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Publication date
2002

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Citation for published version (APA):

de Jong, Y. P. (2002). *Pathogenesis of immune-mediated murine colitis*.

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Chapter 6

Development of chronic colitis is dependent on the cytokine MIF

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ABSTRACT

The cytokine macrophage-migration inhibitory factor (MIF) is secreted by a number of cell types upon induction by lipopolysaccharide (LPS). Because colitis is dependent on interplay between the mucosal immune system and intestinal bacteria, we investigated the role of MIF in experimental colitis. MIF-deficient mice failed to develop disease, but reconstitution of MIF-deficient mice with wild-type innate immune cells restored colitis. In addition, established colitis could be treated with anti-MIF immunoglobulins. Thus, murine colitis is dependent on continuous MIF production by the innate immune system. Because we found increased plasma MIF concentrations in patients with Crohn's disease, these data suggested that MIF is a new target for intervention in Crohn's disease.

INTRODUCTION

Macrophage-migration inhibitory factor (MIF) is a ubiquitously expressed cytokine with a variety of mitogenic and pro-inflammatory functions^{1,2}. There are at least two distinct pro-inflammatory effects of MIF. First, MIF can "override" or counter-regulate the immunosuppressive effects of steroids³. Second, the addition of MIF enhances tumor necrosis factor (TNF) and nitric oxide production by lipopolysaccharide (LPS)-stimulated macrophages. Conversely, MIF-deficient peritoneal macrophages express less of these inflammatory mediators than wild-type macrophages^{4,5}. Because of its strong pro-inflammatory effects and because MIF has been implicated in the pathogenesis of septic shock⁵⁻⁷, we reasoned that this cytokine could be involved in the pathogenesis of inflammatory bowel diseases, that is, Crohn's disease and ulcerative colitis.

In Crohn's disease, as well as in several murine colitis models, inflammation appears to be an interplay between activated T helper 1 (T_H1) cells and antigen-presenting cells⁸⁻¹⁰. The functional importance of T_H1 cells is suggested by the effect of blocking interleukin 12 (IL-12), the cytokine necessary for the induction and maintenance of T_H1 cells, which appears to prevent chronic murine colitis^{11,12}. However, the role of antigen-presenting cells is much less understood. Their importance in experimental colitis is underscored by three sets of observations. First, only blocking the production of cytokines that are produced mainly by macrophages and other innate immune cells—for example, TNF and IL-6—prevented disease, whereas blocking T_H1 cytokines such as interferon- γ (IFN- γ) had little or no effect on experimental colitis¹¹⁻¹⁶. Second, the necessity of a set of well-defined receptor-ligand pairs in the interplay between T_H cells and professional antigen-presenting cells has been shown in several murine colitis models. Blocking interactions between CD40-CD154 and between CD134-OX40L ameliorated disease outcome¹⁷⁻²⁰. Third, bacteria are necessary for the induction of colitis in several experimental models. In addition, to maintain the disease status, T_H1 cells from colitic animals must recognize bacterial antigens^{21,22}.

Because in colitis bacterial products such as LPS chronically stimulate the immune system, we examined plasma MIF concentrations in patients with active Crohn's disease. The results prompted us to test whether MIF is involved in the development of experimental colitis. To this end, MIF-deficient mice were tested for their ability to develop disease in the acute

trinitrobenzene sulfonic acid (TNBS) model and the chronic CD45RB^{hi} T cell transfer model^{23,24}. The latter is a T_H1-dependent mouse model for colitis that shares many features with Crohn's disease, including the involvement of the distal ileum, so-called "skip lesions" and histological changes such as transmural inflammation with extensive ulcerations. In addition, the cell type that produced pathogenic MIF was further defined by studying bone marrow chimeras in the CD45RB^{hi} model. And finally, colitic mice were treated with anti-MIF immunoglobulins to define whether blocking MIF could interfere in established disease.

RESULTS

Elevated plasma MIF in Crohn's patients

To obtain an indication of whether MIF was involved in colitis, plasma concentrations were determined in patients with Crohn's disease. Plasma from patients with an average Crohn's disease activity index (CDAI)²⁵ of 327 ± 45 showed six fold higher MIF concentrations than plasma from healthy controls (Fig. 1). To correlate the high plasma MIF concentrations with the disease state, these same patients were tested after treatment with the anti-TNF Infliximab²⁶. One week after Infliximab treatment, when the average CDAI had decreased to 147 ± 23 , the concentration of MIF in the plasma had decreased concomitantly (that is, 62%) (Fig. 1). This suggested that active disease was responsible for the increased plasma MIF values.

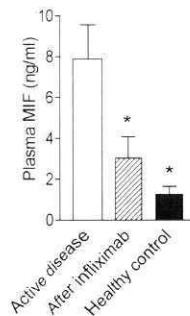


Figure 1. MIF plasma concentrations in patients with Crohn's disease decrease after successful treatment.

