The Journal of Immunology

Cutting Edge: Deficiency of Macrophage Migration Inhibitory Factor Impairs Murine Airway Allergic Responses

Bing Wang, Xiaozhu Huang, Paul J. Wolters, Jiusong Sun, Shiro Kitamoto, Min Yang, Richard Riese, Lin Leng, Harold A. Chapman, Patricia W. Finn, John R. David, Richard Bucala and Guo-Ping Shi

J Immunol 2006; 177:5779-5784; ;
doi: 10.4049/jimmunol.177.9.5779
http://www.jimmunol.org/content/177/9/5779

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Deficiency of Macrophage Migration Inhibitory Factor Impairs Murine Airway Allergic Responses[1]

Bing Wang,[2] Xiaozhu Huang,[2†] Paul J. Wolters,[†] Jiusong Sun,* Shiro Kitamoto,* Min Yang,*[‡] Richard Riese,* Lin Leng,[§] Harold A. Chapman,[†] Patricia W. Finn,[3]* John R. David,[¶] Richard Bucala,[§] and Guo-Ping Shi[4]**

Increased levels of macrophage migration inhibitory factor (MIF) in serum, sputum, and bronchioalveolar lavage fluid (BALF) from asthmatic patients and time/dose-dependent expression of MIF in eosinophils in response to phorbol myristate acetate suggest the participation of MIF in airway inflammation. In this study, we examined inflammation in OVA-sensitized mouse lungs in wild-type and MIF-deficient mice (**MIF$^{-/-}$**). We report increased MIF in the lung and BALF of sensitized wild-type mice.** MIF$^{-/-}$ **mice demonstrated significant reductions in serum IgE and alveolar inflammatory cell recruitment. Reduced Th1/Th2 cytokines and chemokines also were detected in serum or BALF from** MIF$^{-/-}$ **mice. Importantly, alveolar macrophages and mast cells, but not dendritic cells or splenocytes, from** MIF$^{-/-}$ **mice demonstrated impaired CD4$^+$ T cell activation, and the reconstitution of wild-type mast cells in** MIF$^{-/-}$ **mice restored the phenotype of OVA-induced airway inflammation, revealing a novel and essential role of mast cell-derived MIF in experimentally induced airway allergic diseases. The Journal of Immunology, **2006, 177: 5779–5784.**

Macrophage migration inhibitory factor (MIF)[5] was one of the first cytokines described, based on its role in delayed-type hypersensitivity (1). Subsequent investigations demonstrated its participation in various human diseases (2). Although MIF was initially thought to be expressed primarily in T lymphocytes (1), recent investigations have revealed that other tissues or cell types such as pituitary cells, astrocytes, macrophages (Mϕ), smooth muscles cells (SMC), endothelial cells, and mast cells also express this cytokine under inflammatory (3) or antigenic stimulation (4). An involvement of MIF in allergic responses was suggested from observations of increased MIF levels in bronchioalveolar lavage fluid (BALF) from asthmatic patients (5). Eosinophils, the hallmark cells of asthma, release MIF in a time- and concentration-dependent fashion in response to phorbol myristate acetate (5). Further, MIF levels are significantly higher in serum and sputum from asthmatic patients and correlate with the production of eosinophil cationic protein (6), a marker for eosinophilic inflammation of airways in bronchial asthma. It is uncertain, however, whether MIF in asthmatic serum or sputum is a nondiscriminate marker of airway inflammation or directly participates in asthma pathogenesis. In this study, we examined the role of MIF deficiency in a murine model of allergic lung inflammation and demonstrate a pathogenic role for mast cell-derived MIF in airway inflammation and allergy.

Materials and Methods

Animal protocol

BALB/c MIF-deficient mice C.129S4(B6)-Mif^{tm1Dvd} (*MIF*$^{-/-}$) were generated by backcrossing *MIF*$^{-/-}$ mice in C57BL/6J background (7) to BALB/c mice (>12 generations). To induce airway allergic responses, a 12-wk-old mouse was immunized i.p. with 50 μg of OVA in 10 mg of $Al(OH)_3$ on days 0, 7, and 14, followed by intranasal challenge with 1 mg of OVA on days 21, 22, and 23 (8). On day 24, mouse serum collection, BALF harvesting, total leukocyte counting, and Diff-Quik staining (cell typing) were performed as detailed elsewhere (8).

Serum and BALF IgE and cytokine level determination

Serum total IgE was determined with a sandwich ELISA (8). Serum and BALF eotaxin (Cell Sciences), MCP-3 (Cell Sciences), IL-4 (Pierce), IL-5 (Pierce), IL-13 (PeproTech), IFN-γ (BioSource International), TGF-β1 (BioSource International), and TNF-α (BioSource International) were determined using ELISA kits according to the manufacturers' instructions. MIF was measured by a murine-specific, capture ELISA (detection limit of 0.16 ng/ml) (9).

*Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; †Department of Medicine, University of California, San Francisco, CA 94143; ‡Department of Rheumatology, Nanfang Hospital, Southern Medical University, Guangzhou, China; §Department of Internal Medicine, Yale University, New Haven, CT 06520; ¶Department of Immunology and Infectious Disease, Harvard School of Public Health, Boston, MA 02115

Received for publication October 19, 2005. Accepted for publication August 23, 2006.

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.

[1] This work was partially supported by a grant from the Sandler Award for Basic Science (to G.-P.S.) and National Heart, Lung, and Blood Institute Grants BI HL60942 (to G.-P.S.), HL075026 (to P.W.F.), and HL48621 (to H.A.C.), and National Institutes of Health Grant AR49610 (to R.B.).

