Cutting Edge: Macrophage Migration Inhibitory Factor Is Necessary for Progression of Experimental Autoimmune Encephalomyelitis

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Macrophage migration inhibitory factor (MIF) has been shown to up-regulate proinflammatory cytokine production and antagonize glucocorticoid activity. These properties suggest that MIF may serve as a therapeutic target in the treatment of inflammatory autoimmune disease. MIF is a pleiotropic cytokine that is produced during inflammatory responses by many cells, including activated T cells, macrophages, and pituitary cells (1–3). MIF is constitutively expressed and is stored and released from intracellular pools. Unlike other proinflammatory cytokines, MIF secretion is induced, rather than inhibited, by glucocorticoid release (4).

Evidence suggests that glucocorticoids can influence the clinical course of inflammatory autoimmune diseases. Steroids have been widely used to treat relapses of multiple sclerosis (MS) and high doses of glucocorticoids administered during murine experimental autoimmune encephalomyelitis (EAE) relapses promote amelioration of EAE (5, 6). We have previously reported that mice producing high levels of corticosterone (CORT) in- creased MIF secretion. MIF blocks CORT abrogated this effect. Glucocorticoids have been shown to potentiate a shift from Th1 cytokine production (IL-2 and IFN-γ) to Th2 cytokines (IL-4 and IL-10), which have protective effects in MS and EAE (7–10).

Studies in MS and EAE suggest that MIF may play a role in regulation of disease severity as well as in exacerbations of disease. Levels of MIF measured in the cerebrospinal fluid (CSF) of MS patients during relapse were significantly elevated relative to levels during remission (11). Increased levels of MIF were also found in the plasma of patients with sepsis and inflammatory bowel disease and were correlated with the severity of symptoms (12, 13). Importantly, MIF−/− mice were shown to be resistant to sepsis and inflammatory bowel disease (13–15). In EAE, Denkinger et al. (16) reported that anti-MIF Ab treatment reduced the severity of clinical signs and accelerated recovery. Blockade of MIF activity by Ab impaired the migration of neuroantigen reactive T cells to the CNS by down-regulation of VCAM-1. In our study, a role for MIF in the acute as well as the progressive phases of EAE was examined using mice genetically depleted of MIF. We show here that MIF promotes EAE progression via glucocorticoid antagonism and regulation of inflammatory cytokine production.

Materials and Methods

Induction and assessment of EAE

MIF−/− mice (B6;129S4-Mif−/−), which were generated as previously described (14), or wild-type (WT) mice of the same strain combination (B6129) (Taconic Farms) were injected s.c. over four sites on the back with 100 μl containing 200 μg of myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide (Princeton Biomolecules) combined with complete Freund’s adjuvant (containing 200 μg of heat-killed Mycobacterium tuberculosis Jamaica strain). Pertussis toxin (200 ng) (List Biological Laboratories) was injected i.p. in 0.2 ml of PBS at the time of immunization and 48 h later. All animals were observed daily for clinical signs and scored as described previously (5). Brains and spinal cords were examined for inflammatory cell infiltration by H&E staining. All animal procedures were performed in accordance with approved university protocols.

Measurement of CORT levels

Blood was obtained from the retroorbital plexus of anesthetized mice using heparinized tubes on days 10, 17, and 25 after MOG peptide immunization at the same time each day. Serum was collected and stored at −20°C. CORT levels were determined by a validated CORT assay provided by PRISM Diagnostics (Waltham, MA). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. © 2005 by The American Association of Immunologists, Inc. 0022-1767/05/$20.00.
Cumulative periods of disease worsening in the MIF\(^{-/-}\) group was significantly lower compared with the WT mice, although the overall incidence of disease was not different between the two groups. We also examined the character and severity of CNS inflammation. Infiltrates were observed in the CNS of WT controls at day 17 but not in MIF\(^{-/-}\) mice. Interestingly, the extent of CNS inflammation was similar at day 25 (data not shown). Denkinger et al. (16) also showed a significant decrease in CNS infiltration in anti-MIF-treated mice compared with untreated controls. Our data suggest that MIF is less important for the induction phase of EAE but is a necessary factor for disease progression.