Plasma concentrations of MIF in patients with active Crohn's disease ($n=18$) were sixfold higher than in healthy individuals ($n=7$). After successful Infliximab treatment, MIF concentrations decreased 62% but were still slightly

increased over values in healthy controls ($P < 0.01$, between patients with Crohn's disease and healthy or successfully treated individuals).

To exclude the possibility that exogenous glucocorticoids were responsible for the increased plasma MIF concentrations in Crohn's disease³, patients with active Crohn's disease that were treated with glucocorticoids were compared to patients treated with other anti-inflammatory drugs. No difference in plasma MIF concentrations was observed between these two groups (data not shown).

MIF-deficient mice protected from colitis

To further study the role of MIF in colitis, acute and chronic mouse models were used. The acute TNBS model allows for a rapid evaluation of whether MIF is involved in colon inflammation, whereas the chronic CD45RB^{hi} transfer model permits an assessment of the relative contributions of pathogenic T cells and the reciprocal innate immune system. To this end, MIF-deficient (MIF^{-/-})⁵ mice were crossed with T cell-deficient recombination-activating gene 2-deficient (RAG-2^{-/-}) animals to generate double mutant MIF^{-/-}RAG-2^{-/-} mice. Then, CD45RB^{hi} T cells from H-2-matched wild-type or MIF^{-/-} mice were transferred into either MIF^{-/-}RAG-2^{-/-} or RAG-2^{-/-} recipients (Fig. 2a).

In the CD45RB^{hi} transfer model, two distinct phenotypes were observed in the four experimental groups. MIF^{-/-}RAG-2^{-/-} recipients remained largely unaffected by the transfer of disease-causing T cells, whereas RAG-2^{-/-} recipients lost weight (Fig. 2b). Whether donor T cells had the ability to produce MIF did not affect the weight loss (Fig. 2b). Similarly, MIF^{-/-}RAG-2^{-/-} recipients were less sick than RAG-2^{-/-} mice, as judged by the disease activity score, which included signs of wasting, diarrhea and colitis (Fig. 2c). The results were confirmed by statistical analysis of histological colitis scores (Fig. 2d). There was a marked difference in the pathology of colons from wild-type CD45RB^{hi} cells transferred into RAG-2^{-/-} and MIF^{-/-}RAG-2^{-/-} mice (Fig. 2e,f, respectively).

