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Monocyte Chemoattractant Protein-1 (CCL2) in Inflammatory Disease and Adaptive Immunity: Therapeutic Opportunities and Controversies

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ABSTRACT

Monocyte chemoattractant protein (MCP)-1 (CCL2) specifically attracts monocytes and memory T cells. Its expression occurs in a variety of diseases characterized by mononuclear cell infiltration, and there is substantial biological and genetic evidence for its essential role in atherosclerosis and multiple sclerosis. Despite intensive screening, there are as yet no small-molecule antagonists of the receptor of MCP-1/CCL2, CCR2. However, biological agents, including antibodies and inhibitory peptides, have been developed and may be useful for these indications. Recent evidence from genetically modified mice indicates that MCP-1 and CCR2 have unanticipated effects on T helper (Th) cell development. However, unlike the identical phenotypes of *MCP-1/CCL2*$^{-/-}$ and *CCR2*$^{-/-}$ mice in inflammatory diseases, the phenotypes of these mice are disparate in adaptive immunity: MCP-1 stimulates Th2 polarization, whereas CCR2 activation stimulates Th1 polarization. This presents both a challenge and an opportunity for targeting the MCP-1/CCL2/CCR2 axis in disease.
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INTRODUCTION

Monocyte chemoattractant protein (MCP)-1 (CCL2) was only the third chemokine to be purified to homogeneity after platelet factor 4 and interleukin (IL)-8 (42,71). Its ability to attract monocytes, but not neutrophils, *in vitro* was one of the early indications that chemokines would have activities that might explain the trafficking patterns of leukocytes in inflammation. This prediction has been fulfilled by demonstrations of the critical role that MCP-1/CCL2 plays in inflammatory diseases that involve mononuclear cell infiltration. In fact, the striking phenotypes of mice deficient for MCP-1/CCL2, or for its receptor, CCR2, in inflammatory models provide strong arguments against concerns about functional redundancy in the chemokine system *in vivo*, and for the benefits of pharmacological targeting of this ligand/receptor pair in disease. Early results have suggested that there is tremendous promise in this therapeutic approach.

However, MCP-1/CCL2 expression also occurs in a variety of settings in addition to inflammation, and as investigators have begun to examine its functions in these contexts, they have been confronted by unexpected results. For example, activities attributed to MCP-1/CCL2 cannot always be symmetrically matched by activities of CCR2, even though no other functional receptors for MCP-1/CCL2 have been identified. Furthermore, three additional MCPs in humans and four additional MCPs in mice can activate CCR2, and their contributions to normal physiology and disease are just now being revealed. Thus, on closer inspection, the deceptively simple MCP-1/CCL2/CCR2 system has revealed itself to be much

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more complicated. This review will attempt to summarize what we know about MCP-1/CCL2 and CCR2 not only in areas where the data support a clear-cut interpretation of their functions, but also where rapidly evolving information makes the ground feel a little less firm.

IN VITRO PROPERTIES AND RECEPTOR ACTIVATION

The eponymous function of MCP-1/CCL2 is, of course, its ability to attract monocytes in chemotaxis chambers *in vitro* (42,71). Its potency, with an eliciting of 50% of the maximal response (EC_{50}) of 1 nM, is similar to that of many other chemokines in analogous assays. In addition, MCP-1/CCL2 has been shown to attract memory T cells (8), although CCR2 levels are low in resting cells. It also attracts basophils and stimulates histamine release (2,4,38). Given the fact that CCR2 is a G protein-coupled receptor, it is not surprising that MCP-1/CCL2 can induce a variety of other physiological responses from cells expressing the receptor. These include a rise in intracellular calcium, the induction of the respiratory burst, and a new gene expression (32,58). Because CCR2 can be expressed by cells other than leukocytes (e.g., neurons [3,12]), it is likely that MCP-1/CCL2-mediated activation of nonhematopoietic cells will elicit more specialized responses.

