Macrophage migration inhibitory factor promotes the migration of dendritic cells through CD74 and the activation of the Src/PI3K/myosin II pathway

Annette Ives | Didier Le Roy | Charlotte Théroude | Jürgen Bernhagen

Thierry Roger | Thierry Calandra

1Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland
2Chair of Vascular Biology, Institute for Stroke and Dementia Research (ISD), Klinikum der Universität München (KUM), Ludwig-Maximilians-University (LMU), Munich, Germany
3Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Abstract

Constitutively expressed by innate immune cells, the cytokine macrophage migration inhibitory factor (MIF) initiates host immune responses and drives pathogenic responses in infectious, inflammatory, and autoimmune diseases. Dendritic cells (DCs) express high levels of MIF, but the role of MIF in DC function remains poorly characterized. As migration is critical for DC immune surveillance, we investigated whether MIF promoted the migration of DCs. In classical transwell experiments, MIF−/− bone marrow-derived DCs (BMDCs) or MIF+/+ BMDCs treated with ISO-1, an inhibitor of MIF, showed markedly reduced spontaneous migration and chemotaxis. CD74−/− BMDCs that are deficient in the ligand-binding component of the cognate MIF receptor exhibited a migration defect similar to that of MIF−/− BMDCs. Adoptive transfer experiments of LPS-matured MIF+/+ and MIF−/− and of CD74+/+ and CD74−/− BMDCs injected into the hind footpads of homologous or heterologous mice showed that the autocrine and paracrine MIF activity acting via CD74 contributed to the recruitment of DCs to the draining lymph nodes. Mechanistically, MIF activated the Src/PI3K signaling pathway and myosin II complexes, which were required for the migration of BMDCs. Altogether, these data show that the cytokine MIF exerts chemokine-like activity for DC motility and trafficking.

KEYWORDS
chemokine, cytokine, dendritic cell, innate immunity, MIF, motility

Abbreviations: BMDCs, bone marrow-derived dendritic cells; CCL, chemokine (C-C motif) ligand; CCR, C-C motif chemokine receptor; CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C motif chemokine receptor; DCs, dendritic cells; D-DT, D-dopachrome tautomerase; Itg, integrins; LPS, lipopolysaccharide; MII, myosin II; MIF, macrophage migration inhibitory factor; MLCH, myosin II light chain.

Thierry Roger and Thierry Calandra contributed equally to this work.
Migration of leukocytes from the bloodstream into tissues is essential for the maintenance of homeostasis, body surveillance, and the mounting of host responses to danger signals sensed by sentinel innate immune cells in injured tissues. Inflammatory mediators released by innate immune cells trigger complex leukocyte-endothelial interactions resulting in the trans-endothelial migration, extravasation, and navigation of leukocytes into the interstitium. Microbial products, extracellular matrix, chemokines and cytokines, and lipid mediators are key drivers of adhesion-dependent and adhesion-independent leukocyte migration. The binding of chemokines to cognate G-protein-coupled receptors of leukocytes activates intracellular signaling pathways including the Rho family of GTPases, Ca^{2+} signaling, phosphoinositide 3-kinase (PI3K)-Akt, and mitogen-activated protein kinases (MAPKs) that generate a bipolar mechanosensory state for cell migration.

Macrophages and dendritic cells (DCs) are the main sentinel cells of the innate immune system that patrol peripheral tissues. They play a fundamental role in the recruitment of leukocytes following exposure to harmful environmental compounds, microbial products, or endogenous danger molecules. Tissue macrophages are an abundant source of a broad array of cytokines and chemokines that stimulate the migration of leukocytes into peripheral tissues. DCs are a heterogeneous group of hematopoietic cells bridging innate and adaptive immunity. Beyond the production of cytokines, one key function of the classical (also called conventional) population of DCs is their ability to capture antigens in peripheral tissues and transport them via the lymphatic vessels into the draining lymph nodes where they present antigens to naïve T cells. Following the endocytosis of foreign or self-antigens, DCs undergo a maturation process and up-regulate the expression of C-C chemokine receptor (CCR) 7, which results in an increased motility and haptotaxis into the lymphatic vasculature through the interaction of CCR7 with chemokine (C-C motif) ligand (CCL) 21 expressed by the lymphatic endothelium. Upon arrival in the sub-capsular sinus of the draining lymph nodes, a CCL21 gradient guides classical DCs to the T-cell–rich zone where they support the activation, maturation, and development of effector functions of antigen-specific CD4+ and CD8+ T cells.

Cytokines are crucial effector molecules of innate immunity that play an essential role in the activation of phagocytes, the recruitment of leucocytes, and the maturation and migration of DCs. Within the superfamily of cytokines, macrophage migration inhibitory factor (MIF) occupies a special place. MIF and its close relative D-dopachrome tautomerase (D-DT, also coined MIF-2) are the only identified members of this cytokine family. MIF acts as an enzyme and a hormone, a unique feature among cytokines. Other distinctive MIF traits are its constitutive expression and circulation at high concentration in the bloodstream and body fluids. MIF is released promptly by a broad range of immune and endocrine cells in response to a vast array of stimuli and stress hormones including glucocorticoids. Within the innate immune system, monocytes, macrophages, and DCs express copious amounts of MIF, which they further upregulate during acute inflammation to support robust innate immune responses. MIF mediates these effects via a positive regulation of the expression of Toll-like receptor 4 (TLR4), the inhibition of p53, and the counter-regulation of the immune suppressive effects of glucocorticoids. Hence, MIF acts as an initiator or regulator of infectious, inflammatory, and autoimmune diseases and represents a target for the management of pathological conditions. Extracellular MIF signals through a multicomponent receptor complex composed of CD74 and CD44. CD74 also works in association with C-X-C motif chemokine receptor (CXCR) 2, CXCR4, or CXCR7. By contrast, the intracellular MIF acts through an interaction with p53, COP9 signalosome subunit 5/c-Jun-activation domain-binding protein 1 (CSN5/JAB-1), thioredoxin-interacting protein, and ribosomal protein S19. Downstream signaling pathways activated by MIF include the extracellular signal-regulated kinase (ERK1/2), p38, and c-jun N-terminal kinase (JNK), MAPKs and PI3K/Akt.