[2] B.W. and X.H. contributed equally to this study.

[3] Current address: Department of Medicine, University of California, San Diego, CA 92103.

[4] Address correspondence and reprint requests to Dr. Guo-Ping Shi, Cardiovascular Medicine, NRB-7, 77th Avenue Louis Pasteur, Boston, MA 02115. E-mail address: gshi@rics.bwh.harvard.edu

[5] Abbreviations used in this paper: MIF, migration inhibitory factor; BALF, bronchioalveolar lavage fluid; BMMC, bone marrow-derived mast cell; DC, dendritic cell; Mϕ, macrophage; PMN, polymorphonuclear; SMC, smooth muscle cell.

0022-1767/06/$02.00

Preparation of lung dendritic cells, splenocytes, $CD4^+$ T cells, and mast cells

Mouse lung dendritic cells (DC) were isolated as described previously (10). DC were verified by FACS analysis for CD11c, DEC-205, I-A^d, CD11b, CD14, F4/80, and Gr-1. Isolated lung DC showed positive staining for the cell surface markers of CD11c, DEC-205, and I-A^d, and negative cell surface staining for CD11b, CD14, F4/80, and GR-1. Splenocytes and $CD4^+$ T cells were isolated from C57BL/6J or DO11.10 mouse spleens as we reported previously (11). Mast cells were derived from bone marrow from both $MIF^{+/+}$ and $MIF^{-/-}$ mice in the presence of murine IL-3 (PeproTech) as previously reported (12). PWM-stimulated mast cells were prepared by differentiating bone marrow cells in medium containing PWM-stimulated, splenocyte-conditioned medium as described (13).

Mast cell reconstitution

Bone marrow-derived mast cells (BMMC) from $MIF^{+/+}$ mice were washed with calcium-free PBS and resuspended in PBS. Cells were immediately injected into the tail veins of 5-wk-old $MIF^{-/-}$ mice (1×10^7 cells/mouse). These mice were used in the allergic response model 7 wk after BMMC reconstitution, when BMMC appeared in most recipient mouse lungs (14). Reconstituted BMMC were examined using rabbit anti-murine MIF polyclonal Ab-mediated immunostaining.

Preparation of mouse MIF polyclonal Abs

Full-length mouse MIF cDNA was subcloned into pCRT7/NT-TOPO vector and expressed in BL21 (Invitrogen Life Technologies). Poly(His)$_6$-MIF fusion proteins were purified over a His•Bind Quick column (Novagen), and purified proteins were used for immunizing rabbits to produce polyclonal Abs (Proteintech Group). Ab specificity was verified by Mϕ lysate immunoblot analysis and immunostaining of lung paraffin sections from both $MIF^{+/+}$ and $MIF^{-/-}$ mice.

Lung histology

Mouse lungs were removed and fixed in 10% buffered formalin. Paraffin sections (4 μm) were prepared and used for immunostaining of MIF (rabbit anti-mouse MIF polyclonal Ab; 1/2000) and cell proliferation marker Ki67 (rabbit anti-mouse polyclonal Ab; 1/1000, NovoCastra).

MLR and Ag presentation

MLR was carried as previously described (11) in RPMI 1640 on 96-well plates with 2×10^5 T cells per well from C57BL/6J mice and different amounts APC, including lung DC, alveolar Mϕ, resting BMMC, and PWM-stimulated BMMC from BALB/c mice. Ag presentation was performed with 2×10^5 DO11.10 T cells mixed with 2 μg/ml OVA$_{323–339}$ peptide and different amount of APC. Plates were incubated for 2 days followed by the addition of 1 μCi of [^{3}H]thymidine and another 6 h of incubation before harvesting.

Results and Discussion

MIF deficiency reduces airway allergic responses

In accord with what has been found in human asthmatic patients (5), the BALF of OVA-sensitized $MIF^{+/+}$ mice contained higher amounts of MIF than the saline-sensitized controls (Fig. 1*A*), whereas the BALF of OVA-sensitized $MIF^{-/-}$ mice contained no MIF (data not shown). Immunohistochemical analysis using an anti-murine MIF polyclonal Ab also revealed increased production of MIF in the airway epithelial cells and in peribronchial inflammatory cells, including mast cells. There was a clearly increased density of peribronchial inflammatory cells in the experimentally induced lungs (Fig. 1*B*). These initial data suggested a correlation between MIF expression and murine airway inflammation and that a lack of MIF reduces such allergic responses. This hypothesis was examined using $MIF^{-/-}$ mice. In the same model, OVA-sensitized $MIF^{-/-}$ mice showed evidence of reduced total leukocyte infiltration (Fig. 1*C*), including eosinophils (Fig. 1*D*) and polymorphonuclear (PMN) cells (Fig. 1*E*) in the BALF when compared with those in OVA-sensitized $MIF^{+/+}$ mice. The reductions in Mϕ (Fig. 1*F*) and lymphocytes (Fig. 1*G*) were not statistically significant (ANOVA *t* test), suggesting that MIF is not essential for Mϕ or lymphocyte recruitment but plays a more dominant role in lymphocyte activation and cytokine production. Eosinophils play a critical role in allergic airway remodeling (15) and