One area of controversy in the chemokine field concerns the mechanism whereby MCP-1/CCL2 activates CCR2. Like most other chemokines, the crystal and solution structures of MCP-1/CCL2 indicate that it forms multimers (27). In addition to the by now familiar CC chemokine dimer structure, MCP-1/CCL2 also forms stable tetramers, in which the dimer of dimers interface recapitulates aspects of the CXC dimer structure (40). Initially, these structural findings led to questions about the form in which MCP-1/CCL2 activates CCR2 (i.e., does MCP-1/CCL2 activate its receptor as a monomer or multimer?). One argument for obligate multimer-mediated activation came from analyzing an N-terminally truncated MCP-1/CCL2 variant that behaves as an MCP-1/CCL2 antagonist. This variant, called 7ND (73), lacks amino acids 2–8 and is similar to the N-terminal deletion variant called MCP-1/CCL2(9-76) (19), except that 7ND retains its N-terminal pyroglutamate. Because of the possibility that MCP-1/CCL2 might activate CCR2 as a multimer, one possible mechanism of the inhibition by 7ND of wild-type MCP-1/CCL2 might occur via the formation of inactive heteromultimers. Consistent with that model was the observation that 7ND was unable to inhibit the chemoattractant activity of covalently cross-linked MCP-1/CCL2 homodimers (72). This was interpreted to mean that 7ND had to be able to bind free MCP-1/CCL2 monomers and to create inactive multimers to exert its inhibitory activity. That interpretation, in turn, was taken to mean that MCP-1/CCL2 activates its receptor in a multimeric form.

Provocative data that might be consistent with this model come from the group of Martinez and colleagues (57) in Madrid. Their observations indicate that some chemokine receptors, including CCR2, undergo ligand-driven multimerization, and that this process may be necessary for signal transduction (57). Thus, by analogy to receptor tyrosine kinases and their ligands, dimeric chemokine ligands might be required for inducing receptor dimerization. However, unlike the situation with receptor tyrosine kinases, there has been no demonstration yet that enforced receptor dimerization in the absence of the ligand can generate a cytoplasmic signal.

Although the foregoing data tend to support a model of multimeric MCP-1/CCL2 interaction with CCR2, direct measurements of MCP-1/CCL2 monomer affinities have indicated that the dissociation constant, K_d, for dimer dissociation is on the order of 3–5 μM (52). This concentration is nearly 1000-fold higher than the EC_{50} for chemoattractant activity, suggesting that, at physiological concentrations, essentially all free MCP-1/CCL2 is monomeric. Furthermore, elegant work by the group of Handel and colleagues (51) at the University of California at Berkeley demonstrated that the substitution of proline-8 by alanine produced an obligate monomer that nonetheless had wild-type chemoattractant potency *in vitro*. This would seem to settle the issue in favor of monomeric MCP-1/CCL2 activating its receptor, although these data do not rule out the possibility of two obligate monomers simultaneously binding to CCR2 to accomplish activation.

Ultimately, the discrepancy between monomer and multimer models of MCP-1/CCL2 action may be resolved by recent findings from the group of Proudfoot and colleagues at Serono, indicating that the obligate monomer, while active *in vitro*, is inactive *in vivo* (A. Proudfoot and T. Handel; personal communication). Thus, multimers may be required for the more complicated interactions that occur when leukocytes are attracted *in vivo*. For example, multimers may be required for interactions with glycosaminoglycans that "present" chemokines to leu-

kocytes as they roll on the surface of vascular endothelial cells (44).

MCP-1/CCL2 IN INFLAMMATORY DISEASE

Much less controversial are the findings relating MCP-1/CCL2 and CCR2 to inflammatory disease. Although the discovery that this chemokine/chemokine receptor axis plays an important role in inflammatory settings was somewhat predictable, the topic is worthy of review because of the near-term likelihood that relevant clinical interventions will be forthcoming. Admittedly, MCP-1/CCL2 expression occurs in many different diseases that are characterized by the accumulation of mononuclear cells and will probably be found to contribute to the pathobiology of these diseases. However, the disorders for which the most complete data exist are atherosclerosis, or related vasculopathies, and multiple sclerosis (MS).

Atherosclerosis

The presumption that MCP-1/CCL2 would be involved in atherogenesis stems from the inflammatory model of atherosclerosis, which was gaining credence at about the same time that the fundamentals of chemokine biology were emerging (11,59). Because the earliest steps in the atherogenic cascade involve the infiltration of circulating monocytes into the arterial subendothelium, MCP-1/CCL2 was considered to be an excellent candidate for the signal that elicited their accumulation. In fact, all of the cellular elements of the arterial wall are capable of MCP-1/CCL2 secretion (13,55,64), and potent inducers of MCP-1/CCL2 include pathophysiologically relevant stimuli such as oxidized lipid (i.e., minimally modified low-density lipoprotein [LDL]) (13) and shear stress. In addition, MCP-1/CCL2 expression occurs in human plaques (48,70).

