Critical role of MCP-1 in the pathogenesis of experimental colitis in the context of immune and enterochromaffin cells


Critical role of MCP-1 in the pathogenesis of experimental colitis in the context of immune and enterochromaffin cells. Am J Physiol Gastrointest Liver Physiol 291: G803–G811, 2006. First published May 25, 2006; doi:10.1152/ajpgi.00069.2006.—Mucosal changes in inflammatory bowel disease (IBD) are characterized by ulcerative lesions accompanied by a prominent infiltrate of inflammatory cells including lymphocytes, macrophages, and neutrophils and alterations in 5-hydroxytryptamine (5-HT)-producing enterochromaffin (EC) cells. Mechanisms involved in recruiting and activating these cells are thought to involve a complex interplay of inflammatory mediators. Studies in clinical and experimental IBD have shown the upregulation of various chemokines including monocyte chemotactrant protein (MCP-1) in mucosal tissues. However, precise information on the roles of this chemokine or the mechanisms by which it takes part in the pathogenesis of IBD are not clear. In this study, we investigated the role of MCP-1 in the development of hapten-induced experimental colitis in mice deficient in MCP-1. Our results showed a significant reduction in the severity of colitis both macroscopically and histologically along with a decrease in mortality in MCP-1-deficient mice compared with wild-type control mice. This was correlated with a downregulation of myeloperoxidase activity, IL-1β, IL-12p40, and IFN-γ production, and infiltration of CD3+ T cells and macrophages in the colonic mucosa. In addition, we observed significantly lower numbers of 5-HT-expressing EC cells in the colon of MCP-1-deficient mice compared with those in wild-type mice after dinitrobenzenesulfonic acid. These results provide evidence for a critical role of MCP-1 in the development of colonic inflammation in this model in the context of immune and enteric endocrine cells.

monocyte chemotactrant protein-1; dinitrobenzenesulfonic acid; CD3-positive cells; 5-hydroxytryptamine; serotonin

INFLAMMATORY BOWEL DISEASE (IBD) includes two chronic gastrointestinal (GI) diseases, ulcerative colitis (UC) and Crohn’s disease (CD), which are relapsing inflammatory conditions of unknown etiology in the GI tract. IBD is the most common and serious chronic inflammatory condition of the human bowel (42). The causes of IBD are unknown, but epidemiological and laboratory works (11, 12, 21, 22, 39) have suggested that environmental and genetic factors are important in the pathogenesis of the IBD, which is associated with dysregulation of the mucosal immune system. Mucosal changes in IBD are characterized by ulcerative lesions accompanied by a prominent infiltrate of inflammatory cells including T lymphocytes, macrophages, neutrophils, and plasma cells. The mechanisms involved in recruiting and activating these inflammatory cells are thought to involve a complex interplay of inflammatory mediators.

The intensity of the inflammatory response in both clinical and experimental IBD is determined both by the local expression of growth factors and proinflammatory cytokines within the mucosa and by coordinated mechanisms of cellular recruitment, involving the upregulation of both vascular adhesion molecules and chemokine expression (40). Chemokines, a group of protein messengers (1, 5, 6, 33, 48), play a major role in the maintenance of inflammatory processes, and the final composition of leukocytes present in the inflamed intestine is most likely due to both secreted chemokines and the relative expression of specific chemokine cell surface receptors on different cell types. The production of chemokines within the intestine establishes a chemotactic gradient capable of increasing the migration of monocytes/macrophages, granulocytes, and lymphocytes from the bloodstream through the endothelium into both the mucosa and submucosa during chronic IBD.

T cells constitute an important part of many immune responses, including those associated with IBD. The chronic inflammation of CD is maintained by a T helper 1 (Th1)-driven immune response. T cells isolated from the colon of patients suffering from CD produce large amounts of IFN-γ and TNF-α and little IL-4 or IL-10. Experimental models represent a powerful tool to dissect these various components to better understand their individual contribution to disease pathogenesis. Many mouse models of experimental colitis are associated with a Th1-type immune response, as reflected by infiltration of IFN-γ-producing T cells in the colon. Chemokines are also considered important in attracting antigen-presenting cells (APCs) to sites of inflammation, directing APCs to lymphatic vessels, bringing APCs and lymphocytes together within the lymphoid organ, and recruiting the appropriate effector T cells to sites of inflammation (19, 44).