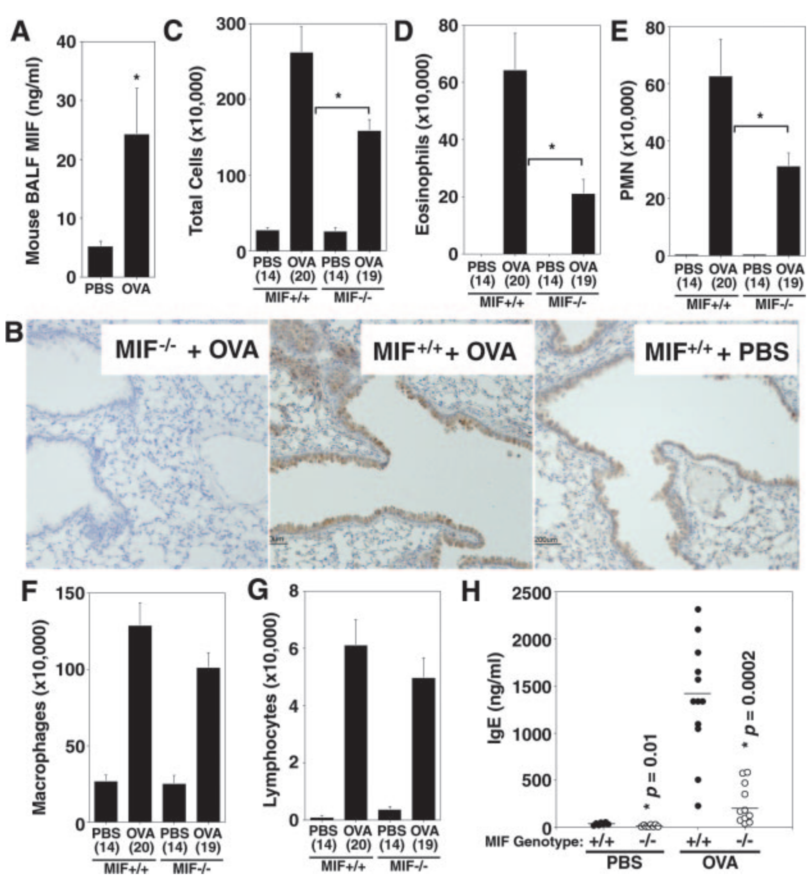

FIGURE 1. Impaired allergic responses in $MIF^{-/-}$ mice. *A*, BALF MIF ELISA. OVA-sensitized $MIF^{+/+}$ mouse BALF contained higher amounts of MIF than BALF from PBS-treated mice. *B*, Immunohistology. Rabbit anti-mouse MIF polyclonal Ab-mediated immunostaining demonstrated higher numbers of MIF-positive cells in airway epithelial and peribronchial connective tissues in OVA-sensitized lungs (*middle panel*) than in those sensitized with PBS (*right panel*), whereas OVA-sensitized $MIF^{-/-}$ lungs were negative for this Ab (*left panel*). *C–G*, Compared with OVA-sensitized $MIF^{-/-}$ BALF, OVA-sensitized $MIF^{+/+}$ mouse BALF contained significantly higher numbers of total leukocytes (*C*), eosinophils (*D*), and PMN cells (*E*), although reductions in Mϕ (*F*) and lymphocytes (*G*) were not significant. *H*, After OVA immunization, $MIF^{-/-}$ mice developed significantly less total IgE than $MIF^{+/+}$ mice. *, $p < 0.05$.

often serve as hallmarks of allergic airway inflammation. Reduced eosinophil infiltration in $MIF^{-/-}$ mice thus suggested a direct participation of MIF in airway allergic responses. Along with reduced eosinophil infiltration, serum total IgE levels also were significantly lower in $MIF^{-/-}$ mice after OVA immunization (Fig. 1*H*). Reduced IgE was not due to altered B cell function, because splenocytes from $MIF^{-/-}$ mice showed similar levels of cell surface I-A^d expression, LPS-induced proliferation, and DO11.10 T cell activation as those from $MIF^{+/+}$ mice (data not shown). These data in $MIF^{-/-}$ mice unequivocally demonstrated that MIF is not simply a molecule marker of inflammation but rather plays an essential role in the pathogenesis of allergic airway diseases.

MIF-deficient BALF or serum contains reduced levels of chemokines

Reduced inflammatory cell infiltration in $MIF^{-/-}$ mice suggested reductions in chemoattractant production. We anticipated that MIF may affect the production of chemoattractants and indirectly impair leukocyte or eosinophil infiltration into the lung. To test this hypothesis, we measured both eotaxin and MCP-3 in serum and BALF. Eotaxin acts via the CCR3 receptor to mediate the chemotaxis of both eosinophils and mast cells in allergen-sensitized airway inflammation and hyperresponsiveness (16). An eotaxin ELISA demonstrated a significant reduction in this chemokine in BALF, but not in serum, from OVA-sensitized $MIF^{-/-}$ mice as compared with OVA-sensitized $MIF^{+/+}$ mice (Fig. 2*A*). MCP-3 is important for eosinophil infiltration and activation and acts via CCR1, CCR2, and CCR3 (17). Consistently, serum but not BALF from OVA-sensitized $MIF^{-/-}$ mice showed a significant reduction of this chemokine (Fig. 2*B*). Therefore, MIF appears to regulate the production of chemokines to control migration and activation of eosinophils (Fig. 1*D*) or other leukocytes such as PMN (Fig. 1*E*).