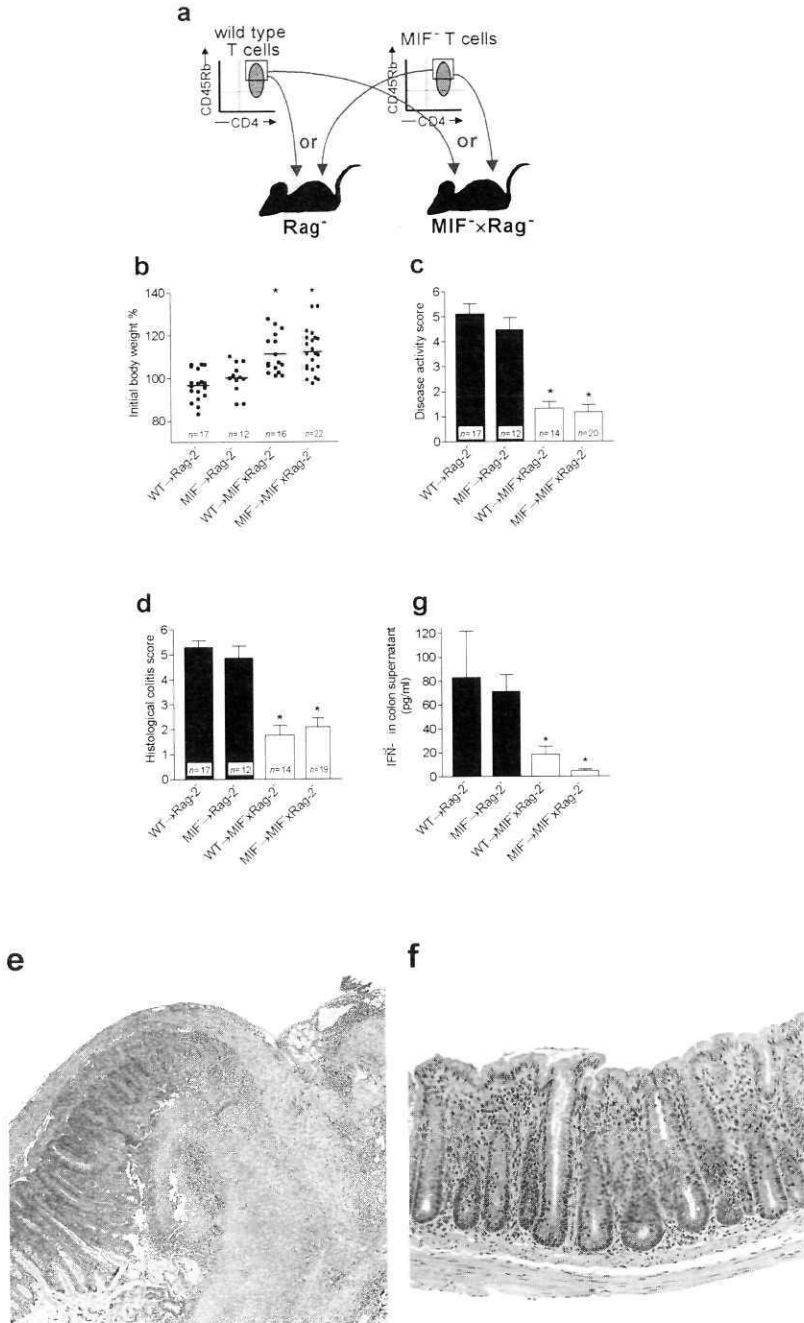


Figure 2. MIF-deficient mice do not develop severe colitis.

(a) Disease-causing CD45RB^{hi} T cells were taken from either wild-type (WT) or MIF^{-/-} (MIF) donor mice and transferred into either RAG-2^{-/-} (Rag-2) or MIF^{-/-}RAG-2^{-/-} (MIF-Rag-2) recipient mice to create four different experimental groups. (b) Four to six weeks after T cell transfer, MIF^{-/-}RAG-2^{-/-} recipients did not suffer weight loss as the RAG-2^{-/-} recipients did. (c) They also showed fewer clinical signs of colitis or (d) histological abnormalities in their colon. Disease severity was independent of the source of donor T cells, that is, from wild-type mice or from MIF-deficient mice. (e) WT→RAG-2^{-/-} mice showed severe colitis with crypt elongation (score 3), inflammatory infiltrate (score 3), ulcers and transmural inflammation, which was reminiscent of Crohn's disease. (f) In contrast, none of the MIF^{-/-}RAG-2^{-/-} recipients showed these severe changes, although some mice still had mild crypt elongation (score 1) and mild inflammation (score 1). (g) MIF^{-/-}RAG-2^{-/-} recipients, that were less sick than RAG-2^{-/-} recipients, had fewer activated T cells in their colon; this was reflected by the lower amounts of IFN- γ produced. (b-e) * $P < 0.001$, comparing columns 1 or 2 to columns 3 or 4. (e) * $P < 0.05$ comparing columns 1 or 2 to columns 3 or 4.

To determine whether MIF contributed to colitis in another model, MIF^{-/-} mice were infused with TNBS and colitis was scored 3 days later. Like MIF^{-/-}RAG-2^{-/-} mice in the CD45RB^{hi} transfer model, MIF^{-/-} mice were protected from TNBS-induced colitis. This was apparent from both the difference in end weights (MIF^{-/-} mice were 97.8±2.2% and wild-type mice were 90.9±4.0% of their start weight, $n=7$ per group, $P=0.16$) and the gross colitis score (MIF^{-/-} mice, 1.4±0.3; wild-type mice, 3.9±1.0, $P < 0.05$).

To investigate whether the absence of MIF had changed the T_H cell phenotype in the CD45RB^{hi} transfer model, T cells from the lamina propria of colitic mice were stained for intracellular cytokines. No difference was observed in the percentage IFN- γ - or TNF-producing T cells (data not shown). However, the number of cells in colons from MIF^{-/-}RAG-2^{-/-} mice was less than half of that found in RAG-2^{-/-} mice. Less IFN- γ was produced in colons from MIF^{-/-}RAG-2^{-/-} than from RAG-2^{-/-} recipients (Fig. 2g); in addition, no IL-4 could be detected (data not shown). Thus the absence of MIF impaired T cell expansion without affecting the T_H1 polarization.

Taken together, these data showed that MIF was required for disease development in both an acute and a chronic colitis model. In addition, colitis depended on MIF production in the recipient animal and not by the donor T cells.

MIF produced by innate immune cells

Having established that murine colitis was dependent on MIF from sources other than T cells, we sought to make a distinction between ubiquitously expressed MIF and MIF produced by the nonlymphocyte immune system. To this end, lethally irradiated MIF^{-/-}RAG-2^{-/-} mice were first transplanted with RAG-2^{-/-} bone marrow to generate bone marrow chimeras in which part of the nonlymphocyte hematopoietic cells could express MIF, whereas the rest of the mouse remained MIF-deficient. In control experiments, this resulted in approximately 50–75% of blood and spleen macrophages originating from donor bone marrow (see Methods). Four weeks later, the MIF^{-/-}RAG-2^{-/-} bone marrow chimeras received either wild-type or MIF^{-/-} CD45RB^{hi} T cells (Fig. 3a).