Monocyte chemotactrant protein (MCP)-1 is produced by a variety of cells including dendritic cells, macrophages, endothelial cells, and fibroblasts, and its expression is upregulated after exposure to inflammatory stimuli such as IL-1 and TNF-α (32). MCP-1 was originally identified as a monocyte-specific chemoattractant but was later shown to act on T cells, mast cells, basophils, and natural killer cells (2, 9, 14, 29). Elevation of MCP-1 is observed in mucosal tissues from patients with CD and UC (7, 36, 40) and also in experimental models of colitis (45, 47). However, precise information on the role of...
this chemokine or the mechanisms by which it take part in the pathogenesis of IBD remain to be determined.

The most well-characterized subset of GI endocrine cells are enterochromaffin (EC) cells, which are dispersed throughout the GI mucosa and are the main source of 5-hydroxytryptamine (5-HT; serotonin) in the gut (15). 5-HT is considered to be important in maintaining intestinal homeostasis, and it has been implicated in the pathophysiology of a number of GI diseases including functional disorders like IBS (8, 13, 16). Alterations in colonic EC cells have been also observed in animal models of colitis (30, 38). However, the mechanisms regulating the changes in EC cells in the GI tract in enteric inflammation and the precise role of 5-HT in host defense are still not clear. Because of the strategic location of EC cells in the GI mucosa and are the main source of 5-hydroxytryptamine (5-HT; serotonin) in the gut (15). 5-HT is considered to be important in maintaining intestinal homeostasis, and it has been implicated in the pathophysiology of a number of GI diseases including functional disorders like IBS (8, 13, 16). Alterations in colonic EC cells have been also observed in animal models of colitis (30, 38). However, the mechanisms regulating the changes in EC cells in the GI tract in enteric inflammation and the precise role of 5-HT in host defense are still not clear. Because of the strategic location of EC cells in the GI mucosa, inflammation-induced changes in EC cells are likely to be modulated by the immune system as a component of host defense in GI inflammation. It has been reported that human MCP-1 plays a fundamental role in histamine and 5-HT reduction in the number of CD3+ T cells, F4/80+ macrophages, and 5-HT expressing EC cells, implying a critical role for this chemokine in the pathogenesis of inflammation in this model of experimental colitis.

MATERIALS AND METHODS

Animals. Breeding pairs of MCP-1−/− mice on a C57BL/6 background (31) were provided by B. Rollins (Dana-Farber Cancer Institute, Boston, MA) and were kept and bred under specific pathogen-free conditions at the animal facilities of McMaster University (Hamilton, ON, Canada). Appropriate control C57BL/6 (MCP-1+/+) mice were obtained from the Jackson Laboratories. All animals were kept in sterilized, filter-topped cages and fed autoclaved food; only male mice of 8–10 wk of age were used. The protocols employed were in direct accordance with guidelines drafted by the McMaster University Animal Care Committee and the Canadian Council on the Use of Laboratory Animals.

Induction of colitis. Colitis was induced by an intracolonial administration of DNBS (ICN, Aurora, OH) as described previously (42). In brief, a stock solution of DNBS was made by dissolving 50 mg of DNBS per 1 ml of 50% ethanol. Mice anesthetized with 2% enflurane were injected in the distal 4 cm of the colon with 100 μl of this solution, containing 5 mg of DNBS, using a 1-ml tuberculin syringe (Becton Dickinson, Franklin Lakes, NJ) and polyethylene-90 tubing (Clay Adams, Parsippany, NJ). Mice were given 8% sucrose in 0.2% saline to prevent dehydration during the first week after DNBS administration. Control mice were killed 3 days after the intracolonial administration with 50% ethanol.

Assessment of colonic damage. The colon was removed and opened longitudinally, and the damage was assessed macroscopically and histologically using previously published criteria (42). Briefly, the macroscopic criteria included macroscopic mucosal damage (assessed with a scale), thickening of the colonic wall, the presence of adhesions between the colon and other intra-abdominal organs, the consistency of fecal material (as an indicator of diarrhea), and the presence of hyperemia. Microscopic criteria for damage and inflammation were investigated by light microscopy on hematoxylin-eosin-stained histological sections obtained from gut segments taken from a region of the inflamed colon immediately adjacent to gross macroscopical damage. Histological criteria were based on the following: degree of mucosal architectural changes, cellular infiltration, goblet cell depletion, and presence of crypt abscesses. Macroscopic and histological damage were recorded and scored for each mouse by two different investigators who were blinded to the treatment condition.