Another hallmark of airway inflammation is increased cell proliferation mediated by TGF-β from eosinophils (18) or airway SMC (19). In vitro experiments demonstrated that TGF-β induces MIF expression in murine colon carcinoma cells (20) and, in turn, MIF regulates TGF-β expression in mesangial cells (21). Therefore, it is possible that reduced numbers of eosinophils in $MIF^{-/-}$ mice may lead to a reduction of BALF TGF-β levels, which may explain reduced peribronchial inflammatory cell densities in $MIF^{-/-}$ mice (Fig. 1*B*) by an impairment in their proliferation. A TGF-β1 ELISA affirmed this hypothesis and demonstrated a significant reduction of this multifunctional growth factor in BALF but not in the serum of saline- or OVA-sensitized $MIF^{-/-}$ mice (Fig. 2*C*). These data suggest a role for MIF in eosinophil and/or airway SMC TGF-β production and consequent airway inflammatory cell proliferation. Indeed, the numbers of Ki67-positive cells were reduced in OVA-sensitized $MIF^{-/-}$ lungs relative to OVA-sensitized $MIF^{+/+}$ lungs (Fig. 2*D*).

MIF deficiency impairs Th1 and Th2 cytokine production

In addition to leukocyte infiltration, T cell activation and cytokine production also play critical roles in the pathogenesis of allergic airway diseases such as asthma. Although asthma is mediated by Th2-type T cells, which produce a repertoire of cytokines including IL-4, IL-5, and IL-13, there is a clear role for Th1 cytokines such as IFN-γ in both the establishment and direction of the allergic phenotype. Increased MIF levels in human asthmatic serum, sputum, and BALF (5, 6), as well as OVA-sensitized $MIF^{+/+}$ mouse BALF (Fig. 1*A*), suggest a role of MIF in maintaining airway inflammation and T cell activities. Therefore, a lack of MIF may lower airway inflammation, as reflected by a reduction of BALF total leukocytes (Fig. 1*C*) or eosinophils (Fig. 1*D*) and reduce T cell activity. To test this hypothesis, we measured both the Th1 cytokines IFN-γ and TNF-α and the Th2 cytokines IL-4, IL-5, and IL-13. BALF but not serum IFN-γ levels were significantly reduced in $MIF^{-/-}$ mice (Fig. 3*A*), although BALF TNF-α was undetectable (data not shown) and serum TNF-α levels remained the same (Fig. 3*B*). Whereas serum levels of IL-4 were undetectable (data not

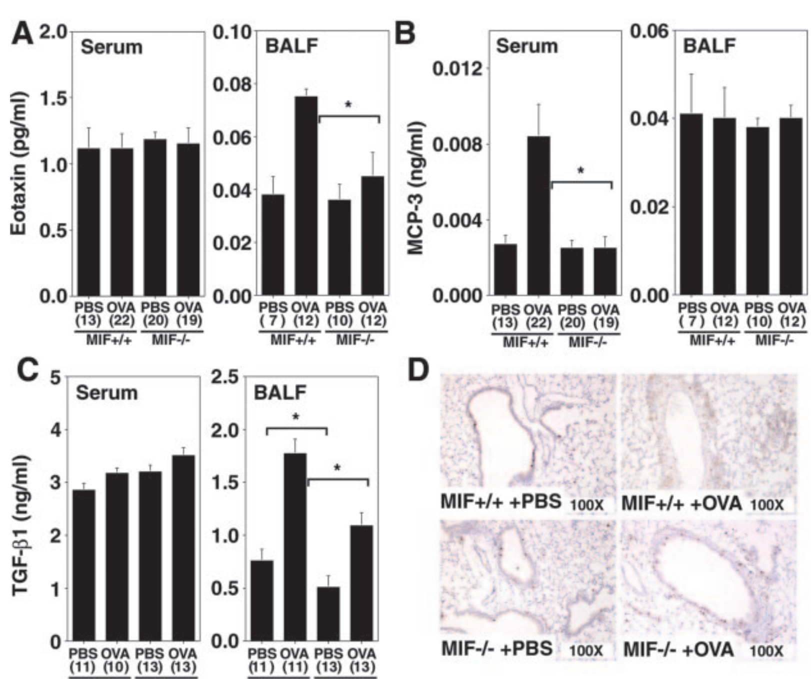

FIGURE 2. MIF-deficiency reduced chemokine and TGF-β1 levels and airway inflammatory cell proliferation. *A*, OVA-sensitized $MIF^{-/-}$ mouse BALF but not serum contained lower levels of eotaxin. *B*, Decreased serum but not decreased BALF MCP-3 levels were detected in $MIF^{-/-}$ mice. *C*, TGF-β1 levels also were reduced in $MIF^{-/-}$ mouse BALF but not in serum. *D*, Ki67-positive peribronchial inflammatory cells were much less in OVA-sensitized $MIF^{-/-}$ mouse lungs. *, $p < 0.05$.