Several theories have been advanced to explain the transition from acute or progressive disease to remission or recovery. First, increased levels of steroid hormones, whether produced endogenously or introduced exogenously, can lead to amelioration of disease flares (5, 18). Because MIF has been shown to be a potent antagonist of glucocorticoid activity, it is possible that the absence of MIF in MIF\(^{-/-}\) animals allows for elevations in glucocorticoid hormones contributing to protection from disease. We measured CORT in the serum of MIF\(^{-/-}\) and WT mice at various times during the EAE disease course: before neuroantigen immunization (day −1), before the onset of clinical signs (day 10), during acute disease (day 17), and during the progressive phase of EAE (day 25). MIF\(^{-/-}\) animals display significantly higher levels of serum CORT compared with WT mice before and during acute EAE (Fig. 2), with elevations of CORT appearing as early as 3 days postimmunization (data not shown). To rule out the possibility that the dramatic difference in CORT levels between the MIF\(^{-/-}\) and WT animals could be due to a naturally increased hormone level in the MIF\(^{-/-}\) animals, levels of CORT in the serum of unimmunized MIF\(^{-/-}\) and WT mice were tested. Both MIF\(^{-/-}\) and WT mice had negligible basal levels of serum CORT (Fig. 2). As with high-dose glucocorticoid therapy, it is possible that the increased levels of endogenous glucocorticoids may have rendered MIF\(^{-/-}\) animals resistant to glucocorticoids effects. To determine whether animals were CORT resistant, we tested the proliferative capacity of mitogen-stimulated spleen cells from WT and MIF\(^{-/-}\) animals in the presence of a range of doses of CORT. Lymphocytes from both MIF\(^{-/-}\) and WT groups showed decreased proliferative responses upon addition of CORT (data not shown). These results suggest that MIF is contributing to disease exacerbations via glucocorticoid antagonism.

A second theory to explain the shift from acute or progressive disease to a state of recovery or remission involves the balance of

![Fig. 1](image-url). MIF\(^{-/-}\) mice exhibit significantly reduced signs of progressive EAE. WT (○) and MIF\(^{-/-}\) (△) mice were immunized with MOG\(_{35-55}\) peptide + CFA and pertussis toxin and were scored daily for signs of EAE. Ten mice per group were used, and these data are representative of four separate experiments.

### Results and Discussion

Increased levels of MIF can be detected during periods of inflammation and exacerbation of disease; notably, elevation in MIF was detected in the CSF of MS patients during disease relapse but not during remission (11–13). To explore a causative role for MIF in relapse or progression of disease, we studied MIF during the course of EAE. B6129 WT mice and MIF-deficient mice (MIF\(^{-/-}\)) were immunized with MOG\(_{35-55}\) and adjuvants. As shown in Fig. 1, WT mice exhibit a progressive course of EAE. In contrast, MIF\(^{-/-}\) mice show signs of disease during the acute disease period but recover around day 20 postimmunization. As shown in Table 1, MIF\(^{-/-}\) mice show no difference in clinical score relative to controls during the acute disease period. However, the cumulative disease score is significantly lower in the MIF\(^{-/-}\) group compared with the WT group. Furthermore, the number of cumulative periods of disease worsening in the MIF\(^{-/-}\) group was significantly lower compared with the WT mice, although the overall incidence of disease was not different between the two groups. We also examined the character and severity of CNS inflammation. Infiltrates were observed in the CNS of WT controls at day 17 but not in MIF\(^{-/-}\) mice. Interestingly, the extent of CNS inflammation was similar at day 25 (data not shown). Denkinger et al. (16) also showed a significant decrease in CNS infiltration in anti-MIF-treated mice compared with untreated controls. Our data suggest that MIF is less important for the induction phase of EAE but is a necessary factor for disease progression.

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### Table 1: A significant reduction in disease severity and progression in MIF\(^{-/-}\) mice

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Day of Onset</th>
<th>Acute CDS(^b)</th>
<th>CDS</th>
<th>No. of Cumulative Periods of Disease Worsening(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT 9/10 (90%)</td>
<td>12.8 ± 1.2</td>
<td>14.1 ± 0.2</td>
<td>66.9 ± 0.1</td>
<td>22.0 ± 0.4</td>
</tr>
<tr>
<td>MIF(^{-/-}) 10/10 (100%)</td>
<td>14.0 ± 0.8</td>
<td>11.2 ± 0.2</td>
<td>31.2 ± 0.1(^d)</td>
<td>8.0 ± 0.3(^e)</td>
</tr>
</tbody>
</table>

\(^a\) WT mice and MIF\(^{-/-}\) mice were immunized with MOG\(_{35-55}\) + CFA and pertussis toxin and were scored daily for EAE signs for 45 days. Representative results from four experiments are shown.

\(^b\) Cumulative disease score (CDS) is defined as the sum of daily clinical scores for individual animals.