Unexpectedly given its name, MIF displayed chemokine-like function as a non-cognate ligand for CXCR2 and CXCR4. Subsequent work indicated that MIF promotes the chemotaxis of neutrophils, B cells, eosinophils, and myeloid-derived suppressor cells through the engagement of one or several of its receptors, that is, CD74, CRXR2, CXCR4, and CXCR7, as well as indirectly through the chemokine (C-C motif) ligand (CCL) 2/MCP-1. Given that MIF is expressed abundantly in DCs and that migration is a key feature of DCs, we explored whether MIF was a bona fide chemotactic factor for DCs using genetic and pharmacological approaches.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

Animal experiments were approved by the Service des Affaires Vétérinaires, Direction Générale de l’Agriculture, de la Viticulture et des Affaires Vétérinaires (DGAV), état de Vaud (Épalinges, Switzerland) under authorizations n° 876.9, 877.8, and 877.9 to TR and were performed according to Swiss and ARRIVE guidelines (http://www.nc3rs.org.uk/arrive-guidelines).
2.2 | Mice and cells

Eight to twelve-week-old BALB/cAnNCrl and C57BL/6N mice were purchased from Charles River Laboratories (L'Arbresle, France). MIF−/− BALB/c mice and MIF−/− C57BL/6N mice were backcrossed at least eight times onto BALB/cAnNCrl and C57BL/6N genetic backgrounds. CD74−/− C57BL/6N mice were obtained from Prof Richard Bucala (Yale University School of Medicine, New Haven, CT). Mice were housed under specific pathogen-free conditions in the animal facility of the Centre des Laboratoires d’Epalinges (Switzerland, license number VD-H04) at 22°C, with 70% humidity in ambient air and 12-hour light/dark cycles. Colonies were free of norovirus and mouse hepatitis virus. Bone marrow cells were cultured in IMDM containing 2 μM 2-mercaptoethanol (βME), 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen, San Diego, CA), and 10% of heat-inactivated FCS (Biochrom AG, Berlin, Germany) supplemented with 50 ng/mL of granulocyte-macrophage colony-stimulating factor and 20 ng/mL of IL-4 (ProSpec, East Brunswick, NJ). Loosely adherent bone marrow-derived dendritic cells (BMDCs) were collected after seven days. BMDCs were incubated 36 hours with 10 ng/mL Ultrapure Salmonella minnesota LPS (List Biologicals Laboratories, Campbell, CA) to generate activated DCs.

2.3 | Cell migration assay

Cell migration was assessed using Corning Costar Transwell cell culture inserts or Corning Transwell-COL collagen-coated membrane inserts (Corning Life Sciences BV) with 5 μm pore size (Corning BV Life Sciences, Amsterdam, NL). Briefly, MIF+/+ and MIF−/− BMDCs were washed with PBS and incubated for 1 hour in RPMI 1640 medium (Invitrogen) containing 0.1% BSA (Sigma-Aldrich, Buchs, Switzerland). In some experiments, BMDCs were preincubated for 1 hour with recombinant MIF (rMIF) or inhibitors of MIF [4,5-dihydro-3-(4-hydroxyphenyl)-5-isoxazoleacetic acid methyl ester, known as ISO-1, 100 μM], CXCR2 (SB225002, 100 μM), CXCR4 (AMD3100, 100 μg/mL), MEK-1/2 (U0126, 10 μM), myosin II (blebbistatin, 100 μM), PI3K (Ly29002, 10 μM; wortmannin, 1 μM), ROCK (Y27632, 5 μM), or SRC (PP2, 10 μM; Src inhibitor-1, 5 nM). Five × 10^5 cells were transferred to the transwell inserts. The lower chamber of the transwell device contained medium with or without recombinant CCL5 (500 ng/mL), CCL19 (250 ng/mL), CCL20 (100 ng/mL), CCL21 (250 ng/mL), or CXCL12 (250 ng/mL). The number of cells migrating into the lower chamber was assessed after 6 hours. MIF was prepared as described previously. Chemokines were from Peprotech (Rocky Hill, NJ), and other reagents were from Sigma-Aldrich (Buchs, Switzerland) to generate activated DCs.

2.4 | RNA analysis

Total RNA was isolated, reversed transcribed, and used in real-time quantitative PCR conducted with a QuantStudio 12K Flex system (Life Technologies, Carlsbad, CA). Primer pairs are listed in Table S1. Relative gene specific expression levels were calculated with the 2^ΔΔCT method using Hprt as a reference gene.

2.5 | Flow cytometry analysis

BMDCs were incubated with the 2.4G2 antibody (BD Biosciences, Erembodegem, Belgium) to block non-specific binding and stained with antibodies listed in Table S2. Dead cells were excluded following 7-ADD staining. Data were acquired using a LSR II flow cytometer (BD Biosciences) and analyzed using the FlowJo 10.2 software (FlowJo LLC, Ashland OR).

2.6 | Adherence to fibronectin-coated glass slides

BMDCs were seeded onto fibronectin-coated (5-6 μg/cm^2) glass multi-well microscope slides at a density of 1.5 × 10^5 cells/cm^2. After 1 hour, slides were washed with PBS, stained with DIFF QUICK (Sigma-Aldrich), and mounted with coverslips. The number of adherent cells was determined in a semi-automated manner using the Image J software.