Evidence for the pathogenetic involvement of MCP-1/CCL2 was obtained primarily and most convincingly through the analysis of genetically modified mice that were deficient for MCP-1/CCL2 or CCR2. For example, MCP-1/CCL2-deficient mice in an LDL receptor-deficient background were fed a cholate-free, high-cholesterol diet and were assessed for aortic lesion formation (24). After 12 weeks, the MCP-1/CCL2-deficient mice had 83% less lipid deposition throughout their aortic trees than did LDL receptor-deficient mice with intact MCP-1/CCL2 genes. This difference persisted for as long as 20 weeks of feeding on the high-cholesterol diet, during which time the extent of disease in the *MCP-1/CCL2*$^{-/-}$ mice did not vary, suggesting that their diminished plaque formation was a fixed phenotype. A very similar outcome was described for MCP-1/CCL2 deficiency in an apoB transgenic atherosclerosis model (21). Conversely, gain-of-function experiments have supported the notion of the contribution of MCP-1/CCL2 to atherosclerosis. For example, engineered overexpression of MCP-1/CCL2 in the arterial wall of hypercholesterolemic rabbits produced accelerated atherosclerosis (47), as did transplantation of *apoE*$^{-/-}$ mice using bone marrow cells transgenically overexpressing MCP-1/CCL2 (1). On the receptor side, CCR2 deficiency in an apoE-deficient background also provided substantial protection against plaque formation (7).

In all of the cases involving knockout mice, diminished atherosclerosis correlated with diminished macrophage content in the arterial wall, and in the gain-of-function models, disease severity correlated with increased macrophage content. Thus, the general model that these findings support is that MCP-1/CCL2 is secreted by endothelial and arterial smooth muscle cells in response to vascular insults such as hyperlipidemia. This MCP-1/CCL2 then attracts circulating monocytes, which accumulate in the subendothelium, differentiate into macrophages, continue to take up lipids, and become foam cells of the fatty streak.

Interestingly, a series of recent observations have suggested that the function of MCP-1/CCL2 in this setting is quite circumscribed and limited to the diapedesis step of monocyte infiltration. The earlier step of this process, the firm arrest of monocytes on endothelial cells, is mediated by CXCR2 and its ligands. The first hint of this function for CXCR2 came from the observation that *apoE*$^{-/-}$ mice transplanted with bone marrow cells from mice deficient for the murine CXCR2-like receptor had less lesion formation (5). Since then, CXCR2 has been shown to induce firm monocyte adhesion to endothelial cells (18). Furthermore, CXCR2 ligands, and not MCP-1/CCL2, induce the arrest of monocytes on carotid arterial explants under flow conditions (30). Thus, CXCR2 and CCR2 subserve different functions in monocyte accumulation in atherosclerosis, both of which are necessary for cellular infiltration into the subendothelium.

There is some human genetic support for the importance of MCP-1/CCL2/CCR2 in atherosclerosis. Genotyping for a polymorphism at position –2518 in the MCP-1/CCL2 promoter revealed that the prevalence of G/G homozygotes was significantly higher among patients with coronary artery disease than

control subjects (odds ratio: 2.2) (65). A CCR2 polymorphism in which isoleucine appears at position 64 in place of the more frequent valine, is associated with decreased cardiovascular risk (23). This variant has been reported to signal less efficiently, thus providing a plausible mechanistic basis for the protective effect of the allele.

Because the absence of MCP-1/CCL2 or CCR2 in genetically modified mice had no effect on total cholesterol or individual plasma lipoprotein levels, these results suggest that therapies directed against MCP-1/CCL2 or CCR2 might be beneficial in patients with refractory hypercholesterolemia, or even as an adjunct to therapy with lipid-lowering agents. However, it now seems that lipid-lowering agents may interact already with this pathway. For example, one of the anti-inflammatory activities of the statins is the suppression of MCP-1/CCL2 expression (35). Statin administration to humans also has produced lower plasma MCP-1/CCL2 levels (37), suggesting that these agents exert their cardioprotective effects both by lowering cholesterol and by dampening monocyte recruitment to injured vessels.