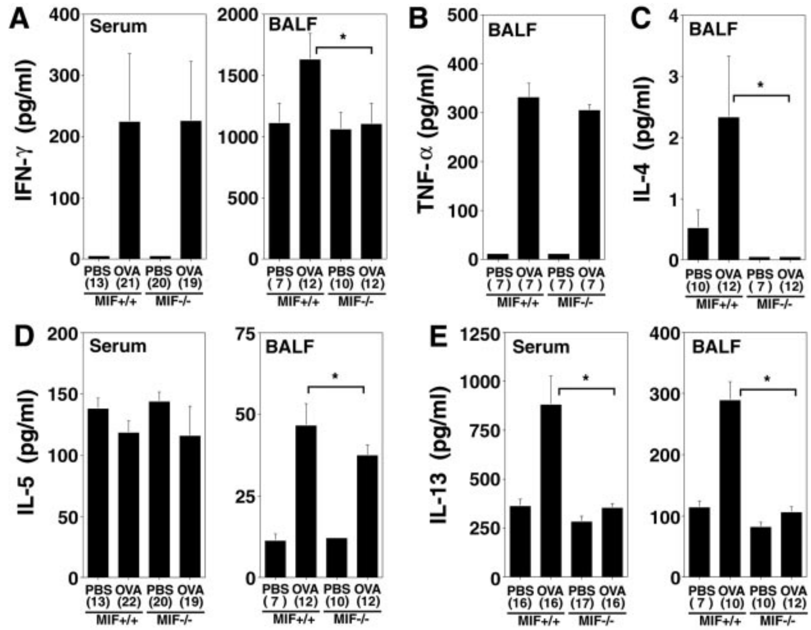

FIGURE 3. Th1 and Th2 cytokine profiles. *A*, IFN-γ levels were reduced in OVA-sensitized $MIF^{-/-}$ mouse BALF but not in serum. *B*, Serum TNF-α levels were undetectable and BALF TNF-α levels showed no differences between OVA-sensitized $MIF^{+/+}$ and $MIF^{-/-}$ mice. *C*, IL-4 levels were reduced in OVA-sensitized $MIF^{-/-}$ mouse BALF, whereas serum IL-4 levels were undetectable. *D*, IL-5 levels also were reduced in OVA-sensitized $MIF^{-/-}$ mouse BALF but not in serum. *E*, Both serum and BALF IL-13 were reduced in OVA-sensitized $MIF^{-/-}$ mice. *, $p < 0.05$.

shown) and those of IL-5 were the same between $MIF^{+/+}$ and $MIF^{-/-}$ mice (Fig. 3*D*), BALF IL-4 (Fig. 3*C*), BALF IL-5 (Fig. 3*D*), and serum and BALF IL-13 (Fig. 3*E*) were all reduced in $MIF^{-/-}$ mice. Thus, MIF appears to act on both Th1- and Th2-type cells in this model of airway inflammation.

MIF deficiency reduces T cell activation

Th1- and Th2-type T cells play essential roles in airway allergic responses (22). Increased levels of Th1- and/or Th2-type cytokines in the serum or BALF of subjects with asthma or Ag-sensitized airway inflammation suggest an enhancement of T cell activation. Thus, reduced levels of T cell cytokines in $MIF^{-/-}$ mice (Fig. 3) suggested a suppression of T cell activation presumably due to the lack of MIF. Indeed, MIF is well known to stimulate T cells both in vitro and in vivo (4). T cell activation can be mediated directly by cytokines or via APC such as B cells, DC, Mϕ, and mast cells. To test these possibilities, we performed MLR using C57BL/6J $CD4^+$ T cells and Ag presentation using

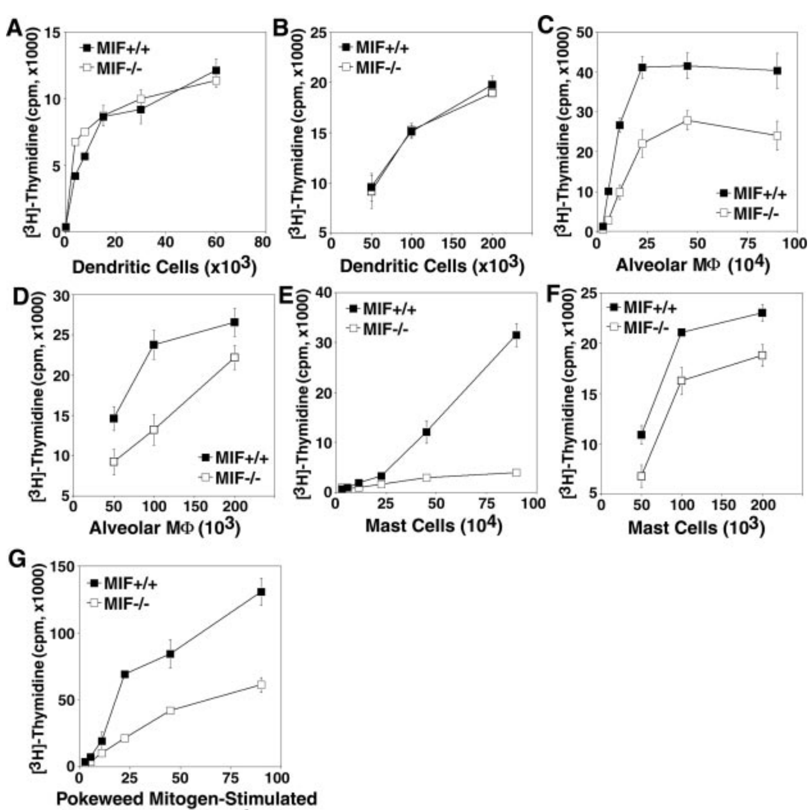

FIGURE 4. MLR (*A*, *C*, *E*, and *G*) and Ag presentation (*B*, *D*, and *F*). Lung DC from $MIF^{+/+}$ and $MIF^{-/-}$ mice were equally potent in activating C57BL/6J $CD4^+$ T cells (*A*) and DO11.10 $CD4^+$ T cells (*B*). In contrast, compared with those from $MIF^{+/+}$ mice, alveolar Mϕ (*C* and *D*) and BMMC (*E* and *F*) from $MIF^{-/-}$ mice showed impaired capability in activating C57BL/6J $CD4^+$ T cells in MLR assay (*C* and *E*) and $OVA_{323-339}$ peptide-mediated DO11.10 $CD4^+$ T cell activation (*D* and *F*). PWM-stimulated BMMC from $MIF^{-/-}$ also demonstrated reduced $CD4^+$ T cell proliferation relative to those from $MIF^{+/+}$ mice (*G*).