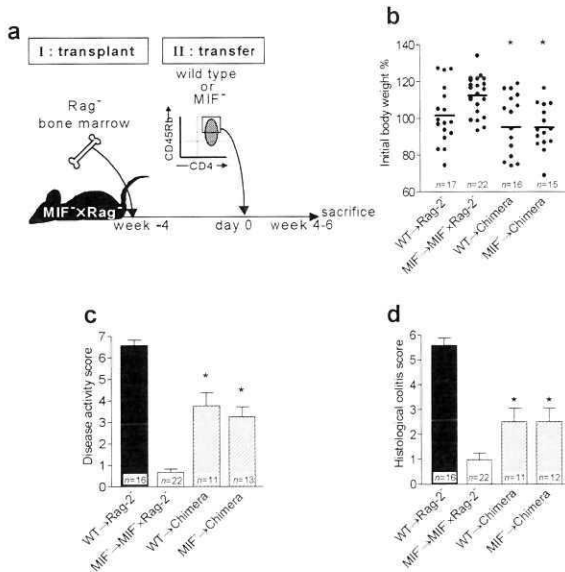


Figure 3. Experimental colitis depends on MIF produced by non-T hematopoietic cells.

(a) Four weeks before T cell transfer, MIF^{-/-}RAG-2^{-/-} recipients were transplanted with RAG-2^{-/-} bone marrow to create chimeras in which only nonlymphocyte hematopoietic cells produced MIF. (b) The presence of these MIF-producing cells caused disease, as judged by the ability of the bone marrow chimeras to lose as much weight as RAG-2^{-/-} recipients. In addition, the chimeras showed (c) significantly more clinical signs of colitis and (d) had more histological

abnormalities than nonchimeric $MIF^{-/-}$ RAG-2 $^{-/-}$ recipients did. (b-d) This was not dependent on MIF produced by T cells (see columns 3 and 4). (b-d) * $P < 0.001$ comparing column 2 to columns 3 or 4.

Reconstitution of part of the nonlymphocyte immune system with MIF-producing cells resulted in the same amount of weight loss as was observed with the wild-type transfer situation (Fig. 3b). Similarly, statistical analyses of the disease activity score (Fig. 3c) and histological colitis score (Fig. 3d) in the bone marrow chimeras were higher than those in $MIF^{-/-} \rightarrow MIF^{-/-}$ RAG-2 $^{-/-}$ mice. Notably, the control group in these experiments, the $MIF^{-/-} \rightarrow MIF^{-/-}$ RAG-2 $^{-/-}$ mice, showed even less disease than in the earlier set of experiments (Fig. 2). Severe pathology with extensive crypt elongation, crypt destruction and massive inflammatory infiltrate was found in some of the bone marrow chimeras (Fig. 4a). To assess whether MIF production by nonlymphocyte immune cells could be detected in inflamed tissue, colons of bone marrow chimeras that had received $MIF^{-/-}$ CD45RB hi T cells were stained for MIF. Large numbers of mononuclear cells produced MIF (Fig. 4b,c), which showed that nonlymphocyte immune cells produced this cytokine *in situ*.

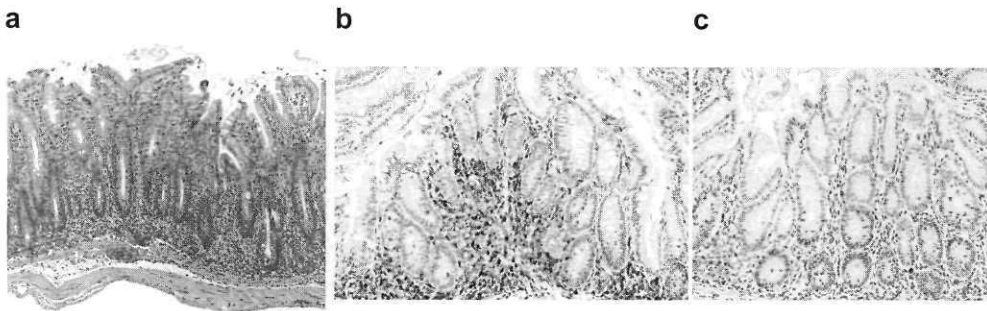


Figure 4. Differences in colon pathology between $MIF^{-/-}$ RAG-2 $^{-/-}$ and RAG-2 $^{-/-}$ mice after the introduction of colitis-inducing T cells.