In addition to statins, however, a direct anti-MCP-1/CCL2 therapy has been shown to be effective in rodent atherosclerosis models. The therapeutic agent in this case is the N-terminally truncated MCP-1/CCL2 variant 7ND, which was mentioned earlier. A group in Japan has pioneered the delivery of 7ND by expression plasmid transduction into skeletal muscle, which results in the synthesis and secretion of substantial amounts of 7ND that can be measured in plasma. This "gene therapy" approach has been used in an L-N(G)-nitro-L-arginine methylester hypertensive rat model (14) and in apoE-deficient mice (49). In both cases, animals treated with 7ND had substantially less lesion formation in their coronary arteries and aortas. As in the MCP-1/CCL2 and CCR2 knockout mice, decreased disease correlated with decreased macrophage influx into the arterial wall. These studies provide a mechanistic confirmation of the importance of MCP-1/CCL2 in atherosclerosis that nicely complements the work in genetically modified animals.

Currently available therapeutic agents include 7ND and antibodies directed against MCP-1 and CCR2. It may be impractical to consider lifelong administration of biological agents like these for preventing atherosclerosis, but there are other settings in which this may make sense. One example is restenosis after balloon angioplasty and stent placement. In the early 1990s, it was shown that balloon injury of the rabbit aorta induced abundant local MCP-1/CCL2 expression, which is consistent with the general idea of MCP-1/CCL2 being produced in response to vascular injury (66). Considering the role of MCP-1/CCL2 in recruiting macrophages and the abundance of smooth muscle cell growth factors secreted by those cells, it was hypothesized that angioplasty-induced MCP-1/CCL2 might contribute to restenosis. This has been confirmed in two different ways. First, the 7ND gene delivery approach was used in a model of femoral artery cuff injury in mice and monkeys (15), and in a carotid artery balloon injury model in hypercholesterolemic rabbits (45). In both cases, 7ND significantly limited neointimal hyperplasia, and in the rabbit model it prevented negative remodeling. In a second approach, anti-CCR2 antibodies prevented in-stent restenosis after iliac arterial angioplasty and stent placement in primates (28). From a therapeutic viewpoint, it would be practical to administer these biological agents during and immediately after stent placement to prevent restenosis.

The animal models provide compelling evidence that MCP-1/CCL2 may play a pathogenetic role in restenosis, but are there any human clinical data to support this idea? Two studies have examined the predictive power of plasma MCP-1/CCL2 levels after revascularization procedures. In one (10), MCP-1/CCL2 levels were measured frequently after balloon angioplasty in patients who then underwent repeat angiography at 6 months. Although MCP-1/CCL2 levels were the same before angioplasty both in patients who did and did not develop restenosis, patients in the former group had significantly higher MCP-1/CCL2 levels 1 day after undergoing angioplasty (10). The second study (50) examined patients who had undergone stent placement. Again, before stent placement, MCP-1/CCL2 levels were the same in all patients with angina, but patients who had evidence of restenosis at 6 months had higher levels of MCP-1/CCL2 soon after the procedure. To be fair, although these studies provide some epidemiological support for the role of MCP-1/CCL2 in restenosis, they do not constitute evidence for a causal relationship.

Multiple Sclerosis

As in atherosclerosis, the pathogenesis of MS involves the infiltration of effector mononuclear cells into target tissues, and, again, MCP-1/CCL2 is likely to play a major role in attracting these cells into the central nervous system (CNS). In fact, correlations have been observed between MCP-1/CCL2 expression and disease activity. Several groups have shown

that, in human disease, astrocytes express MCP-1/CCL2 in demyelinating lesions (43,63,68), and in rodent models of experimental allergic encephalomyelitis (EAE) MCP-1/CCL2 expression occurs during disease flares (56).