2.7 | Western blot analysis

Total cell extracts were obtained by incubating BMDCs in 10 mM HEPES pH 7.9, 10 mM KCl, 0.1 M EDTA, 0.1 mM EGTA, 1 mM DDT, 2.5 mM PMSF, 0.6% NP-40, Complete, Mini Protease, and PhosSTOP phosphatase inhibitor cocktails (Roche Applied Science, Basel, Switzerland) for 10 minutes on ice. Proteins were fractioned through 8-12% PAGE and transferred onto nitrocellulose membranes (Schleicher & Schuell, Keene, NH). Membranes were incubated with antibodies described in Table S2. The signals were detected using the ECL system (GE Healthcare, Little Chalfont, UK), and the images were recorded using a Fusion Fx system (Vilber Lourmat, Collégien, France). Full-size western blots and quantification data are shown in Figures S3 and S4.

2.8 | In vivo migration assay

C57BL/6N MIF+/+ and MIF−/− BMDCs and CD74+/+ and CD74−/− BMDCs were differentiated for 36 hours
with 10 ng/mL Ultrapure LPS, labeled with either 5-carboxyfluorescein diacetate succinimidyl ester (CFSE) (CellTrace CFSE Cell Proliferation Kit, Invitrogen) or CellVue Claret Far-red fluorescent cell linker (Sigma-Aldrich), washed, mixed at a 1:1 ratio (5 × 10^6 cells of each preparation), and injected into the left hind footpad of either MIF⁺/⁺ or MIF⁻/⁻ mice. Twenty-four hours later, popliteal lymph nodes were collected and analyzed by flow cytometry using antibodies directed against CD3, CD11c, CD19, and MHC-II (Table S2).

### 2.9 Graphical representation and statistical analyses

Graphs were plotted and statistical analyses were performed using Prism 8.3.0 (GraphPad Software, Inc). Violin plots show the 25th and 75th percentile, and the median. The bars depict mean ± SD. Comparisons between different groups were performed using the analysis of variance followed by parametric (two-tailed unpaired Student's t-test) and non-parametric (two-tailed Mann-Whitney test) statistical tests. P values less than .05 were considered to indicate statistical significance (*P < .05; **P < .01; ***P < .005).

### 3 RESULTS

#### 3.1 MIF promotes spontaneous and chemokine-induced migration of DCs

To determine whether MIF regulates the migration of DCs, BMDCs derived from MIF⁺/⁺ and MIF⁻/⁻ mice were subjected to transwell migration assays performed in the presence or in the absence of homeostatic (CCL19, CCL21), inflammatory (CCL5, CCL20), or mixed homeostatic/inflammatory (CXCL12) chemokines acting through CCR7 (CCL19 and CCL21), CCR1/3/4/5 (CCL5), CCR6 (CCL20), and CXCR4/ CXCR7 (CXCL12). The number of migrating cells was assessed after 6 hours of incubation. As shown in Figure 1A, the spontaneous migration of MIF⁻/⁻ BMDCs was 1.9-fold (C57BL/6) and 1.6-fold (BALB/c) lower than that of MIF⁺/⁺ BMDCs, respectively (P = .0017 and P = .026). Chemokines increased the migration of MIF⁺/⁺ BMDCs by a factor of 2 to 3. MIF⁻/⁻ C57BL/6 BMDCs exhibited severely impaired migration upon exposure to CCL19, CCL21, CCL5, or CCL20 (1.6-, 1.6-, 2.0-, and 1.9-fold reduction; P = .053, .001, .02, and .04, respectively) (Figure 1B). Interestingly, migration of MIF⁻/⁻ BMDCs was not impaired when it was induced by CXCL12 (Figure 1B). Spontaneous migration and CCL21-induced chemotaxis of MIF⁻/⁻ C57BL/6 BMDCs matured for 3 days with LPS were also markedly lower (2.6- to 3.1-fold) than that of MIF⁺/⁺ C57BL/6 BMDCs (Figure S1A).

Unless specified otherwise, all subsequent experiments were performed with cells derived from C57BL/6 mice.

We also performed transwell migration assays with wild-type BMDCs treated with ISO-1, an inhibitor of MIF. ISO-1 reduced the spontaneous and the CCL21-induced migration of MIF⁺/⁺ BMDCs (2-fold and 1.5-fold; P = .041 and P = .016) (Figure 1C). Next, we used a recombinant mouse MIF (rMIF) for add-back experiments in MIF-deficient BMDCs. BMDCs were incubated with rMIF for 1 hour before being used in transwell migration assays. rMIF increased in a dose-dependent manner spontaneous and CCL21-induced migration of MIF⁻/⁻ BMDCs (2.7-fold and 5.5-fold) (Figure 1D) indicative of a paracrine effect of MIF on spontaneous and chemokine-induced migration of BMDCs.

We then examined the contribution of MIF receptors to the migration of BMDCs using CD74⁺/⁺ and CD74⁻/⁻ BMDCs and pharmacological inhibitors of CXCR2 (SB225002) and CXCR4 (AMD3100) (Figure 1E,F). Like MIF⁻/⁻ BMDCs, CD74⁻/⁻ BMDCs exhibited a marked decrease of spontaneous and chemokine-induced (CCL19 or CCL21) migration (1.8-, 2.0-, and 1.9-fold; P = .001, .011, and .09, respectively) (Figure 1E). The CXCR4 inhibitor AMD3100 exhibited a small effect on CCL21-induced but not on spontaneous chemotaxis of MIF⁺/⁺ BMDCs (P = .016). The CXCR2 inhibitor SB225002 did not affect migration (Figure 1F).

Taken together, these results show that MIF plays an important role in the spontaneous migration and in the chemotaxis of DCs and that it exerts its effects predominantly in a CD74-dependent manner.

