interaction among auto-reactive T cells, B cells, fibroblast-like synoviocytes (FLSs), neutrophils, and macrophages stimulated by a variety of inflammatory cytokines [167–169]. Th2 lymphocytes are assumed to play a pivotal role in the initiation of RA and activation of macrophages and FLSs. Infiltrating monocytes, resident macrophages, and FLSs are known as the dominant source of CCL2 [170, 171]. Previous evidence suggested that CCL2, IL-1β, IL-6, and TNF-α are expressed abundantly in synovial fluid and cells derived from patients with RA [172]. Therefore, therapies directed against CCL2 could alleviate joint damage and be useful in the treatment of RA [173]. Several drugs including dexamethasone, methotrexate [174], sulphasalazine [175], anti-TNF monoclonal antibodies [176], tenidap [177], KE-298 [178], and bindarit [179] have shown inhibitory effects on CCL2 experimentally or clinically. In addition, administration of anti-CCL2 antibodies in animal models of collagen-induced arthritis (CIA) has improved joint swelling and reduced monocyte recruitment into the joint space [180]. The salutary effects of statins on monocytemediated diseases such as RA have been investigated previously. Experimental research on monocytes revealed that treatment with atorvastatin or simvastatin significantly inhibited monocyte migration, CRP-induced secretion of CCL2, CCL3, and CCL4, and up-regulation of ICAM-1 [80]. Likewise, simvastatin attenuated mRNA expression of CCL2 in FLSs derived from RA patients [81]. In an animal model of adjuvant-induced arthritis (AIA), atorvastatin markedly reduced neutrophil influx as well as the concentrations of CCL2, CCL5, TNF-α, IL-6, and IL-1β [82]. The effects of pravastatin and simvastatin on CCL2 expression in CIA were reported by two independent researchers. The pravastatin-treated mice had lower synovial expression of CCL2 mRNA, delayed onset of CIA, and decreased synovial inflammatory cells. Furthermore, CCL2, IL-6, TNF-α, and IFN-γ levels were found to be reduced in the pravastatin-cultured spleen cells [83]. Another investigation by He et al., on CIA animal models demonstrated that simvastatin inhibited NF-κB binding to MCP-1-induced protein (MCPIP) gene enhancer, alleviated aortic levels of MCPIP which in turn ameliorated endothelial dysfunction [84].

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The effect of statin therapy on CCL2 in neuroinflammation

Neuroinflammation is a crucial player in the development of a wide range of CNS pathologies such as neurodegenerative, autoimmune, and infectious diseases [186–188]. CCL2 is involved in CNS inflammation via monocyte recruitment. CCR2 is also broadly expressed throughout the CNS structures including the cerebral cortex, the cerebellum, the limbic system, and the striatum [189–191]. Thus, CCL2/CCR2 inhibition could represent a promising strategy in the treatment of various
neurologic diseases. In this section, we aimed to summarize recent advances in CCL2-targeted therapies in disorders associated with neuroinflammation with a major focus on the roles of statins. Although the effects of statins on CCL2 levels were evaluated in several neuroinflammatory disorders such as Alzheimer’s disease, their roles in many neuroinflammatory conditions such as multiple sclerosis, Parkinson’s disease, and epilepsy have not yet been investigated.

Blood–brain barrier (BBB) dysfunction

The BBB is a dynamic structure consisting of specialized endothelial cells (ECs), astrocytes, and pericytes [192]. Tight junctional integrity of cerebral endothelium is essential to maintain the brain microenvironment in stable circumstances. The BBB disruption occurs in a variety of neurologic disorders, especially those associated with inflammation and angiogenesis, and aggravates the pathological condition [193]. CCL2 is one of the key chemokines inducing alternations in BBB permeability [189]. CCL2 is virtually undetectable in normal conditions however, in neuroinflammatory diseases, CCL2 secretion by astrocytes and ECs makes a chemotactic gradient that attracts monocytes, macrophages, and activated lymphocytes into the CNS [194]. Besides, increased endothelial permeability followed by ECs’ migration toward the CCL2 gradient eventually leads to angiogenesis [195]. Experimental studies applying the CCL2-neutralizing antibodies reported decreased BBB permeability and redistribution of tight junction proteins [196]. The neuroprotective properties of statins and their effects on CCL2 expression were evaluated in several in vitro models of the BBB. In an inflammatory-mediated model of BBB breakdown, the binding of activated T cells to endothelium was strongly prevented by atorvastatin. Besides, atorvastatin pretreatment dose-dependently reduced the upregulation of CCL2 but not of IL-8 [187]. These findings support the previous reports by Ifergan et al., on BBB-derived ECs. They have similarly found that the migration of monocytes and lymphocytes across the BBB was significantly restricted by simvastatin and lovastatin. This effect was associated with a specific reduction in the secretion of CCL2 and CXCL10 by BBB-ECs [197]. As noted in a recent investigation, lipopolysaccharide (LPS) enhanced the production of cytokines such as CCL2 and IL-6 in BBB which have been alleviated by pitavastatin [189].