Support for the contribution of MCP-1/CCL2 to the pathogenesis of EAE came from three sources. First, the antibody neutralization of MCP-1/CCL2 prevented disease relapses in a model of adoptive transfer of EAE in which myelin-sensitized T cells were infused into SJL recipients (33). Interestingly, the neutralization of another chemokine, MIP-1α, prevented the acute disease phase that precedes relapses in this model. Second, mice deficient for CCR2 did not develop EAE after active myelin immunization or after the adoptive transfer of sensitized T cells (16,31). Finally, MCP-1/CCL2-deficient mice failed to develop active EAE in two different myelin immunization models (29). Notably, even though *MCP-1/CCL2*$^{-/-}$ mice did not develop EAE after being immunized with myelin antigens, T cells from these mice were able to produce disease when transferred into wild-type, but otherwise syngeneic, recipients. Conversely, T cells from sensitized wild-type mice were unable to produce disease when transferred into MCP-1/CCL2$^{-/-}$ recipients. Thus, it seems that MCP-1/CCL2 and CCR2 are not necessary in order for mice to mount an immune response to myelin antigens, but rather they are required for attracting effector cells into the CNS, where they can initiate the processes of demyelination and axonal severing that are characteristic of EAE and MS (67).

So far, there have been no reports of therapeutic approaches to rodent disease such as the administration of 7ND in an active immunization model. However, the efficacy of anti-MCP-1/CCL2 antibodies in the adoptive transfer model suggests that there is sufficient disruption of the blood/brain barrier to permit access of large biological agents to appropriate subanatomic domains. Of course, the clinical utility of interferon-β in human disease suggests that the same is true in patients and that anti-MCP-1/CCL2 or anti-CCR2 therapy using biological agents may be clinically effective.

THE INFLUENCE OF MCP-1/CCL2 AND CCR2 ON T HELPER CELL FUNCTION

A fundamental insight arising from the work on MCP-1/CCL2/CCR2 in inflammatory disease is that the notion of chemokine redundancy, which is based on the apparently promiscuous patterns of chemokine ligand/receptor binding *in vitro*, is not particularly relevant *in vivo*. Despite the fact that CCR2 has five high-affinity ligands in the mouse and that monocytes express other chemokine receptors, antibody-mediated inactivation or genetic disruption of MCP-1/CCL2 or CCR2 alone is sufficient to nearly abrogate disease in the models described above. Furthermore, in simpler models of inflammation such as peritoneal thioglycollate instillation, the loss of MCP-1/CCL2 or CCR2 each by themselves prevented monocyte recruitment (6,39).

These observations support the notion that MCP-1/CCL2 and CCR2 are uniquely and nonredundantly responsible for monocyte recruitment in these inflammatory and disease-based models. Thus, it was assumed by most investigators in the field that this chemokine ligand/receptor pair would exert its influences almost exclusively in the area of innate immunity. However, careful analyses of MCP-1/CCL2-deficient and CCR2-deficient mice have revealed that they also have profound effects on the differentiation of T helper, Th, cells. Surprisingly, unlike the concordant effects of MCP-1/CCL2 and CCR2 deficiency on inflammation, the effects of their loss on Th cell phenotypes are completely disparate.

One of the first clues about the effects of MCP-1/CCL2 on adaptive immunity came from a transgenic mouse model in which MCP-1/CCL2 expression was driven by the mouse mammary tumor virus long terminal repeat (60). In other transgenic models, promoters that directed MCP-1/CCL2 expression to a single tissue site predictably elicited monocyte infiltration into that tissue (17,22,26). In contrast, the mouse mammary tumor virus long terminal repeat transgene produced high levels of systemic MCP-1/CCL2, which desensitized circulating monocytes and prevented their directed migration into organs expressing the transgene. Although this transgenic mouse did not demonstrate monocyte infiltration, it did have the abnormal phenotype of being much more susceptible to intracellular bacteria such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*. One explanation for this phenotype might be the desensitization of monocyte CCR2 that explained the absence of monocyte infiltration into organs expressing transgenic MCP-1/CCL2. However, an alternative explanation could be that these mice had deficient Th1 responses, which would be necessary for eliminating intracellular pathogens. Furthermore, the Th1 deficiency might be the consequence of an MCP-1/CCL2-driven Th2 differentiation.