#### 3.2 MIF deficiency impairs the migration of DCs into draining lymph nodes

To evaluate the effect of autocrine and paracrine MIF on the migration of DCs in vivo, we used a model of adoptive cell transfer. Fluorochrome-labelled, LPS-matured MIF⁺/⁺ and MIF⁻/⁻ BMDCs were injected in the footpads of MIF⁺/⁺ and MIF⁻/⁻ mice, and the number of cells that migrated into the popliteal draining lymph nodes was assessed after 24 hours by flow cytometry. As shown in Figure 2A, MIF⁺/⁺ mice injected with MIF⁺/⁺ BMDCs had the highest number of cells migrating into the draining lymph nodes. Migrating BMDCs were reduced by 36% (P = .004) in MIF⁺/⁺ mice injected with MIF⁻/⁻ BMDCs, by 36% (P = .002) in MIF⁻/⁻ mice injected with MIF⁺/⁺ BMDCs, and by 54% (P = .0006) in MIF⁻/⁻ mice injected with MIF⁻/⁻ BMDCs. Thus, in vivo both autocrine and paracrine MIF contributed to an optimal migration of DCs to the draining lymph nodes. To test whether CD74 was involved in these effects, we quantified the migration of CD74⁺/⁺ and CD74⁻/⁻ BMDCs into the draining lymph nodes of wild-type recipient mice (Figure 2B). The migration of CD74⁻/⁻ BMDCs was reduced by 35% when compared with that of CD74⁺/⁺.
BMDCs ($P = .02$). These results indicate that the paracrine effects of MIF on DC migration are CD74-dependent.

### 3.3 MIF regulates integrin expression and adhesion of DCs to extracellular matrix

Leukocyte integrins (Itg) and adhesion molecules interact with the extracellular matrix including collagen, laminin, and fibronectin. We therefore examined whether MIF deficiency affected the expression of various integrins and adhesion molecules such as Itgα4 (CD49d), Itgβ1 (CD29), Itgβ2 (CD18), intercellular adhesion molecule 1 (Icam1, CD54), DC-specific intercellular adhesion molecule-3-grabbing non-integrin (Design, CD209a), and vascular cell adhesion molecule 1 (Vcam1, CD106). MIF$^{-/-}$ BMDCs expressed lower Itgα4 and Itgβ1 mRNA levels than MIF$^{+/+}$ BMDCs (1.6-fold and 1.4-fold reduction, $P = .04$ and $P = .006$). The mRNA levels of Itgβ2, Icam1, Design, and Vcam1 decreased, but the differences were not statistically significant (Figure 3A). Flow cytometry analyses confirmed a reduced expression of Itgβ1 (CD29), but not of Itgβ2 (CD18), in MIF$^{-/-}$ BMDCs (Figure 3B).
Very late antigen-4 (VLA-4) composed of heterodimers of Itgα4 and Itgβ1 interacts with VCAM1 and fibronectin. To determine whether the decreased expression of CD29 in MIF−/− BMDCs was functionally meaningful, we quantified the adhesion of BMDCs to fibronectin-coated glass slides. Compared to untreated MIF+/+ BMDCs, ISO-1-treated MIF+/+ BMDCs and MIF−/− BMDCs exhibited significant reductions in adherence to fibronectin by 2.3-fold and 2.8-fold (P = .03 and P = .004, respectively) (Figure 3C). Similar results were obtained when comparing MIF+/+ and MIF−/− BMDCs obtained from BALB/c mice (MIF+/+ vs MIF−/− adherent BMDCs: 3393 ± 374 vs. 2324 ± 344, n = 3, P = .01). In agreement with these observations, spontaneous migration and CCL19 and CCL21-stimulated chemotaxis of MIF−/− BMDCs were 2.1- to 3.6-fold lower than those of MIF+/+ BMDCs in migration assays performed with collagen-coated transwells (Figure S1B). These results indicate that the endogenous MIF is required for maximal expression of integrins on BMDCs for an optimal interaction with the extracellular matrix.

3.4 | MIF activates the Src/PI3K pathway

Several signaling pathways including the Src, PI3K/Akt, and MAPK regulate the migration of DCs. Among these signaling modules, the RAF/MEK/ERK pathway suppresses DC migration. As MIF can induce activation of PI3K/Akt, MEK/ERK1/2, and Src pathways, we examined the role of PI3K/Akt, Src, and MAPK pathways in migration of DCs in transwell migration assays using specific pharmacological inhibitors. Inhibitors of PI3K (wortmannin and Ly294002) and Src (Src inhibitor 1) decreased spontaneous migration (PI3K inhibitors) and CCL21-induced chemotaxis (PI3K and Src inhibitors) of MIF+/+ BMDCs, in line with previous observations. In contrast, the inhibitor of MEK1/2 (U0126), the MAPK kinase upstream of ERK1/2, had no impact on the migration of DCs (Figure 4A and Figure S2A). Western blot analyses of the levels of phosphorylated PI3K, phosphorylated Akt, and phosphorylated Src in MIF+/+ and MIF−/− BMDCs stimulated with CCL21 are shown in Figure 4B and Figure S3. CCL21 induced a rapid, robust, and transient increase in phosphorylated Akt and phosphorylated ERK1/2 and a modest increase in phosphorylated PI3K p85 and phosphorylated Src, while phosphorylated PI3K p55 was markedly reduced. Levels of phosphorylated PI3K and phosphorylated Src were reduced in MIF−/− BMDCs. This was not the case for phosphorylated Akt and ERK1/2. These results indicate that MIF supports the activation of kinase cascades (PI3K and Src) implicated in spontaneous and chemokine-induced migration of DCs. Of note, PI3K inhibition with wortmannin also reduced spontaneous and CCL21-induced migration of MIF−/− BMDCs (1.7-fold and 1.6-fold, P = .03 and .02, respectively), suggesting that PI3K-mediated migration of DCs is partly MIF-independent.

3.5 | MIF is involved in myosin II-dependent motility of DCs

Myosin II (MII) is an actin motor protein and essential regulator of cell morphology and cell migration. MII is a hexamer composed of two heavy chains of 230 kDa, two essential light chains of 17 kDa, and two regulatory light chains...
of 20 kDa (MLC II). The activity of MII is dependent on the phosphorylation of MLC II at serine 19 (Ser19) and at threonine 18 (Thr18). The upstream regulators of MII include Rho GTPases and ROCK1/2. Upon activation by RhoA, ROCK1 inhibits the MLC phosphatase, thereby increasing the phosphorylation of MLC II that supports actomyosin assembly. Phosphorylation at Ser19/Thr18 induces conformational changes of MII and stimulates MII filament formation and ATPase activity that is required for cell motility.