Myeloperoxidase activity. The degree of colonic inflammation was investigated by an assay of myeloperoxidase (MPO) activity. Three days after the intrarectal administration of DNBS, the colon was removed, snap frozen in liquid nitrogen, and stored at −70°C. Samples were weighed, and MPO was measured using a technique described previously (46). MPO activity is reported as units of MPO per milligram of wet tissue. One unit of MPO was defined as the quantity of enzyme able to convert 1 μmol of hydrogen peroxide to water in 1 min at room temperature.

Immunohistochemistry. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded samples sectioned at 4 μm. Serial sections were deparaffinized in CitriSolv (Fisher Scientific, Nepean, ON, Canada) and rehydrated through a graded series of ethyl alcohol. Endogenous peroxidase was blocked by an incubation in peroxidase blocking reagent (DakoCytomation, Mississauga, ON, Canada) for 15 min. After being washed, sections were predigested with proteinase K solution (DakoCytomation) for 15 min at room temperature or were subjected to antigen retrieval in citrate buffer (pH 6.0) after being heated in a microwave. After being washed and blocked of nonspecific binding with 1% BSA in PBS, sections were incubated with polyclonal rabbit anti-CD3 antibody (DakoCytomation, 1:500, 1 h at room temperature), monoclonal rat anti-mouse antibody F4/80 (Serotec, Raleigh, NC, 1:100, 18 h at 4°C), or 5-HT rabbit antibody (Immunostar, 1:5,000, 1 h at room temperature). After

Fig. 1. Macroscopic damage score in dinitrobenzenesulfonic acid (DNBS)-induced colitis in wild-type [monocyte chemoattractant protein (MCP)-1+/+] and MCP-1-deficient (MCP-1−/−) mice. Colitis was induced by DNBS (5 mg) administered intracolonically, and macroscopic damage was evaluated 3 and 7 days after the induction of colitis. Control mice were killed 3 days after the intracolonial administration of 50% ethanol. Each bar represents the mean ± SE; n = 6–8 mice/group. Representative data from 3 experiments are shown. *Significantly higher than control; **Significantly lower than DNBS-treated MCP-1+/+ mice.
being washed, sections stained with anti-CD3 antibody and 5-HT antibody were incubated with biotinylated anti-rabbit secondary reagent, DakoCytomation (1:300) for 30 min, and sections for F4/80 immunostaining were incubated with horseradish peroxidase-conjugated streptavidin (DakoCytomation, 1:300) for 30 min. Sections were developed with 3, 3′-diaminobenzidine solution as a chromogen. They were counterstained with Meyer’s hematoxylin (DakoCytomation), dehydrated, cleared, and mounted. Negative controls were prepared by omission of the primary antibodies.

CD3+ cells were counted in five different positions in each section, which were chosen randomly. The results were shown as cell counts per hyper-power field. The F4/80+ area was measured by ImageJ software because it was difficult to delineate single cells. The numbers of 5-HT-positive cells were expressed per 10 crypt units.

Evaluation of intestinal tissue cytokines levels. Frozen intestinal tissues were homogenized in lysis buffer containing protease inhibitor cocktail (Sigma). The homogenates were freeze thawed three times and centrifuged, and the supernatant was collected and stored at −20°C until analyzed.

IL-1β and IL-12p40 levels in the supernatant were measured by an enzyme immunoassay technique using a commercially available kit purchased from R&D Systems (Minneapolis, MN). The concentration of protein in the intestinal tissue was determined by a commercially available DC Protein Assay Kit (Bio-Rad), and amounts of cytokines in the tissues were expressed per milligram of tissue protein.

Evaluation of in vitro cytokine production from spleen cells. Single cell suspensions of spleen tissue were prepared in RPMI-1640 containing 10% fetal calf serum, 5 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, and 0.05 mM 2-mercaptoethanol (all from GIBCO-BRL). Cells (10^7 cells/well) were plated in 24-well plates with or without 5 μg/ml of concavalin A (ConA; Sigma). Culture supernatants were harvested after 48 h, and IL-4 and IFN-γ levels were determined by an enzyme immunoassay technique using commercially available kits purchased from R&D Systems.