DO11.10 T cells in the presence of $OVA_{323-339}$ peptide and different APC obtained from *MIF*$^{-/-}$ mice, including lung DC, alveolar Mϕ, and BMMC. Surprisingly, DC from *MIF*$^{-/-}$ mice activated T cells as well as DC from *MIF*$^{+/+}$ mice in both assays (Fig. 4, *A* and *B*). In contrast, both Mϕ (Fig. 4, *C* and *D*) and BMMC (Fig. 4, *E* and *F*) from *MIF*$^{+/+}$ mice and *MIF*$^{-/-}$ mice differed in their ability to elicit T cell activation, although cell surface I-A^{d} levels in DC, Mϕ, and BMMC were not affected by MIF deficiency (data not shown). Further, PWM-stimulated *MIF*$^{-/-}$ BMMC also showed a reduction in T cell activation (Fig. 4*G*). Impaired T cell activation by *MIF*$^{-/-}$ BMMC was not due to an alteration in their intrinsic development or function, as we did not detect significant differences in either morphology or IgE-induced degranulation in BMMC from *MIF*$^{+/+}$ and *MIF*$^{-/-}$ mice according to an established protocol (23) (data not shown). These observations suggested a novel function for mast cell and Mϕ-derived MIF in T cell activation and potentially airway inflammation. However, MIF from other cells such as eosinophils, airway SMC, airway epithelial cells (Fig. 1*B*), and even T cells themselves may be equally important to T cell activation and airway allergic responses.

Mast cell-derived MIF is required for airway inflammation

Our MLR and Ag presentation assays demonstrated that MIF from Mϕ and BMMC, but not from DC, are essential for in vitro $CD4^{+}$ T cell activation (Fig. 4). Nevertheless, lung DC are important in initiating and maintaining allergic airway inflammation by polarizing naive T cells into either Th1 or Th2 effector cells, and they establish T cell memory and tolerance to inhaled Ags (24). It is possible that DC express insufficient MIF and, therefore, the MIF-related effects are not strongly mediated by these cells. Moreover, it is known that other cells such as eosinophils and airway epithelial and SMC also produce MIF (18, 19) (Fig. 1*B*) and, therefore, the role of BMMC- or Mϕ-derived MIF in T cell activation/proliferation observed from our in vitro pure cell population assay (Fig. 4) may be compensated by MIF released from other cell types in vivo.

Mast cells are important cellular effectors in asthma or acute or chronic airway inflammation, and their role in other inflammatory/autoimmune pathologies is gaining increasing prominence (2). Mice lacking mast cells are resistant to allergen-induced airway inflammation, and the reconstitution of BMMC into mast cell-deficient mice restores the inflammatory phenotype (12, 25). It is thought that mast cells contribute to airway inflammation by enhancing proliferation and cytokine production of multiple T cell subsets via direct mast cell-T cell interactions and by undefined soluble factors (26) such as MIF. Indeed, we detected 52 ± 2.1 ng/ml MIF in the conditioned medium of 1×10^{6} resting BMMC, and mast cells in normal lung were also immunoreactive for MIF (data not shown). To examine this hypothesis in vivo, we injected *MIF*$^{+/+}$ BMMC into *MIF*$^{-/-}$ mice and induced airway inflammation. MIF-positive BMMC can be detected within reconstituted and OVA-immunized *MIF*$^{-/-}$ lung parenchyma (Fig. 5*A*) and adjacent to the airway (data not shown), but not in nonreconstituted *MIF*$^{-/-}$ lungs (Fig. 1*B*, *left panel*). OVA-sensitized, BMMC-reconstituted *MIF*$^{-/-}$ lungs contained higher numbers of Ki67-positive cells in the peribronchial tissues than those in nonreconstituted OVA-sensitized *MIF*$^{-/-}$ lungs (Fig. 5*B*), providing direct evidence for mast cell-derived MIF in airway inflammatory cell proliferation. Consistent with increased cell proliferation, BMMC reconstitution also restored the airway inflammatory phenotype, including the recruitment of total leukocytes (Fig. 5*C*), eosinophils (Fig. 5*D*), and PMN cells (Fig. 5*E*), although Mϕ numbers were not affected (Fig. 5*F*). Inversely, BMMC reconstitution reduced total lymphocyte numbers in *MIF*$^{-/-}$ mice (Fig. 5*G*), similar to what was seen previously (25). Such reduction in BALF lymphocytes did not