Alzheimer’s disease (AD)

The presence of abundant neurofibrillary tangles and senile plaques throughout the brain is recognized as the hallmark of AD [197]. Activated microglia, reactive astrocytes, dystrophic neurites, and β-amyloid (Aβ) peptides, are the most important components within a senile plaque [198–201]. Aβ peptides induce secretion of chemokines particularly CCL2, which can contribute to the pathogenesis of AD via chemotraction and activation of inflammatory cells [202, 203]. The levels of CCL2 have been observed to be higher in the serum, urine, and cerebrospinal fluid (CSF) of patients with AD and were correlated with faster progression and greater severity of the disease [204–208]. Despite the possible roles of CCL2 in the development of AD, there is a lack of evidence on CCL2-targeted therapies. An experimental study by Severini et al., suggested the protective effects of bindarit, a CCL2 inhibitor, against Aβ toxicity [209]. In an in vitro study, cultured cerebral ECs and astrocytes were treated with fibrillar Aβ alone and in combination with simvastatin and lovastatin. The cytokine release, cell death, and loss of barrier integrity which were induced by Aβ were effectively prevented by statins. The levels of CCL2, CCL5, IL-6, and IL-8 were significantly lower in the statin-treated group [90]. The effects of atorvastatin (5 mg/kg/day) on NK cell infiltration and cytokine production in aged and Aβ-induced AD rats, were previously established and showed attenuated NK cells in the hippocampus paralleled by a decrease in CCL2 and IFN-γ [91]. Treatment with either 30 mg/kg/day atorvastatin or 3 mg/kg/day pitavastatin demonstrated a significant recovery in the number of CCL2-positive and TNF-α-positive Purkinje cells (PCs) which were reduced in Aβ transgenic mice [92]. In a clinical trial on patients with dyslipidemia and parental history of AD, the effect of atorvastatin on inflammatory markers was compared with a placebo after 18 months. In atorvastatin-treated patients, serum levels of CCL2 were significantly decreased along with a reduction in the levels of L-1β, IL-6, and TNF-α [93].

HIV-associated neurocognitive disorder (HAND)

HIV cell entry is a complex, multistep process involving the chemokine receptors [210]. Higher CCR2 expression on monocytes and memory CD4+ T cells makes them more susceptible to CCL2 and enables them to be recognized as the primary targets for HIV [211]. In the early stage of HIV infection, the presence of even low levels of CCL2 within the CNS parenchyma plays an essential role in the disruption of BBB and transmigration of the HIV-infected leukocytes into the CNS [212]. CCL2 also facilitates the recruitment of uninfected microglia and astrocytes to the sites of active infection, resulting in viral spread within the CNS and severe cognitive impairment [213]. Thus, it could be concluded that CCL2–CCR2 blockade might decelerate HIV infection progression [214]. Despite the considerable role of the CCL2–CCR2 axis in HAND pathogenesis, little is known about therapies targeting this pathway. According to the results of a preclinical study, treatment with simvastatin and atorvastatin modulated monocyte functions associated with HAND. This study reported a decreased proportion of CD16+ monocytes in PMBCs in the presence of statins. Furthermore, simvastatin suppressed CCL2 production by PBMCs and reduced monocyte chemotaxis in vitro [94].

Sepsis-associated cognitive impairment

Sepsis survivors commonly experience long-term neurocognitive disabilities [215]. Although the exact mechanisms are poorly understood, neuroinflammation and BBB dysfunction are involved in the pathogenesis [216]. As discussed in earlier sections, the release of CCL2 could increase endothelial permeability and cause inflammation [189]. However, the potential role of CCL2 in the pathogenesis and further treatment of sepsis-associated cognitive impairment is rarely explored. The results from an in vivo study on mice survivors from sepsis indicated that atorvastatin and simvastatin prevented the microvascular dysfunction, activation of microglia, and the systemic and CNS levels of proinflammatory mediators such as CCL2, IL-1β, and IL-6 [95].

The effect of statin therapy on CCL2 in inflammatory bowel disease (IBD)

Ulcerative lesions with a marked infiltrate of inflammatory cells are the main mucosal changes observed in IBD [217]. Although the current understanding of the disease mechanisms is still evolving, dysregulated immune responses are considered one
of the most important pathogenic factors [218]. It is shown that TNF-α-induced expression of CCL-2 could facilitate the recruitment of monocytes, neutrophils, and lymphocytes into the intestinal space, leading to intestinal inflammation [219]. Immunohistochemical studies also confirmed the production of CCL2 by endothelial cells, smooth muscle cells, and macrophages in IBD [220]. CCL2 upregulation in serum and mucosal tissues has been reported in experimental and clinical studies [220–222].