In fact, independent observations have pointed to a possible association between MCP-1/CCL2 and Th2 responses. *In vivo*, for example, the administration of

anti-MCP-1/CCL2 antibodies reduced the size of pulmonary granulomas elicited by embolized *Schistosoma mansoni* eggs (9). Because these granulomas are filled with eosinophils and type 2 cytokines, it might be inferred that MCP-1/CCL2 helps to drive this Th2-polarized response. This observation has been reproduced in *MCP-1/CCL2*$^{-/-}$ mice (39). *In vitro*, it has been suggested that the addition of MCP-1/CCL2 to naïve T cells in the presence of antigen drives their differentiation in a Th2 direction (34). Finally, of clinical relevance, antibody blockade of MCP-1/CCL2 in allergic airways hypersensitivity models led to decreased cellularity in bronchial alveolar lavage fluid and diminished bronchial hyperreactivity (20). (The same results have been observed in *MCP-1/CCL2*$^{-/-}$ mice [C. Gerard and B. Rollins; unpublished data]). Again, the amelioration of asthma-like disease in the absence of MCP-1/CCL2 suggests that this chemokine is involved in promoting Th2 responses.

A formal test of the effects of MCP-1/CCL2 on Th cell polarization *in vivo* was performed by immunizing MCP-1/CCL2-deficient C57Bl/6 mice using a protocol designed to elicit a mixed population of Th1 and Th2 cells (25). Sensitized T cells from *MCP-1/CCL2*$^{-/-}$ mice were able to secrete normal amounts of interferon-γ and IL-2 in response to *in vitro* antigen challenge, suggesting that Th1 differentiation was intact. However, T cells from these mice were unable to secrete IL-4, IL-5, or IL-10. Furthermore, the subclass of antibodies elicited against the immunizing antigen were almost exclusively immunoglobulin (Ig) G2a and IgG2b in the *MCP-1/CCL2*$^{-/-}$ mice, but included substantial amounts of IgG_1 in wild-type mice. Thus, *MCP-1/CCL2*$^{-/-}$ mice are unable to perform the Ig subclass switch that is characteristic of Th2 responses. The global Th2 defect in *MCP-1/CCL2*$^{-/-}$ mice was further confirmed by the relative resistance of MCP-1/CCL2-deficient Balb/c mice to *Leishmania major* infection (25).

In contrast, CCR2-deficient mice subjected to similar (although not identical) analyses indicated that they had a severe Th1 defect. For example, in response to *in vitro* rechallenge, sensitized T cells from *CCR2*$^{-/-}$ mice produced far less interferon-γ, but equivalent amounts of IL-5 and IL-10 compared to cells from wild-type mice (6,53). Lymph node cultures from the knockout mice had barely detectable levels of IL-12, unlike cultures from wild-type and *MCP-1/CCL2*$^{-/-}$ mice (25). And, in striking contrast to MCP-1/CCL2-deficient mice, CCR2-deficient mice were more susceptible to *L. major* (61), and demonstrated normal (41) or enhanced (36) cellularity and airways hyperreactivity in bronchial hypersensitivity models. The sensitivity to *Leishmania* correlated with a diminished capacity for epidermal Langerhans cells to migrate to regional lymph nodes in response to fluorescein isothiocyanate skin painting (61). A similar defect in antigen-presenting cell (APC) migration to the lung and regional lymph nodes has been implicated in the mechanism underlying the striking sensitivity of *CCR2*$^{-/-}$ mice to *M. tuberculosis* (54).

Although the Th1 deficiency in *CCR2*$^{-/-}$ mice seems to be related to defective APC migration, the precise mechanism whereby this leads to problems in Th1 polarization is still unclear. One might predict, for example, that a paucity of APCs in regional nodes should lead to an overall diminished immune response. However, lymph node cultures from sensitized *CCR2*$^{-/-}$ mice make just as much IL-2 after rechallenge as cultures from sensitized wild-type mice (6). The same is true of Th2-deficient *MCP-1/CCL2*$^{-/-}$ mice (25). Still, purified naïve T cells from *MCP-1/CCL2*$^{-/-}$ and *CCR2*$^{-/-}$ mice are capable of being polarized in either a Th1 or a Th2 direction *in vitro*, indicating that the deficiencies observed *in vivo* are not inherent to T cells (53) (C. Daly and B. Rollins; unpublished data). This points to a problem involving the migration either of Th0 cells or APCs but, again, the precise manner in which this translates into a polarized Th deficiency is unknown. Perhaps these mice experience selective abnormalities in the trafficking of DC1 and DC2 cells (i.e., APCs that can specifically support the differentiation of Th1 and Th2 cells, respectively).