Given the key role played by MII in the cell movement, we investigated the effects of pharmacological inhibitors of MII (blebbistatin) and ROCK (Y27632) on the migration of BMDCs. Blebbistatin and Y27632 inhibited the spontaneous migration and CCL21-dependent chemotaxis of MIF+/+ BMDCs (blebbistatin: 2.7- and 3.8-fold decrease, \( P = .05 \) and \( P = .012 \); Y27632: 1.4- and 1.5-fold decrease, \( P = .03 \) and \( P = .004 \)) (Figure 5A,B). Blebbistatin was also found to decrease the chemotaxis of MIF+/+ BMDCs upon stimulation with CCL19, CCL5, and CCL20 (Figure S2B). Next, we examined by western blotting the effect of MIF on the phosphorylation of MLC II at Ser19 and Thr18 in MIF+/+ and MIF−/− BMDCs after stimulation with CCL21. CCL21 induced a rapid (peaking after one minute) and persistent (up until 60 minutes) elevation of phosphorylated MLC II in MIF+/+ BMDCs, which was delayed, of lower magnitude and of shorter duration in MIF−/− BMDCs (Figure 5C, Figure S4). These data indicate that MIF promotes MII-dependent motility of DCs.
DISCUSSION

Using genetic and pharmacological approaches, we showed that MIF promotes steady-state migration and chemotaxis of BMDCs in vitro and in vivo in a classical model of adoptive transfer and homing of DCs to the lymph nodes. The impairment of DC migration was especially striking when transferring MIF−/− BMDCs into MIF−/− recipient mice, indicating that MIF is playing an active role in DC recruitment and lymph node trafficking by haptotaxis. Mechanistically, MIF migration stimulating activity was mediated by the promotion of cellular adhesion via the expression of β1 integrin (CD29) and the activation of the Src/PI3K signaling pathway, which induced cellular locomotion through MII-dependent contraction (Figure 6).

Many of the biological effects of MIF require the activation of a receptor complex consisting of CD74, the ligand-binding unit, and CD44, the signal-transducing element. Via a pseudo(E)LR motif and a chemokine-mimicking N-like loop, MIF also functions as a non-canonical ligand for the chemokine receptors CXCR2 and CXCR4. Working in concert with CD74, CXCR2 or CXCR4 mediates MIF chemokine-like activity for monocytes, eosinophils, neutrophils, NKT cells, T cells, or B cells. MIF also facilitates CXCL1-induced neutrophil chemotaxis.

The present data provide genetic evidence that CD74 was required for maximal spontaneous migration and chemotaxis of DCs. In sharp contrast, a previous study reported an increased migration of CD74-deficient DCs. The reasons underlying these diametrically opposed results remain unclear. Our data are consistent with
studies that showed a role for CD74 in the migration of monocytes, NKT cells, B cells, and CLL cells. CD74-dependent promotion of DC migration by MIF relied on the activation of the Src and PI3K kinases, which is in agreement with previous studies, demonstrating the activation of these pathways by MIF.43,45,52,55 Given that CXCR4 inhibition only modestly affected MIF migratory effects and that genetic deficiency or pharmacological inhibition of CXCR4 causes major defect of myelopoiesis and DC maturation and survival,71-75 we did not perform in vivo experiments using CXCR4 targeting approaches. p53 and CSN5/JAB1 have been reported to affect the cell motility in a MIF-independent manner.76-78 We did not investigate whether MIF promoted DC migration via p53 or CSN5/JAB1, but are not aware of previous findings suggesting that it might be the case.

MIF has been involved in the recruitment of antigen-presenting cells in the epidermis or in the dermis. Transwell cell migration experiments carried out with a competitive inhibitor of CXCR2 (SB22502 at 100 nM) resulted in a 34% reduction of the migration of immature human DCs. With the same experimental setting, we found that CXCR2 inhibition with SB22502 tested at wide dose range (30 nM to 300 µM; shown using 100 µM in Figure 1F) had no impact on BMDCs migration. In contrast, inhibition of CXCR4 resulted in a modest but statistically significant reduction of BMDC chemotaxis, in line with studies showing an involvement of the MIF/CXCR4 axis in monocyte and T-cell chemotaxis. Integrins play an essential role in haptotactic migration of leukocytes driven by ligands in the extracellular matrix.1,80 The exploration of the mechanisms involved in MIF-dependent DC migration indicated that MIF modulates the expression of β1 integrin. These findings are consistent with earlier studies in which MIF upregulated the expression of αvβ3 integrin in endometrial adenocarcinoma and chondrosarcoma cells and of β1 integrin in podocytes.81-84 Functionally, integrin expression was associated with increased motility of chondrosarcoma cells and increased adhesion of podocytes.82,84 β2 integrins have been implicated in the arrest of monocytes induced by MIF and in the CCL2-dependent emigration of monocytes out of blood vessels.85 Of note, the activation of CD74 by MIF in CLL cells resulted in the expression of Tap63 and of VLA-4, a heterocomplex of α4 and β1 integrins that enabled the migration of CLL to the bone marrow.86 VLA-4 mediated MIF-induced cellular recruitment of macrophages and the adhesion and arrest of leukocytes on the endothelium through VCAM-1 or fibronectin. In line with these findings, we observed that MIF-modulated Iga4 mRNA expression with the reduced adherence of MIF−/− or ISO-1 treated BMDCs to fibronectin-coated glass slides. Overall,
these data are consistent with the notion that integrins mediate adhesion-dependent migration of DCs in a two-dimensional environment, whereas an actin-protrusive and integrin-independent mode of locomotion is critical for migration in three-dimensional environments.5