Statistical analysis. Data were analyzed using Student’s t-test with P < 0.05 considered as significant. All results are expressed as means ± SE.

RESULTS

Macroscopic evaluation of the colon on days 3 and 7 after the administration of 5 mg of DNBS revealed a significant difference between MCP-1+/+ and MCP-1−/− mice. MCP-1+/+ mice after DNBS administration exhibited massive ulceration, thickening of the colonic wall, hyperemia, and severe adhesions between the colon and other organs. This was accompanied by a 10% mortality rate. In contrast, MCP-1−/− mice after DNBS treatment showed significantly less mucosal damage, less thickening of the colonic wall, and fewer adhesions (Fig. 1). There was no mortality in MCP-1−/− mice after the administration of DNBS. There were no significant differences in macroscopic damage scores between MCP-1−/− mice after DNBS and MCP-1−/− control mice.

Histological examination of the colon in MCP-1+/+ mice after DNBS treatment revealed an intense granulocyte infiltrate extending throughout the mucosa and submucosa, often involving the muscularis propria. There was also marked muco-
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Fig. 3. Measurement of myeloperoxidase (MPO) activity of DNBS-induced colitis in the colon of MCP-1\(^{+/+}\) and MCP-1\(^{-/-}\) mice. Colitis was induced by the intracolonic administration of DNBS (5 mg), and MPO activity from the colon was evaluated on day 3 after the administration of DNBS. Controls were mice that were killed 3 days after the intracolonic administration of 50% ethanol. Each bar represents the mean ± SE of 5 animals. *Significantly lower compared with DNBS-treated MCP-1\(^{+/+}\) mice.

Fig. 4. Accumulation of CD3\(^{+}\) cells in DNBS-induced colitis in MCP-1\(^{+/+}\) and MCP-1\(^{-/-}\) mice. Colitis was induced by DNBS (5 mg) administered via an intracolonic route, and CD3\(^{+}\) cells were evaluated on days 3 and 7 after the induction of colitis. Control mice were treated with 50% ethanol. A: CD3 immunostaining of colon tissue of control MCP-1\(^{+/+}\) mice. B: CD3 immunostaining of colon tissue of control MCP-1\(^{-/-}\) mice. C: CD3 immunostaining of colon tissue of control MCP-1\(^{-/-}\) mice on day 7 after DNBS. D: CD3 immunostaining of colon tissue of MCP-1\(^{-/-}\) mice after DNBS. E: numbers of CD3\(^{+}\) cells in MCP-1\(^{-/-}\) and MCP-1\(^{+/+}\) mice after DNBS. Data are expressed in cell counts per hyper-power field (HPF). Arrows indicate CD3\(^{+}\) cells. *Significantly lower compared with DNBS-treated MCP-1\(^{+/+}\) mice.
ConA-stimulated spleen cells and observed a significantly lower level of IFN-γ production by spleen cells in MCP-1−/− mice compared with that in MCP-1+/+ mice after DNBS administration on day 7 after DNBS (Fig. 7). We failed to detect IFN-γ on day 3 after DNBS and also IL-4 on days 3 and 7 after DNBS in the supernatant of in vitro ConA-stimulated spleen cells in both MCP-1+/+ and MCP-1−/− mice.

In addition, we also investigated 5-HT-expressing EC cells in MCP-1+/+ and MCP-1−/− mice in DNBS-mediated colitis. There was an upregulation in the numbers of 5-HT-expressing EC cells after DNBS administration in MCP-1+/+ mice, but this DNBS-mediated increase in the numbers of EC cells was not evident in MCP-1−/− mice. We observed significantly lower number of 5-HT-expressing EC cells in MCP-1−/− mice compared with those in MCP-1+/+ mice after DNBS.