Despite the mechanistic uncertainties, it is quite clear that *MCP-1/ CCL2*$^{-/-}$ and *CCR2*$^{-/-}$ mice have discrepant phenotypes in several models that depend on Th cell polarization. How can this be explained? There are several possibilities. First, as noted earlier, CCR2 has five distinct high-affinity ligands in mice, and four in humans. If these ligands are not biologically interchangeable, then they may elicit different effects when they bind to CCR2. There is ample precedent for this phenomenon in the activation of CXCR2. This receptor binds all erypoietin glutamate-leucine-arginine (ELR) containing CXC chemokines with nearly identical affinities, yet the various ligands have differing EC_{50} values, depending on the biological outcome being measured (e.g., chemotaxis versus the induction of a respiratory burst) (46,69). If an analogous situation applies to the ligands of CCR2, then it may be possible that MCP-1/CCL2 drives Th2 responses, while, in its absence, another ligand such as MCP-3 might drive Th1 re-

sponses. On the receptor side, the loss of all CCR2 signaling would result in Th1 deficits, regardless of the ligands involved.

Another possible explanation for the difference between *MCP-1/CCL2*$^{-/-}$ and *CCR2*$^{-/-}$ mice in adaptive immunity could be that MCP-1/CCL2 has another, as yet undefined, receptor. Although there is currently no genetic evidence to support this contention, there are some provocative biochemical data pointing to this possibility. Specifically, very low concentrations of MCP-1/CCL2 can induce the expression of tissue factor by human arterial smooth muscle cells, despite the fact that these cells have no detectable CCR2 expression by reverse transcriptase polymerase chain reaction (62). The MCP-1/CCL2 dose–response characteristics indicate that the activation of another chemokine receptor (e.g., CCR1) by MCP-1/CCL2 is an unlikely explanation. Rather, the data point to the possibility that these cells express another non-CCR2 receptor for MCP-1/CCL2. If the existence of a second receptor were confirmed, then one might consider the possibility that the activation of this presumably MCP-1/CCL2-specific receptor could drive Th2 polarization, whereas CCR2 activation drives Th1 polarization. The activation by MCP-1/CCL2 of this putative receptor would have to trump its activation of CCR2 when both are present in the setting of adaptive immune responses. Obviously, the confirmation of this model awaits the definitive identification and cloning of a second receptor.

Regardless of the explanation, the disparity between *MCP-1/CCL2*$^{-/-}$ and *CCR2*$^{-/-}$ mice in adaptive immunity carries an important implication, namely, that the effects of MCP-1/CCL2 depend on the context in which it is activating cells. For example, in the afferent arm of the adaptive immune response, naïve T cells encounter APCs loaded with antigen in the T-cell zone of regional lymph nodes. MCP-1/CCL2 is secreted in this subanatomic area (25) and serves in an as yet obscure manner to polarize T cells in a Th2 direction (Fig. 1). Whether this occurs by means of attracting DC2 cells, as indicated in Fig. 1, or by some other mechanism is currently unknown.

In contrast, in the efferent arm of the immune response, for example in end-organ inflammation, MCP-1/CCL2 serves a different purpose. In these settings, MCP-1/CCL2 acts to attract effector cells (Fig. 1). Because of the distribution of CCR2 among circulating leukocytes, these cells will be macrophages and memory effector T cells (i.e., abundant