Upon binding of extracellular matrix proteins, integrin ligands, chemokines, cytokines, and growth factors to their cognate receptors, the family of Rho GTPases activates a signaling cascade that drives MII-dependent actomyosin assembly, cell protrusion, and motility of leukocytes including DCs.1,7 The Rho-associated protein kinases (ROCK1 and ROCK2) inhibit the activity of MLC phosphatase, thus augmenting the state of MLC II phosphorylation by MLC kinase, which supports DC migration. We found that inhibition of ROCK or of MII reduced the steady-state migration and chemotaxis of BMDCs. Mechanistically, MIF exerted its effects through the phosphorylation of the MLC II chains at Thr18 and Ser19. In a similar fashion, MIF promoted the activity of the Rho GTPase Rac1 and the migration of human lung adenocarcinoma cells and the induction of MLC kinase activity in fibroblasts.8,7,88

Taken together with previous work conducted in tumor and immune cells, our data indicate that MIF affects leukocyte trafficking in an integrin-dependent (transendothelial migration) or integrin-independent (migration into lymphoid organs) manner mainly via an interaction with the CD74 receptor and the activation of a Src/PI3K signaling pathway (Figure 6). This is the first report unraveling the signaling pathway, whereby MIF drives the activation of cell motility in immune cells. These observations reinforce the view that MIF plays a central role in promoting inflammatory and immune responses and that targeting MIF or its receptors are attractive immunotherapeutic approaches for the management of pathological conditions.

ACKNOWLEDGMENTS
The authors were supported by funding from the Swiss National Science Foundation (SNSF) grant number 310030_138488 and CRSII3_147662S (TC) and 310030_145014 and 310030_173123 (TR), the Société Académique Vaudoise (Lausanne, Switzerland) (TR), the Wilhelm-Sander Foundation grant number 2017.009.1 (JB), and the Deutsche Forschungsgemeinschaft (DFG) grant SFB1123/A3 (JB). CT was supported by the European Sepsis Academy Horizon 2020 Marie Skłodowska-Curie Action: Innovative Training Network (MSCA-ESA-ITN, grant number 676129) and received a scholarship from the Société Académique Vaudoise (Lausanne, Switzerland). We thank Ms Amiel Olivoz-Ortiz for her involvement in the present study and Simona Gerra for help with the rMIF preparations. We thank Prof. Richard Bucala (Yale University School of Medicine, New Haven, CT) for the generous gift of CD74−/− knockout mice.

CONFLICT OF INTEREST
All authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Study conception and design: T. Roger and T. Calandra. Acquisition of data: A. Ives, D. Le Roy, C. Théroude, and T. Roger. Analysis and interpretation of data: A. Ives, D. Le Roy, C. Théroude, J. Bernhagen, T. Roger, and T. Calandra. Drafting of manuscript: A. Ives, T. Roger, and T. Calandra. Final approval of the submitted manuscript: all authors.

ORCID
Jürgen Bernhagen https://orcid.org/0000-0003-2996-2652
Thierry Roger https://orcid.org/0000-0002-9358-0109
Thierry Calandra https://orcid.org/0000-0003-3051-1285

REFERENCES
1. Weninger W, Biro M, Jain R. Leukocyte migration in the interstitial space of non-lymphoid organs. Nat Rev Immunol. 2014;14:232-246.
2. Schulz O, Hammerschmidt SI, Moschovakis GL, Forster R. Chemokines and chemokine receptors in lymphoid tissue dynamics. Annu Rev Immunol. 2016;34:203-242.

3. Worbs T, Hammerschmidt SI, Forster R. Dendritic cell migration in health and disease. Nat Rev Immunol. 2017;17:30-48.

4. Vestweber D. How leukocytes cross the vascular endothelium. Nat Rev Immunol. 2015;15:692-704.

5. Lammermann T, Bader BL, Monksley SJ, et al. Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature. 2008;453:51-55.

6. Huse M. Mechanical forces in the immune system. Nat Rev Immunol. 2017;17:679-690.

7. Bros M, Haas K, Moll L, Grabbe S. RhoA as a key regulator of innate and adaptive immunity. Cells. 2019;8(7):733.

8. See P, Dutertre CA, Chen J, et al. Mapping the human DC lineage and terminal maturation induced by lipopolysaccharide or CD40 ligation. J Exp Med. 1994;179:1895-1902.

9. Morelli AE, Zahorack AF, Larregina AT, et al. Cytokine production by mouse myeloid dendritic cells in relation to differentiation and terminal maturation induced by lipopolysaccharide or CD40 ligation. Blood. 2001;98:1512-1523.

10. Worbs T, Ding X, Chanson AL, Renner P, Calandra T. Regulation of constitutive and microbial pathogen-induced human macrophage migration inhibitory factor (MIF) gene expression. Eur J Immunol. 2007;37:3509-3521.

11. Merk M, Mitchell RA, Endres S, Bucala R. D-dopachrome tautomerase (D-DT or MIF-2): doubling the MIF cytokine family. Cytokine. 2012;59:10-17.

12. Altan-Bonnet G, Mukherjee R. Cytokine-mediated communication: a quantitative appraisal of immune complexity. Nat Rev Immunol. 2019;19:205-217.

13. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol. 2003;3:791-800.

14. Luger J, Bucala R. The immunobiology of MIF: function, genetics and prospects for precision medicine. Nat Rev Rheumatol. 2019;15:427-437.

15. Engblom D, Sandberg M, Olsson CA, Rege J, Nicolson GL. Macrophage migration inhibitory factor (MIF) as a therapeutic target for rheumatoid arthritis. FASEB J. 2016;26:907-916.

16. Roger T, Schlaphach LJ, Schneider A, et al. Plasma levels of macrophage migration inhibitory factor and d-dopachrome tautomerase show a highly specific profile in early life. Front Immunol. 2017;8:26.