(a) The presence of wild-type nonlymphocyte immune cells in bone marrow chimeras substantially increased pathological features with moderate inflammatory cell infiltration (score 2) and marked crypt elongation (score 2.5) in a number of mice. (b) Colons of chimeras that had received $MIF^{-/-}$ CD45RB hi T cells showed high numbers of MIF-producing mononuclear cells; (c) in contrast, no marked signal was picked up by control antibody staining.

Taken together, these observations showed that the impaired disease development in the MIF^{-/-} RAG-2^{-/-} recipient mice was predominantly due to the inability of nonlymphocyte hematopoietic cells to produce MIF.

Anti-MIF suppresses established colitis

The dependence of murine colitis on MIF produced by hematopoietic cells suggested that blockade of MIF might have a therapeutic effect in colitis. We therefore tested whether administration of a monoclonal antibody (mAb) to MIF prevented disease. In the first experiments, WT→RAG-2^{-/-} mice were treated with anti-MIF from the start of the experiment (Fig. 5a). As a positive control, anti-TNF administration was used because it suppresses chronic colitis when administered from the start of the experiment^{13,14}. We found that anti-MIF-treated mice developed only mild disease with a disease activity score of 2.1±0.3, similar to anti-TNF-treated animals (1.5±0.3), whereas control antibody-treated mice developed severe disease (4.7±0.7). This result was also apparent after histological examination of the colons (Fig. 5b). Thus, in concordance with the results obtained in MIF-deficient mice, anti-MIF substantially prevented disease development.

Having confirmed that anti-MIF treatment prevented the progression and severity of colitis, we attempted to ameliorate established disease with anti-MIF treatment in a second group of experiments. For this therapeutic treatment, the same transfer experiments were done but antibody was not administered until 21 days after T cell transfer (Fig. 5a). At the start of the treatment, WT→RAG-2^{-/-} mice had lost weight, on average 6.5% more than untransplanted RAG-2^{-/-} littermates ($P<0.001$), and some mice already had colitis (histology score 2.3±1.0, Fig. 5b). On day 21, the WT±RAG-2^{-/-} mice received either anti-MIF or control antibody for 15–17 days (Fig. 5a). Mice that received anti-MIF showed few signs of disease (disease activity score 1.3±0.5), whereas most control antibody recipients had severe disease (disease activity score 5.2±0.9, Fig. 5b). When the tissues were examined histologically, these differences were even more apparent. Mice that had moderate colitis at the start of the treatment (Fig. 5c) had little to no crypt elongation with very low numbers of infiltrating lymphocytes after anti-MIF administration (Fig. 5d). In contrast, most control antibody-treated mice progressed to severe inflammation (Fig. 5e). These experiments showed that anti-MIF was able to suppress established colitis.