\(^c\) Period of disease worsening is defined as a >1 increase in clinical score for 2 consecutive days.

\(^d\) \(p < 0.05\) by Student’s t test.

\(^e\) \(p < 0.004\) by Student’s t test.
Th1 and Th2 cytokines. A progressive course of EAE is correlated with the production of proinflammatory Th1 cytokines such as IFN-γ and TNF-α (19, 20). In contrast, Th2 cytokines, such as IL-4 and IL-10, in the CNS as well as in the periphery have been associated with reduced disease severity and recovery (21–23). Because MIF up-regulates proinflammatory Th1 cytokines, it is possible that the lack of MIF would promote a shift to a more Th2-like cytokine profile. We analyzed cytokine production in MIF−/− and WT mice before the onset of disease, during acute EAE, and during the progressive phase of disease. As shown in Fig. 3, lymphoid cells from MIF−/− animals produced IL-4 at a much higher frequency than WT lymphoid cells during acute EAE (Fig. 3A) in response to MOG35–55 stimulation. In addition, we also observed an elevated frequency of cells producing IL-10 during the progressive phase of EAE (Fig. 3B). In contrast, levels of proinflammatory cytokines such as IFN-γ (Fig. 4A) and TNF-α (Fig. 4B) were significantly decreased in MIF−/− animals before the onset of disease and during acute disease, respectively. In addition, IL-6 (Fig. 4A) and TNF-α (Fig. 4B) levels in the MIF−/− mice were significantly decreased during disease progression. These data show that MIF may be influencing disease progression through the up-regulation of Th1 cytokines.

When analyzing the clinical, hormonal, and cytokine data together, two events seem to be critically important. First, the lack of MIF antagonism of CORT leads to increased levels of CORT beginning soon after MOG35–55 peptide immunization. The increase in CORT levels leads to the second important event, which is a profound decrease in IFN-γ by day 17 p.i. A reciprocal relationship between CORT and IFN-γ is also seen later when decreasing levels of CORT in the MIF−/− animals at day 25 correlate with an elevation in IFN-γ. Interestingly, IFN-γ has also been shown to be important in the control and regulation of EAE exacerbation (24–26). Elevated levels of glucocorticoids during acute EAE in the MIF−/− mice also likely contribute to increases in IL-10, which is important for EAE recovery. In addition, IL-6 and TNF-α, two cytokines that stimulate the synthesis of glucocorticoids and in turn are inhibited by glucocorticoids (27, 28), are significantly decreased after CORT elevation in MIF−/− animals. Our data indicate that the relationship between MIF and glucocorticoids may have a significant influence on the course of disease and that the elevation in glucocorticoids produces an alteration in disease progression due to modulation of cytokine production. Glucocorticoids are an essential part of the homeostatic mechanism regulating the immune and endocrine systems. Those individuals with chronic inflammatory disorders, such as MS, have been successfully treated with glucocorticoids for a finite period of time. However, no long-term benefit has been identified and dose-limiting side effects are consequences of long-term glucocorticoid therapy (29). In EAE, exogenous administration of glucocorticoids results in suppression of EAE, a decrease in lymphoid cell numbers, and a decrease in Th1 cytokine production (5). In this study, we...
propose that a relationship exists between MIF and glucocorticoid hormone levels. It has been shown previously by Fingerle-Rowson et al. (30) that exogenous glucocorticoids administered to rats induce increased levels of MIF in the blood and tissue. Conversely, adrenalectomized rats showed no dysregulation in MIF production, indicating that MIF is affected by the presence of increased glucocorticoids but is not affected by the absence of endogenous glucocorticoids (30). To further understand the effects of MIF on EAE, the levels of MIF during the normal course of disease as well as in animals treated with CORT need to be determined. Although levels of MIF in the CSF have been shown to be elevated in patients during MS relapse, the levels of MIF after glucocorticoid treatment have not been determined in relapsing or progressive MS patients.

Collectively, our findings suggest that MIF plays a crucial role in mediating the progression of EAE. The mechanism by which MIF exerts its effects appears to be via glucocorticoid antagonism and by promotion of Th1 cytokines such as IL-6, IFN-γ, and TNF-α. Our data also suggest a significant role for glucocorticoids and Th2 cytokines in the pathway to EAE remission and recovery. High levels of glucocorticoids, in the absence of MIF, influence both the clinical disease course and cytokine profiles in EAE affected animals. Therapeutic strategies targeting MIF regulation may provide new insights into the treatment of MS.

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Disclosures
The authors have no financial conflict of interest.

References