17. Calandra T, Bernhagen J, Metz CN, et al. MIF as a glucocorticoid-induced modulator of cytokine production. Nature. 1995;377:68-71.

18. Lugrin J, Ding X, Le Roy D, et al. Histone deacetylase inhibitors repress macrophage migration inhibitory factor (MIF) expression by targeting MIF gene transcription through a local chromatin deacetylation. Biochim Biophys Acta. 2009;1793:1749-1758.

19. Calandra T, Bernhagen J, Mitchell RA, Bucala R. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J Exp Med. 1994;179:1895-1902.

20. Morelli AE, Zahorack AF, Larregina AT, et al. Cytokine production by mouse myeloid dendritic cells in relation to differentiation and terminal maturation induced by lipopolysaccharide or CD40 ligation. Blood. 2001;98:1512-1523.

21. Roger T, Ding X, Chanson AL, Renner P, Calandra T. Regulation of constitutive and microbial pathogen-induced human macrophage migration inhibitory factor (MIF) gene expression. Eur J Immunol. 2007;37:3509-3521.

22. Roger T, David J, Glauser MP, Calandra T. MIF regulates innate immune responses through modulation of Toll-like receptor 4. Nature. 2001;414:920-924.
38. Kerschbaumer RJ, Rieger M, Volkel D, et al. Neutralization of macrophage migration inhibitory factor (MIF) by fully human antibodies correlates with their specificity for the beta-sheet structure of MIF. *J Biol Chem*. 2012;287:7446-7455.

39. Ouertati-Sakouhi H, El-Turk F, Fauvet B, et al. Identification and characterization of novel classes of macrophage migration inhibitory factor (MIF) inhibitors with distinct mechanisms of action. *J Biol Chem*. 2010;285:26581-26598.

40. Ouertati-Sakouhi H, El-Turk F, Fauvet B, et al. A new class of isoquinocyanate-based irreversible inhibitors of macrophage migration inhibitory factor. *Biochemistry*. 2009;48:9858-9870.

41. Gunther S, Fagone P, Jalce G, Atanasov AG, Guignabert C, Nicoletti F. Role of MIF and D-DT in immune-inflammatory, autoimmune, and chronic respiratory diseases: from pathogenic factors to therapeutic targets. *Drug Discov Today*. 2019;24:428-439.

42. Leng L, Metz CN, Fang Y, et al. MIF signal transduction initiated by binding to CD74. *J Exp Med*. 2003;197:1467-1476.

43. Shi X, Leng W, Wang T, et al. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. *Immunity*. 2006;25:595-606.

44. Bernhagen J, Krohn R, Lue H, et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat Med*. 2007;13:587-596.

45. Schwartz V, Kruttgen A, Weiss J, et al. Role for CD74 and CXCR4 in clathrin-dependent endocytosis of the cytokine MIF. *Eur J Cell Biol*. 2012;91:435-449.

46. Alampour-Rajabi S, El Bounkari O, Rot A, et al. MIF inter-...

47. Alampour-Rajabi S, El Bounkari O, Rot A, et al. MIF interacts with CXCR7 to promote receptor internalization, ERK1/2 and ZAP-70 signaling, and lymphocyte chemotaxis. *FASEB J*. 2015;29:4497-4511.

48. Hudson JD, Shoabi MA, Maestro R, Carnero A, Hannon GJ, Beach AH. A proinflammatory cytokine inhibits p53 tumor suppressor activity. *J Exp Med*. 1999;190:1375-1382.

49. Filip AM, Klug J, Cayli S, et al. Ribosomal protein S19 interacts with macrophage migration inhibitory factor and attenuates its pro-inflammatory function. *J Biol Chem*. 2009;284:7977-7985.

50. Kim MJ, Kim WS, Kim DO, et al. Macrophage migration inhibitory factor interacts with thioredoxin-interacting protein and induces NF-kappaB activity. *Cell Signal*. 2017;34:110-120.

51. Kleemann R, Haussner A, Geiger G, et al. Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. *Oncogene*. 2007;26:5046-5059.

52. Santos LL, Lacey D, Yang Y, Leech M, Morand EF. Activation of synovial cell p38 MAP kinase by macrophage migration inhibitory factor. *J Rheumatol*. 2004;31:1038-1043.

53. Santos LL, Lacey D, Yang Y, Leech M, Morand EF. Activation of synovial cell p38 MAP kinase by macrophage migration inhibitory factor. *J Rheumatol*. 2004;31:1038-1043.

54. Roger T, Chanson AL, Knaup-Reymond M, Calandra T. Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1. *Eur J Immunol*. 2005;35:3405-3413.

55. Lue H, Kapurniotu A, Fingerle-Rowson G, et al. Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. *Cell Signal*. 2006;18:688-703.

56. Santos LL, Fan H, Hall P, et al. Macrophage migration inhibitory factor regulates neutrophil chemotactic responses in inflammatory arthritis in mice. *Arthritis Rheum*. 2011;63:960-970.

57. Klasen C, Ohl K, Sternkopf M, et al. MIF promotes B cell chemotaxis through the receptors CXCR4 and CD74 and ZAP-70 signaling. *J Immunol*. 2014;192:5273-5284.

58. de Souza HS, Tortori CA, Lintomen L, et al. Macrophage migration inhibitory factor promotes eosinophil accumulation and tissue remodeling in eosinophilic esophagitis. *Mucosal Immunol*. 2015;8:1154-1165.

59. Zhang H, Ye YL, Li MX, et al. CXCL2/MIF-CXCR2 signaling promotes the recruitment of myeloid-derived suppressor cells and is correlated with prognosis in bladder cancer. *Oncogene*. 2017;36:2095-2104.

60. Bozza M, Satoskar AR, Lin G, et al. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. *J Exp Med*. 1999;189:341-346.