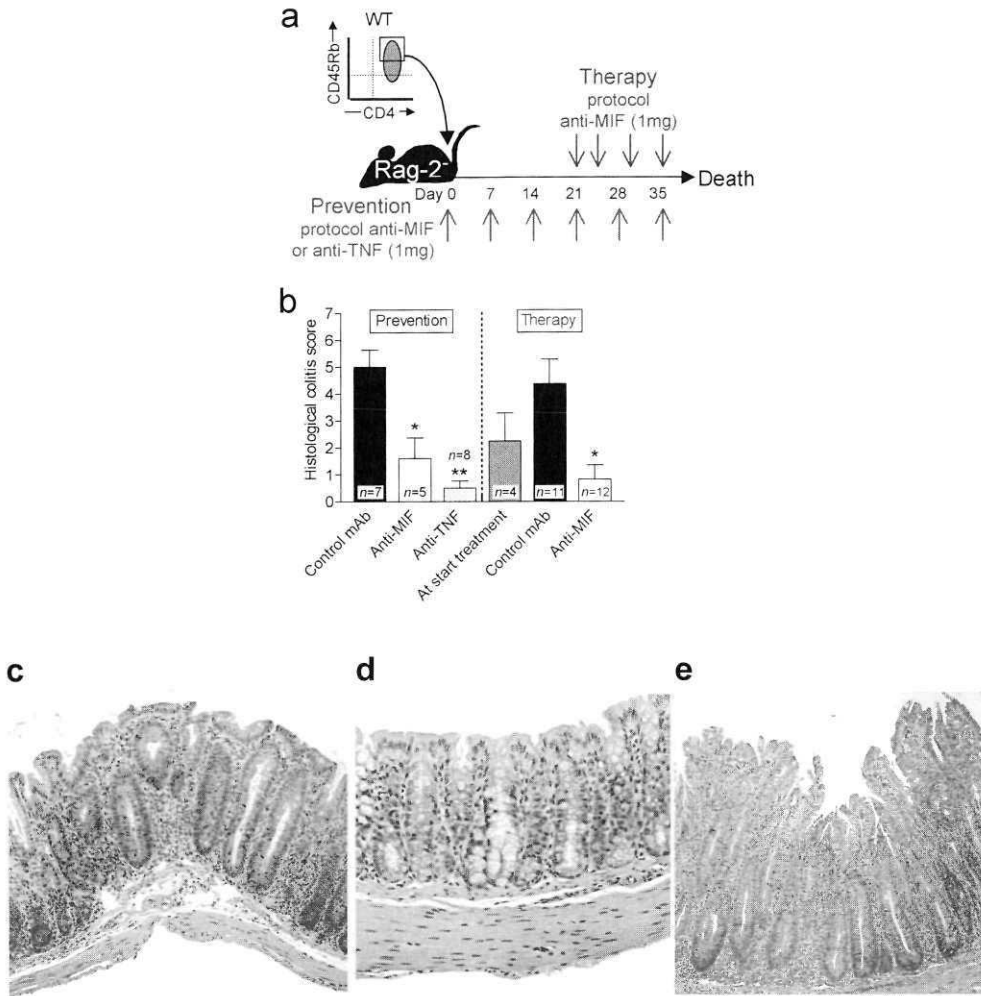


Figure 5. Anti-MIF therapy can abrogate the development of experimental colitis.

(a) Wild-type T cells were transplanted into RAG-2^{-/-} mice that received either 1 mg of anti-MIF or anti-TNF from the start (blue arrows, "prevention") or 1 mg of anti-MIF 3 weeks into the experiment (red arrows, "therapy"). (b) Preventive treatment with both anti-MIF and anti-TNF impaired the development of colitis. Therapeutic treatment with anti-MIF could suppress colitis to a degree that was comparable to that at the start of antibody treatment. * $P < 0.01$ comparing column 1 to column 2 and comparing column 5 to column 6; ** $P < 0.001$ comparing column 1 to column 3. (c) Histological examination showed that on day 21, before antibody therapy was started, some animals showed signs of

colitis. (d) When mice were treated with anti-MIF, they had little crypt elongation or goblet cell depletion and few inflammatory cells. (e) In contrast, most animals that received control antibody progressed to severe colitis with marked lymphocyte invasion and crypt elongation.

MIF activates macrophages

Because of the strong protection from disease by anti-MIF administration and the fact that this model depended on MIF produced by nonlymphocyte hematopoietic cells, the effect of MIF on peritoneal macrophages was examined. Addition of MIF alone was able to specifically induce IL-6 and IL-12 production (Fig. 6a). In contrast to earlier studies that stimulated peritoneal macrophages with LPS + MIF^{4,5}, no TNF production was observed with MIF alone (data not shown). Similarly, no IL-18 could be detected after addition of MIF (data not shown).

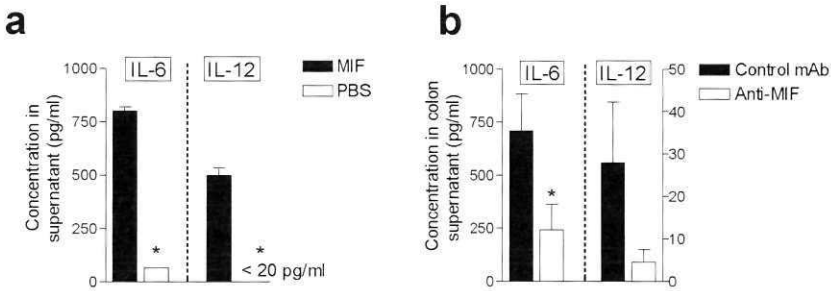


Figure 6. MIF induces IL-6 and IL-12 production in macrophages.

(a) Addition of recombinant MIF to peritoneal macrophages induced IL-6 and IL-12 production; these cytokines were produced in very low amounts after addition of PBS alone. * $P < 0.001$ (b) To test whether antigen-presenting cells in the colon also produced these cytokines in disease, colon supernatants from therapeutically anti-MIF- and control mAb-treated mice were measured. Both IL-6 and IL-12 production was impaired by anti-MIF administration.; * $P < 0.05$.

The lack of IL-12 production by macrophages could account for the disappearance of infiltrating lymphocytes in the therapeutically treated mice (Fig. 5d). To test whether professional antigen-presenting cells *in situ* also produced less IL-12, cytokines in total colon supernatant were measured. Like *in vitro*-stimulated macrophages, lower amounts of IL-6 and IL-12 were detected in colons from anti-MIF- than control antibody-treated mice (Fig. 6b). These results show that

therapeutically administered anti-MIF can interfere with IL-6 and IL-12 production in the colon, explaining the decreased numbers of activated T_H1 cells in anti-MIF-treated mice.