Fig. 5. Accumulation of F4/80+ cells in DNBS-induced colitis in MCP-1+/+ and MCP-1−/− mice. Colitis was induced by DNBS (5 mg) administered via an intracolonic route, and F4/80+ cells were evaluated on days 3 and 7 after the induction of colitis. Control mice were treated with 50% ethanol. A: F4/80 immunostaining of colon tissue of control MCP-1+/+ mice. B: F4/80 immunostaining of colon tissue of MCP-1+/+ mice on day 7 after DNBS. C: F4/80 immunostaining of colon tissue of control MCP-1−/− mice. D: F4/80 immunostaining of colon tissue of MCP-1−/− mice on day 7 after DNBS. E: F4/80+ area. Data are expressed as the percentages of the positive staining areas of the total area. *F4/80+ areas were significantly lower in MCP-1−/− mice after DNBS compared with those in MCP-1+/+ mice after DNBS. Original magnification: ×100 in A and C and ×200 in B and D.

Fig. 6. IL-1β and IL-12p40 levels in colonic tissues from MCP-1+/+ and MCP-1−/− mice in DNBS-induced colitis. Colitis was induced by the intracolonic administration of DNBS (5 mg), and IL-1β (A) and IL-12p40 (B) levels in colon tissues were evaluated on day 3 after the administration of DNBS. Controls were mice that received an intracolonic administration of 50% ethanol. Each value (in pg/ml) represents the mean ± SE from 5 mice. *Significantly lower compared with DNBS-treated MCP-1+/+ mice.
compared with those in MCP-1+/+ mice on day 7 after DNBS (Fig. 8). There were no significant differences in 5-HT-expressing EC cells between control MCP-1+/+ and control MCP-1−/− mice.

DISCUSSION

Under normal conditions, the mucosal immune system in the GI tract is responsible for the downregulation of the heightened host defense system due to the presence of microorganisms and dietary antigens. The inflammatory cells and mediators must be kept in control so they do not damage the host tissue. IBD presumably arise from the dysregulation of the immunomodulating mechanisms, which allows the normally downregulated responses to run uncontrolled into a chronic, nonspecific inflammatory condition with intermittent periods of acute exacerbation. The persistent release of inflammatory chemokines and cytokines promotes adhesion, migration, and activation of inflammatory cells and causes tissue damage. IBD is characterized by intense mucosal recruitment of activated cells from both the innate and adaptive immune systems. Recent studies on IBD have focused on the identification of major cellular components and mediators in this chronic inflammatory condition. The characteristics of granulocytes, T cells, monocyte/macrophages, and lymphocytes in the inflamed lesion as well as a wide variety of cytokines, adhesion molecules, and free radicals that regulate cell functions in IBD are being intensely pursued (34). In addition, studies are being carried on the contribution of EC cells and 5-HT signaling in both experimental and clinical studies (8, 13, 16, 30, 38). The concept that chemokines could contribute to the pathogenesis of IBD generates from a series of clinical studies published almost a decade ago, in which rectal biopsies of patients with active UC
or CD were observed to produce high levels of the human chemokine CXCL8/IL-8 (4, 26, 27). Subsequent studies by Mazzucchelli et al. (35) and Daig et al. (20) showed that the expression of CXCL8/IL-8 correlated with the severity of inflammation and with leukocyte chemotactic activity in the inflamed colon. Although recent studies in clinical and experimental IBD have demonstrated an upregulation of MCP-1 production, little is known about the precise role of this chemokine in the pathogenesis of IBD. The present study is the first evidence of a critical role of MCP-1 in the pathogenesis of hapten-mediated experimental colitis.

DNBS-induced colitis is a well-characterized Th1-driven transmural inflammation of the colon and may be considered a model of CD, which is also characterized by transmural inflammation and with the development of a Th1-type immune response (28, 42). In the present study, we have shown that deficiency in the MCP-1 gene in mice not only attenuated the mortality and severity of inflammation associated with DNBS-induced colitis but also reduced the production of IFN-γ.

The amelioration of DNBS-induced colonic inflammation in MCP-1−/− mice was observed in all the parameters examined, including both the macroscopical and microscopical indexes as well as MPO activity, and was evident on both on days 3 and 7 after colitis. MPO is an enzyme contained in azurophilic granules of neutrophils as well in other myeloid cells and is commonly used as an index of neutrophil infiltration and inflammation (46). Previous studies have reported an extensive accumulation of neutrophils and a significant increase of MPO in DNBS-induced colitis (42, 46). In the present study, we observed significantly lower MPO activity in MCP-1−/− mice compared with that in MCP-1+/+ mice after DNBS administration.