In a mice model of colitis, treatment with bindarit, a CCL2 inhibitor, was shown to decrease the severity of the disease [223]. Two studies were conducted by Grip et al., to investigate the effects of atorvastatin on Crohn’s disease (CD). In the first one, treating PBMCs obtained from patients with CD with atorvastatin showed suppressed levels of CCL2 and TNF-α [96]. However, the other study reported significantly reduced plasma levels of CXCL10 but not CCL2 in 10 patients with CD [97].

The effect of statin therapy on CCL2 in gynecologic disorders

In the past two decades, many authors have been investigating the roles of chemokines in endometriosis, polycystic ovarian syndrome (PCOS), and infertility [224–227]. Due to the similarities among endometriosis, malignancies, and also autoimmune diseases, CCL2 involvement in the pathogenesis, diagnosis, and treatment of endometriosis has attracted increasing attention [227]. Higher levels of CCL2 have been observed in the peritoneal fluid of patients with endometriosis and were correlated with disease severity [228]. CCL2 in combination with CA125 and CCR1 mRNA has been recognized as a high-sensitive diagnostic test for endometriosis [229]. An experimental work by Cakmak et al., emphasized the ability of simvastatin to reduce CCL2 levels dose-dependently in endometriotic implants of a mouse model. Likewise, in cultured endometriotic cells, both simvastatin and mevastatin exerted inhibitory effects on CCL2 mRNA expression [98]. Contrary to the expectations, a prospective RCT on 40 women with endometriosis who were scheduled for laparoscopic surgery revealed that simvastatin therapy (20 mg/day) did not reduce the expression of CCL2, and thus could not be used as an ideal treatment [99]. In a recently published RCT, 40 medication-naive obese patients with PCOS were treated with 20 mg/day atorvastatin for 12 weeks and were compared with a placebo group. Both study groups were treated with metformin for further 12 weeks. The results demonstrated that the makers of adipose tissue inflammation such as CCL2 and IL-6 significantly decreased in atorvastatin-treated participants [100]. It has been lately reported that simvastatin decreased the preterm birth (PTB) incidence in mice, inhibited myometrial cell contraction, and reduced the expression of proinflammatory markers including CCL2, CXCL1, IL-6, and IL-8 [101]. In a rat preeclampsia model, treatment with pravastatin resulted in decreased blood pressure, urine protein level, and fetal growth restriction. Also, serum levels of CCL2 and IL-6 were decreased following pravastatin administration. These data highlighted the role of statins in preventing preeclampsia [102].

Statin dosage and possible adverse effects in non-cardiovascular conditions

Various factors could influence the potential effect of statin on CCL2 levels in non-cardiovascular diseases. The dosage and type of statin administered, other agents used in a combination therapy with statin, the different pathogenesis of each non-cardiovascular condition, and the type and design of the study are several factors that could likely impact the observed effects of statin. As shown in Tables 1 and 2, the statin dosage employed in preclinical and clinical studies is rather heterogeneous. Atorvastatin in doses of 20, 40, and 80 mg/day have been used in clinical models of asthma, AD, Crohn’s disease, and PCOS. Administering 40 mg/day atorvastatin in asthma and AD, as well as 20 mg/day in patients with PCOS has been reported to be effective in reducing CCL2 levels [59, 93, 100]. However, 80 mg/day atorvastatin did not affect the levels of CCL2 in patients with Crohn’s disease [97].

To our best knowledge, a few studies which are reviewed here reported serious adverse effects of statins; as Gaugler et al. showed that 1000 μM pravastatin in vitro resulted in cell toxicity [66]. However, it is of notion that despite growing literature supporting the efficacy of statins in the treatment of several noncardiovascular diseases, several studies reported undesirable side effect concerning taking this medication. For instance, investigations suggested that statins are associated with increased development of diabetes mellitus and elevations in hepatic transaminases, but not peripheral neuropathy and carcinogenesis [230–232]. Furthermore, prescription of statins for noncardiovascular disorders might cause unwanted drug–drug interactions that could emerge as a possible challenge.

Concluding remarks and outlook

This review collates the existing evidence regarding the effects of statins on the CCL2–CCR2 signaling pathway in various non-cardiovascular pathologies. Although most studies demonstrated the inhibitory effects of statins on CCL2, CCL2 expression levels have been reported as consistent or higher in several studies. The capacity of statins to target the CCL2–CCR2 axis is still mainly inferred from cellular studies or research on animal models, and in many cases, is based upon guilt by association rather than hard clinical and experimental literature. More clinical trials with larger scales and extended follow-up duration are required to explore the effects of statins alone or in combination with the standard treatment protocol on the levels of CCL2. Also, further efforts are needed to clarify if the type and dosage of statins is associated with their effects on CCL2; and if the advantages of using statin surpass the disadvantages. Future investigations will help to further clarify whether statins could be clinically implicated beyond cardiovascular diseases by inhibiting the CCL2–CCR2 axis.

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**References**


Targeting the CCL2–CCR2 signaling pathway