DISCUSSION

The data presented here show that MIF plays an essential role in the pathogenesis of murine colitis, a model for Crohn's disease. Although originally discovered as a factor secreted by T cells^{27, 28}, MIF is now known to be ubiquitously expressed with high amounts being secreted from the pituitary gland, T cells, macrophages and several other cell types¹. We have shown that neither MIF secreted by T cells nor MIF produced by the pituitary gland plays a dominant role in the CD45RB^{hi} colitis model. From our studies, we cannot specifically say what subset(s) of the innate immune system or from which organs contribute to experimental colitis. However, it is likely that macrophages, which produce high amounts of MIF⁴ and can be found abundantly in experimental colitis, are the hematopoietic cell type responsible for the restoration of disease in our bone marrow chimera studies.

Macrophages readily produce MIF upon activation with a number of different stimuli. Thus, IFN- γ , TNF and glucocorticoids as well as bacterial products such as LPS and exotoxins from Gram-positive bacteria are potent inducers of MIF^{4, 29}. Experimental colitis is dependent on aggravated T_H1 cells, which could be detected early in the disease¹⁸. These IFN- γ -producing T_H1 cells together with large amounts of bacterial products such as LPS in the lumen of the colon will provide ample stimulation to macrophages in the lamina propria. Based on our observations, we speculate that in response to these stimuli, gut macrophages produce MIF that has two distinct pro-inflammatory effects. On the one hand, MIF will further contribute to IL-6 and IL-12 production: these two cytokines, whose importance in the pathogenesis of experimental colitis has been shown^{11, 16}, will then activate other inflammatory cells, for example T_H1 cells and neutrophils. On the other hand, macrophage-produced MIF—in combination with LPS from colonic bacteria—will augment TNF and nitric oxide production by innate immune cells.

Treatment for Crohn's disease depends largely on the chronic use of immunosuppressive reagents that can result in severe side effects. In the last 5 years, a number of more specific targets for

potential treatment have been discovered in murine colitis models^{11, 14, 16, 30}. This has so far lead to the introduction of an anti-TNF treatment for patients with Crohn's disease that, although effective, does not suppress disease in all patients²⁶. We have shown here that anti-MIF doesn't only prevent but also suppresses established murine colitis. This contrasts with anti-TNF treatment in mice, with which we were never able to show any therapeutic effectiveness even though anti-TNF was able to prevent disease^{13, 14}. A similar observation has been made with regard to the treatment of established peritonitis after coecal ligation and puncture in mice: in contrast to anti-IL-1 or anti-TNF, anti-MIF increased the survival of mice when administered 8 h after the induction of septic shock³¹. Along with the data reported here, these observations could be explained by the hypothesis that anti-MIF is more potent at interfering with a wider array of pro-inflammatory mediators, for example, both the IL-6 and IL-12 as well as the TNF and nitric oxide pathways. Further investigations on how MIF signals and how its gene expression is regulated should provide answers to these speculations.

Based on the potent immunosuppressive effects of anti-MIF in mice and the increased plasma concentrations in patients, we speculate that intervention in MIF signaling could form a future target for the treatment of Crohn's disease.

METHODS

Patients. Eighteen patients (14 female, 4 male) with therapy-refractory active Crohn's disease (CDAI>220) received a single intravenous administration of the anti-TNF Infliximab²⁶ (5 mg/kg) as a 2 h infusion. Blood samples were obtained by venipuncture in citrate decoagulation tubes before Infliximab infusion and 1 week afterwards. Fourteen Prednisone-treated patients (CDAI=276±49) were compared to 20 patients (CDAI= 235±78) that had not received glucocorticoid therapy (6-mercaptopurine or azathioprine, *n*=9; methotrexate, *n*=2; no anti-inflammatory drugs, *n*=9). Patients had given informed consent for these studies.

Mice. MIF-deficient mice⁵ on a B6×129 background were crossed with T cell-deficient 129 RAG-2-deficient mice (Taconic, Germantown, NY). F₂ mice with the following genotypes: MIF^{-/-}RAG-2^{-/-}, MIF^{+/-}RAG-2^{-/-} (RAG-2^{-/-}), MIF^{-/-}RAG-2^{+/-} (MIF^{-/-}) and MIF^{+/+}RAG-2^{+/+} (wild-type) were interbred and used for experiments. For antibody treatment experiments, 129SvEv wild-

type CD45RB^{hi} T cells were injected into 129 RAG-2^{-/-} mice (both from Taconic). For TNBS experiments, MIF^{-/-} mice were compared to (B6×129)F₁ wild-type mice (Jackson Labs, Bar Harbor, ME).