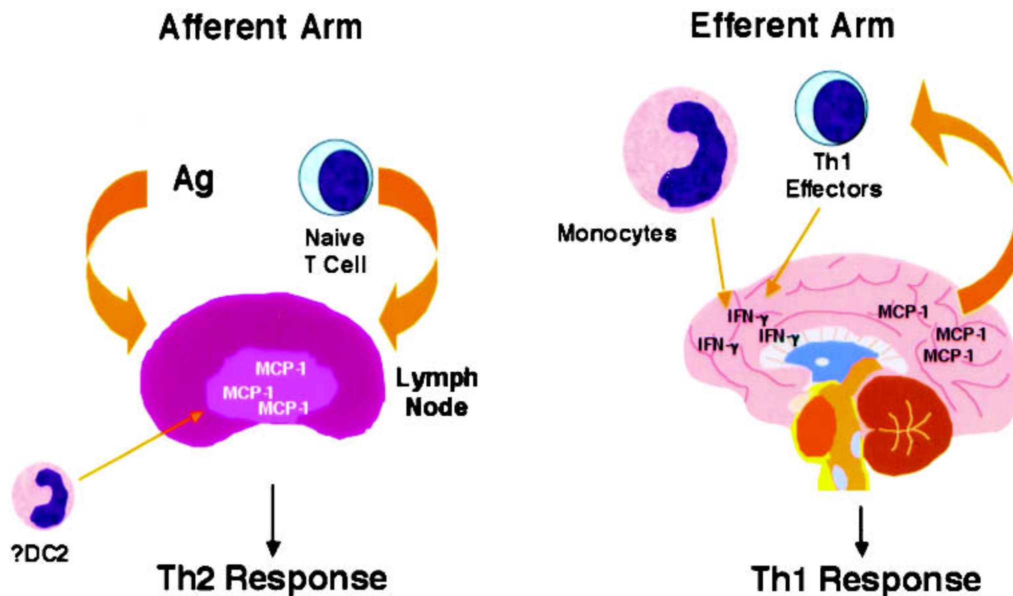

Figure 1. The effects of MCP-1/CCL2 in adaptive immunity depend on context. *Left*: During immunization (i.e., the afferent arm of the immune response), MCP-1/CCL2 is secreted by cells in the T-cell zone of the lymph node, and this chemokine stimulates Th2 polarization of a naïve T cell. MCP-1/CCL2 is shown as possibly attracting DC2 cells to accomplish this, but the mechanism whereby MCP-1/CCL2 stimulates Th2 polarization in this setting is unknown. *Right*: During end-organ inflammation (i.e., the efferent arm of the immune response) shown here as CNS inflammation, MCP-1 is secreted by resident cells to attract effector cells. These will be cells that secrete interferon-γ, thereby producing a functionally Th1 outcome.

sources of interferon-γ). Here, MCP-1/CCL2 will seem to be driving Th1 responses, although what it is really doing is eliciting effector cells that are already Th1-polarized. For example, EAE immunization uses antigens and adjuvants that stimulate strongly Th1 polarized responses in a manner that is completely MCP-1/CCL2-independent, as described above (29). Thus, when MCP-1/CCL2 is expressed in the CNS during relapses, the cells it attracts are Th1 cells and the targets of Th1 cells such as macrophages. This explains why the phenotype of MCP-1/CCL2-deficient mice is identical to the phenotype of CCR2-deficient mice in EAE. A similar situation occurs in atherosclerosis models, another setting in which the phenotypes of *MCP-1/CCL2*$^{-/-}$ and *CCR2*$^{-/-}$ mice are the same.

SUMMARY

In several inflammatory diseases marked by mononuclear cell infiltration, there is now solid genetic evidence for the importance of the MCP-1/CCL2/CCR2 axis in pathobiology, if not pathogenesis. The only debate that remains is how best to target this system therapeutically. Despite a decade of screening for small-molecule antagonists, none has been identified yet that will move into the clinic in the near future. Biological agents such as antibodies and inhibitory peptides have been developed, and some conceivably could be developed into therapies. However, considering the cost of their development and the complicated delivery strategies they will require, most companies will be reluctant to pursue these agents so long as the promise of small-molecule antagonists exists. Nonetheless, the effectiveness of these biological agents in animal models suggests that they may provide therapeutic opportunities until "real" drugs come along.

Meanwhile, there is also substantial evidence that MCP-1/CCL2 and CCR2 can have profound effects both on innate and adaptive immune responses. Therapeutic targeting of this ligand/receptor pair therefore could have wide-ranging consequences that extend beyond simply blocking monocyte and macrophage migration. But, given the data on the disparate effects of MCP-1/CCL2 and CCR2 on Th cell polarization, the blockade of MCP-1/CCL2 and the blockade of CCR2 are not equivalent strategies. This is particularly relevant to the development of biological agents such as antibodies that will be directed exclusively at one protein or the other. Considerable thought and planning will have to go into making decisions about the proper settings in which to use these agents. Elucidating the mechanistic basis for the effect of MCP-1/CCL2 and CCR2 on adaptive immunity will certainly increase the chances that a rational approach to their blockade will be developed.

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