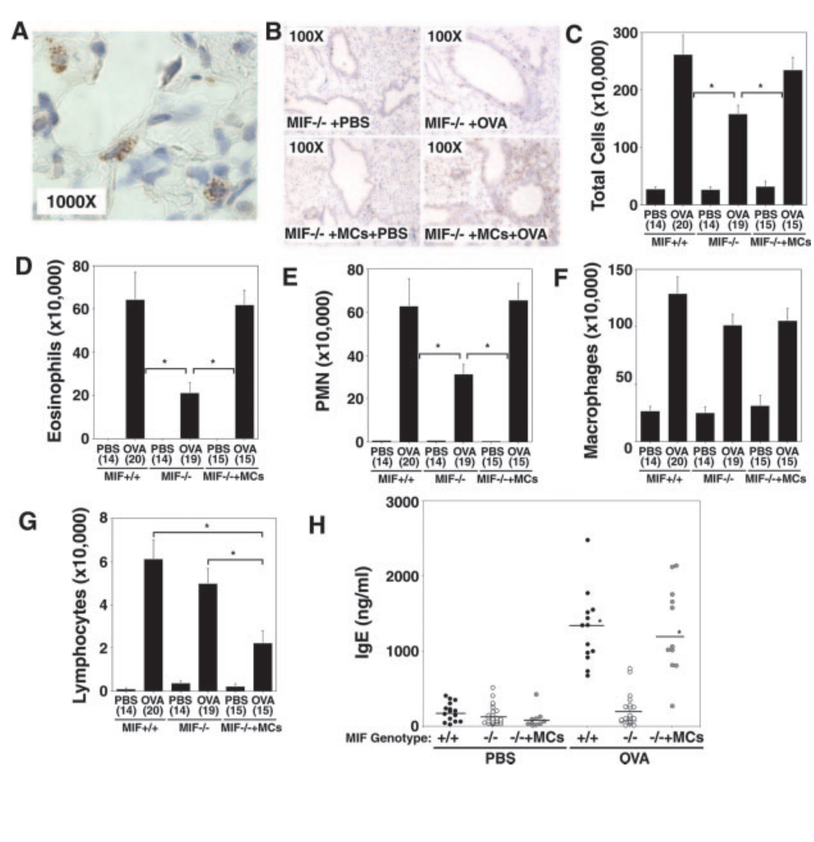

FIGURE 5. BMMC reconstitution. *A*, MIF polyclonal Ab immunostaining demonstrated MIF-positive mast cells in wild-type BMMC-reconstituted *MIF*$^{-/-}$ lung parenchyma. *B*, BMMC reconstitution restored high numbers of Ki67-positive proliferating peribronchial inflammatory cells in *MIF*$^{-/-}$ mice. *C–G*, Total leukocytes (*C*), eosinophils (*D*), and PMN (*E*) were also restored after BMMC reconstitution (+MCs), although the numbers of Mϕ (*F*) were not changed while those of lymphocytes were reduced after BMMC reconstitution (*G*). *H*, BMMC reconstitution significantly restored serum IgE levels in *MIF*$^{-/-}$ mice (*, $p < 0.05$).

affect the Ag-sensitized airway immune responses. Indeed, reconstituted $MIF^{-/-}$ mice produced comparable amounts of serum total IgE as $MIF^{+/+}$ mice (Fig. 5*H*), suggesting that the lymphocytes in BMMC-reconstituted mice acted comparably to those in $MIF^{+/+}$ mice. Phenotype recovery in reconstituted mice was not due to excessive mast cells in their lung. Methylene blue staining demonstrated similar numbers of mast cells per lung section in BMMC-reconstituted mice vs wild-type mice (32 ± 5 vs 38 ± 5; $p = 0.39$, $n = 5$). Therefore, BMMC-derived MIF is sufficient to initiate T cell activation in this murine allergic response model.

It remains to be explained why $MIF^{-/-}$ mice showed impaired airway inflammation after aluminum-conjugated OVA sensitization (Fig. 1), whereas similar models did not yield a similar reduction of airway allergic responses in mast cell-deficient mice (12). Although not conclusive, our data suggest that MIF from both mast cells and Mϕ are critical to T cell activation and, therefore, Mϕ in addition to mast cells may contribute to aluminum-OVA-induced airway inflammation. Further, in contrast to mast cell-deficient mice, mice deficient in the mast cell-activating molecules Igμ (27) and FcεRIα (28) showed impaired eosinophil infiltration relative to their wild-type controls after aluminum-OVA sensitization. Therefore, more complex mechanisms may be involved.

In summary, our study demonstrates that MIF production in the lungs, in part from intrapulmonary mast cells and Mϕ, participates directly in allergic airway inflammation by enhancing inflammatory cell recruitment and activating lymphocytes to promote the release of Th1/Th2 cytokines and chemokines.

Disclosures

The authors have no financial conflict of interest.