Colitis models and bone marrow chimeras. Acute colitis was assessed 3 days after infusion of TNBS. In short, 2 mg of TNBS (Sigma, St Louis, MO) in 50% ethanol was administered in a 100 µl enema²⁴. The CD45RB^{hi} transfer model was generated as described^{18,23} with minor modifications. Sorted CD4⁺CD45RB^{hi} cells, which were 96–99% pure, were resuspended at 2×10^5 in 400 µl of PBS and injected into the tail veins of recipient mice that were 7–10 weeks of age. To make chimeras, 3–4-week-old MIF^{-/-} RAG-2^{-/-} and RAG-2^{-/-} mice were total body-irradiated (10 Gy) before receiving 5×10^6 erythrocyte-depleted bone marrow cells that were extracted from RAG-2^{-/-} mice as described¹¹. Control mice for chimera experiments received autologous bone marrow, that is MIF^{-/-}RAG-2^{-/-} mice received bone marrow from MIF^{-/-}RAG-2^{-/-} mice and RAG-2^{-/-} mice bone marrow from RAG-2^{-/-} donors; 0–33% of mice did not survive this procedure. As an indication of the reconstitution efficiency, cells from control MIF^{-/-}RAG-2^{-/-} and RAG-2^{-/-} mice that had received H-2^d BALB/c RAG-2^{-/-} (Taconic) bone marrow were analyzed. In these mice, $62.5 \pm 18.8\%$ of CD11b⁺ peripheral blood cells and $68.5 \pm 20.0\%$ of CD11b⁺ splenocytes expressed H-2^d, which showed that in a control experiment ± 50 –75% of macrophages and granulocytes in blood and spleen originated from the donor bone marrow.

Cell preparation and stimulation. T cells, isolated from lamina propria, were stimulated with 10 µg/ml of platebound anti-CD3~ (clone 145-2C11, PharMingen, San Diego, CA) for 4 h in RPMI with 10% fetal calf serum (FCS) for intracellular staining¹¹. Resting peritoneal macrophages were isolated by standard techniques and stimulated with either 1 µg/ml (data not shown) or 10 µg/ml of MIF or PBS containing an equivalent amount of LPS. For cytokines in colon supernatant, 100 mg of colon was extensively washed in PBS and cultured in 2.5 ml of RPMI with 10% FCS for 36 h.

Antibodies and cytokines. For *in vivo* studies, anti-mouse MIF (clone III.D.9) and control isotype IgG1 (clone Hb49) were prepared from ascites and purified by anion exchange after a saturated ammonium sulfate cut. Anti-mouse TNF (clone V1q) was provided by D. Shealy (Centocor, Malvern, PA). For MIF immunostaining, a polyclonal rabbit anti-MIF and rabbit control serum were used. Highly purified recombinant MIF was produced as described³² and contained 22 pg of LPS per milligram of MIF. Surface and cytoplasmic staining and

fluorescence-activated cell sorter analysis were done as described¹¹ with the use of isotype-matched control antibody staining as the zero value. The following antibodies from PharMingen were used: anti-CD4 (clone RM4-5), anti-IFN- γ (clone XMG1.2), anti-TNF (clone MP6-XT22) and rat isotype IgG1 control. Red⁶⁷⁰-streptavidin was from Gibco-BRL (Grand Island, NY). IFN- γ , IL-4 (Endogen, Woburn, MA) IL-6, IL-12p70 and IL-18 (PharMingen) were detected with standard ELISA kits.

Disease monitoring and scoring. For the TNBS model, mice were weighed daily and scored by two independent investigators as described²⁴. CD45RB^{hi} T cell recipients were weighed twice a week and monitored for appearance and signs of soft stool and diarrhea. When some mice were moribund, all mice in that experiment were killed and scored on a disease activity score that was the sum of four parameters: hunching and wasting were scored 0 or 1, colon thickening 0–3 and stool consistency 0–3. Three tissue samples from proximal, middle and distal colon were prepared for histological staining with hemoxylin and eosin. For the histological colitis score, the area most affected was scored on a scale of 0–3 in each of three criteria: cell infiltration, crypt elongation and the number of crypt abscesses. Histological grades were assigned in a blinded manner by one pathologist.

Statistical analysis. Weight and cytokine data were analyzed with Prism 3 software (GraphPad, San Diego, CA). *P* values were calculated with a nonpaired *t*-test, except for the Crohn's patient plasma values, which were analyzed with a paired *t*-test. Discrete disease activity and histological scores were analyzed with Mantel-Haenszel χ^2 test with SAS software. Data are mean \pm 1 s.e.m. values.

ACKNOWLEDGEMENTS

We thank D. Podolsky for critically reviewing the manuscript, A. Meinhardt for technical advice and G. Lin for assistance with genotyping mice. Supported by grants from the National Institutes of Health (DK52510 to CT; DK47677 to AKB; AI25532 to JRD; DK43351 to CT and AKB) and the Crohn's and Colitis Foundation of America (YPJ).

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