IL-12 produced by APCs (primarily macrophages and B cells) activates the differentiation of Th cells toward Th1-type responses (23) by stimulating T and natural killer cell production of IFN-γ, and we have previously shown a significant increase in the IL-12 level in DNBS-mediated colitis (28). In this study, we observed significantly lower IL-12 production post-DNBS in MCP-1−/− mice compared with that in MCP-1+/+ mice. IL-1β is a proinflammatory cytokine that has an important role in inflammation and is produced by many cell types of both the peripheral and central immune system, including macrophages and lymphocytes. In this study, we also observed significantly lower IL-1β production in MCP-1−/− mice after DNBS treatment compared with that in MCP-1+/+ mice.

C-C chemokine receptor 2 (CCR2), the receptor for MCP-1, is expressed on monocytes, neutrophils, and lymphocytes (43), and CCR2 signaling has been reported to promote Th1 immune responses in vivo (10, 49, 50). Recently, it has been shown that CCR2-deficient mice were protected from dextran sodium sulfate (DSS)-induced intestinal adhesions and mucosal ulcerations (3). T cells activated in vitro with anti-CD3 upregulated expression of the MCP-1 receptor (41). MCP-1 induces the expression of integrins required for chemotaxis and acts as a potent attractant for inflammatory cells. It has been reported that MCP-1 recruits monocytes and lymphocytes (24), and we have recently shown that transfer and overexpression of the MCP-1 gene in euthymic mice also increased infiltration of CD3+ lymphocytes in the colonic wall (37). In Chlamydia psittaci infection, it has been also demonstrated that inhibition of accumulation of neutrophils in the lungs was associated with downregulation of MCP-1 (25). Recently, a significant increase in CCR2-expressing CD4 T cells has also been reported in CD (17). In the present study, we observed significant reductions in F4/80+ macrophages and CD3+ cells in MCP-1−/− mice after DNBS compared with those in MCP-1+/+ mice. These findings, along with the observation of attenuation in MPO activity, suggest that MCP-1 has an important role in the pathogenesis of colitis in this model by regulating the infiltration of inflammatory cells in the colon.

The present study also demonstrated an important role of MCP-1 in 5-HT-producing EC cells in relation to the pathogenesis of DNBS-mediated colitis. Several clinical and experimental studies have clearly demonstrated an alteration of EC cell numbers and 5-HT content in IBD (8, 16, 30, 38). An increase in 5-HT-immunoreactive cells has been reported in patients with CD (8), whereas a decrease in EC cell numbers and 5-HT content has been observed in patients with UC (16). In the experimental models of colitis induced by both trinitrobenzenesulfonic acid and DSS, increases in 5-HT secretions have been observed (30, 38). Although these studies have demonstrated changes in EC cells and 5-HT content in GI inflammation, the mechanisms involved in the differentiation or proliferation of EC cells and in the synthesis and release of 5-HT remain to be determined. Our study demonstrated an upregulation in the numbers of colonic EC cells after DNBS administration. We observed significantly higher numbers of 5-HT-expressing EC cells in MCP-1−/− mice on day 7 after DNBS. This DNBS-induced increase in EC cells was not evident in the colon of MCP-1−/− mice, suggesting that MCP-1 plays an important role in the proliferation or differentiation of 5-HT-producing EC cells during this inflammatory condition of the gut. MCP-1 may contribute in EC biology by acting either directly on EC cells through the presence of specific receptors on the cells or indirectly via acting on other immune cells like lymphocytes or macrophages. Because the data from the present study clearly demonstrated that deficiency of MCP-1 was associated with a significant reduction in CD3 T cells and F4/80+ macrophages in the colon after DNBS administration, it is very likely that the effects we observed in EC cell numbers after DNBS administration are due to modulation of cytokine production from immune cells. The absence of a significant difference in EC cell number on day 3 after DNBS might be due to hindrance by extensive mucosal damage that is associated with the early acute stage of DNBS-mediated colitis in MCP-1+/+ mice.

Taken together, the results of the present study clearly demonstrate that MCP-1 plays an important role in the pathogenesis of DNBS-mediated colitis in relation to the recruitment of immune and EC cells, and the absence of this chemokine is associated with a significant reduction in inflammation in this model.

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