References

1. David, J. R. 1966. Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc. Natl. Acad. Sci. USA* 56: 72–77.
2. Lue, H., R. Kleemann, T. Calandra, T. Roger, and J. Bernhagen. 2002. Macrophage migration inhibitory factor (MIF): mechanisms of action and role in disease. *Microbes Infect.* 4: 449–460.
3. Galli, S. J., S. Nakae, and M. Tsai. 2005. Mast cells in the development of adaptive immune responses. *Nat. Immunol.* 6: 135–142.
4. Bacher, M., C. N. Metz, T. Calandra, K. Mayer, J. Chesney, M. Lohoff, D. Gemsa, T. Donnelly, and R. Bucala. 1996. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc. Natl. Acad. Sci. USA* 93: 7849–7854.
5. Rossi, A. G., C. Haslett, N. Hirani, A. P. Greening, I. Rahman, C. N. Metz, R. Bucala, and S. C. Donnelly. 1998. Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. *J. Clin. Invest.* 101: 2869–2874.
6. Yamaguchi, E., J. Nishihira, T. Shimizu, T. Takahashi, N. Kitashiro, N. Hizawa, K. Kamishima, and Y. Kawakami. 2000. Macrophage migration inhibitory factor (MIF) in bronchial asthma. *Clin. Exp. Allergy* 30: 1244–1249.
7. Bozza, M., A. R. Satoskar, G. Lin, B. Lu, A. A. Humbles, C. Gerard, and J. R. David. 1999. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. *J. Exp. Med.* 189: 341–346.
8. Kuperman, D. A., X. Huang, L. L. Koth, G. H. Chang, G. M. Dolganov, Z. Zhu, J. A. Elias, D. Sheppard, and D. J. Erle. 2002. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat. Med.* 8: 885–889.
9. Donnelly, S. C., C. Haslett, P. T. Reid, I. S. Grant, W. A. Wallace, C. N. Metz, L. J. Bruce, and R. Bucala. 1997. Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. *Nat. Med.* 3: 320–323.
10. Flano, E., I. J. Kim, J. Moore, D. L. Woodland, and M. A. Blackman. 2003. Differential γ-herpesvirus distribution in distinct anatomical locations and cell subsets during persistent infection in mice. *J. Immunol.* 170: 3828–3834.
11. Shi, G. P., J. A. Villadangos, G. Dranoff, C. Small, L. Gu, K. J. Haley, R. Riese, H. L. Ploegh, and H. A. Chapman. 1999. Cathepsin S required for normal MHC class II peptide loading and germinal center development. *Immunity* 10: 197–206.
12. Williams, C. M., and S. J. Galli. 2000. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J. Exp. Med.* 192: 455–462.
13. Hamaguchi, Y., Y. Kanakura, J. Fujita, S. Takeda, T. Nakano, S. Tarui, T. Honjo, and Y. Kitamura. 1987. Interleukin 4 as an essential factor for in vitro clonal growth of murine connective tissue-type mast cells. *J. Exp. Med.* 165: 268–273.
14. Wolters, P. J., J. Mallen-St Clair, C. C. Lewis, S. A. Villalta, P. Baluk, D. J. Erle, and G. H. Caughey. 2005. Tissue-selective mast cell reconstitution and differential lung gene expression in mast cell-deficient Kit(W-sh)/Kit(W-sh) sash mice. *Clin. Exp. Allergy* 35: 82–88.
15. Humbles, A. A., C. M. Lloyd, S. J. McMillan, D. S. Friend, G. Xanthou, E. E. McKenna, S. Ghiran, N. P. Gerard, C. Yu, S. H. Orkin, and C. Gerard. 2004. A critical role for eosinophils in allergic airways remodeling. *Science* 305: 1776–1779.
16. Humbles, A. A., B. Lu, D. S. Friend, S. Okinaga, J. Lora, A. Al-Garawi, T. R. Martin, N. P. Gerard, and C. Gerard. 2002. The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. *Proc. Natl. Acad. Sci. USA* 99: 1479–1484.
17. Chung, I. Y., Y. H. Kim, M. K. Choi, Y. J. Noh, C. S. Park, Y. K. Do, D. Y. Lee, Y. S. Lee, H. S. Chang, and K. S. Kim. 2004. Eotaxin and monocyte chemotactic protein-3 use different modes of action. *Biochem. Biophys. Res. Commun.* 314: 646–653.
18. Ohno, I., Y. Nitta, K. Yamauchi, H. Hoshi, M. Honma, K. Woolley, P. O'Byrne, G. Tamura, M. Jordana, and K. Shirato. 1996. Transforming growth factor β 1 (TGF β 1) gene expression by eosinophils in asthmatic airway inflammation. *Am. J. Respir. Cell Mol. Biol.* 15: 404–409.
19. Coutts, A., G. Chen, N. Stephens, S. Hirst, D. Douglas, T. Eichholtz, and N. Khalil. 2001. Release of biologically active TGF-β from airway smooth muscle cells induces autocrine synthesis of collagen. *Am. J. Physiol.* 280: L999–L1008.
20. Takahashi, N., J. Nishihira, Y. Sato, M. Kondo, H. Ogawa, T. Ohshima, Y. Une, and S. Todo. 1998. Involvement of macrophage migration inhibitory factor (MIF) in the mechanism of tumor cell growth. *Mol. Med.* 4: 707–714.
21. Leung, J. C., L. Y. Chan, A. W. Tsang, E. W. Liu, M. F. Lam, S. C. Tang, and K. N. Lai. 2004. Anti-macrophage migration inhibitory factor reduces transforming growth factor-β1 expression in experimental IgA nephropathy. *Nephrol. Dial. Transplant.* 19: 1976–1985.
22. Hansen, G., G. Berry, R. H. DeKruyff, and D. T. Umetsu. 1999. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J. Clin. Invest.* 103: 175–183.
23. Wolters, P. J., C. T. Pham, D. J. Muilenburg, T. J. Ley, and G. H. Caughey. 2001. Dipeptidyl peptidase I is essential for activation of mast cell chymases, but not tryptases, in mice. *J. Biol. Chem.* 276: 18551–18556.
24. Lambrecht, B. N. 2005. Dendritic cells and the regulation of the allergic immune response. *Allergy* 60: 271–282.
25. Kobayashi, T., T. Miura, T. Haba, M. Sato, I. Serizawa, H. Nagai, and K. Ishizaka. 2000. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. *J. Immunol.* 164: 3855–3861.
26. Nakae, S., H. Suto, M. Kakurai, J. D. Sedgwick, M. Tsai, and S. J. Galli. 2005. Mast cells enhance T cell activation: importance of mast cell-derived TNF. *Proc. Natl. Acad. Sci. USA* 102: 6467–6472.
27. Korsgren, M., J. S. Erjefalt, O. Korsgren, F. Sundler, and C. G. Persson. 1997. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. *J. Exp. Med.* 185: 885–892.
28. Mayr, S. I., R. I. Zuberi, M. Zhang, J. de Sousa-Hitzler, K. Ngo, Y. Kuwabara, L. Yu, W. P. Fung-Leung, and F. T. Liu. 2002. IgE-dependent mast cell activation potentiates airway responses in murine asthma models. *J. Immunol.* 169: 2061–2068.