61. Fingerle-Rowson G, Petenko O, Metz CN, et al. The p3δ-dependent effects of macrophage migration inhibitory factor revealed by gene targeting. *Proc Natl Acad Sci U S A*. 2003;100:9354-9359.

62. Ciarlo E, Roger T. Screening the impact of sirtuin inhibitors on inflammatory and innate immune responses of macrophages and in a mouse model of endotoxic shock. *Methods Mol Biol*. 2016;1436:313-334.

63. Ciarlo E, Heinonen T, Herderschee J, et al. Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections in vivo. *Sci Rep*. 2016;6:37944.

64. Ciarlo E, Heinonen T, Theroude C, et al. Trained immunity confers broad-spectrum protection against bacterial infections. *J Infect Dis*. 2020;222:1869-1881.

65. Lubetsky JB, Dios A, Han J, et al. The tautomerase active site of macrophage migration inhibitory factor is a potential target for discovery of novel anti-inflammatory agents. *J Biol Chem*. 2002;277:24976-24982.

66. Randolph GJ, Ochando J, Partida-Sanchez S. Migration of dendritic cell subsets and their precursors. *Annu Rev Immunol*. 2008;26:293-316.

67. Hogstad B, Berres ML, Chakraborty R, et al. RAF/MEK/extracellular signal-related kinase pathway suppresses dendritic cell migration and traps dendritic cells in Langerhans cell histiocytosis lesions. *J Exp Med*. 2018;215:319-336.

68. Weber C, Kraemer S, Drechsler M, et al. Structural determinants of MIF functions in CXCR2-mediated inflammatory and atherogenic leukocyte recruitment. *Proc Natl Acad Sci U S A*. 2008;105:16278-16283.

69. Hsieh CY, Chen CL, Lin YS, et al. Macrophage migration inhibitory factor triggers chemotaxis of CD74+CXCR2+ NK T cells in chemically induced IFN-gamma-mediated skin inflammation. *J Immunol*. 2014;193:3693-3703.

70. Faure-Andre G, Vargas P, Yusef MI, et al. Regulation of dendritic cell migration by CD74, the MHC class II-associated invariant chain. *Science*. 2008;322:1705-1710.

71. Lopez MJ, Seyed-Razavi Y, Jamali A, Harris DL, Hamrah P. The chemokine receptor CXCR4 mediates recruitment of CD11c+ conventional dendritic cells into the inflamed murine cornea. *Invest Ophthal Vis Sci*. 2018;59:5671-5681.
72. Umemoto E, Otani K, Ikeno T, et al. Constitutive plasmacytoid dendritic cell migration to the splenic white pulp is cooperatively regulated by CCR7- and CXCR4-mediated signaling. *J Immunol*. 2012;189:191-199.

73. Kabashima K, Sugita K, Shiraishi N, Tamamura H, Fujii N, Tokura Y. CXCR4 engagement promotes dendritic cell survival and maturation. *Biochem Biophys Res Commun*. 2007;361:1012-1016.

74. Kohara H, Omatsu Y, Sugiyama T, Noda M, Fujii N, Nagasawa T. Development of plasmacytoid dendritic cells in bone marrow stromal cell niches requires CXCL12-CXCR4 chemokine signaling. *Blood*. 2007;110:4153-4160.

75. Kontos C, El Bounkari O, Krammer C, et al. Designed CXCR4 mimic acts as a soluble chemokine receptor that blocks atherogenic inflammation by agonist-specific targeting. *Nat Commun*. 2020;11:5981.

76. Mao L, Le S, Jin X, Liu G, Chen J, Hu J. CSN5 promotes the invasion and metastasis of pancreatic cancer by stabilization of FOXM1. *Exp Cell Res*. 2019;374:274-281.

77. Hwang CI, Matoso A, Corney DC, et al. Wild-type p53 controls cell motility and invasion by dual regulation of MET expression. *Proc Natl Acad Sci U S A*. 2011;108:14240-14245.

78. Vogl AM, Phu L, Becerra R, et al. Global site-specific neddylation profiling reveals that NEDDylated coflin regulates actin dynamics. *Nat Struct Mol Biol*. 2020;27:210-220.

79. Brocks T, Fedorchenko O, Schliermann N, et al. Macrophage migration inhibitory factor protects from nonmelanoma epidermal tumors by regulating the number of antigen-presenting cells in skin. *FASEB J*. 2017;31:526-543.

80. Kechagia JZ, Ivaska J, Roca-Cusachs P. Integrins as biomechanical sensors of the microenvironment. *Nat Rev Mol Cell Biol*. 2019;20:457-473.

81. Bondza PK, Metz CN, Akoum A. Macrophage migration inhibitory factor up-regulates alpha(v)beta(3) integrin and vascular endothelial growth factor expression in endometrial adenocarcinoma cell line Ishikawa. *J Reprod Immunol*. 2008;77:142-151.

82. Lee CY, Su MJ, Huang CY, et al. Macrophage migration inhibitory factor increases cell motility and up-regulates alphavbeta3 integrin in human chondrosarcoma cells. *J Cell Biochem*. 2012;113:1590-1598.

83. Khoufache K, Bazin S, Girard K, et al. Macrophage migration inhibitory factor antagonist blocks the development of endometriosis in vivo. *PLoS ONE*. 2012;7:e37264.

84. Chen CA, Chang JM, Yang YL, Chang EE, Chen HC. Macrophage migration inhibitory factor regulates integrin-beta1 and cyclin D1 expression via ERK pathway in podocytes. *Biochem Pharmacother*. 2020;124:109892.

85. Gregory JL, Morand EF, McKeown SJ, et al. Macrophage migration inhibitory factor induces macrophage recruitment via CC chemokine ligand 2. *J Immunol*. 2006;177:8072-8079.

86. Binsky I, Lantner F, Grabovsky V, et al. TAp63 regulates VLA-4 expression and chronic lymphocytic leukemia cell migration to the bone marrow in a CD74-dependent manner. *J Immunol*. 2010;184:4761-4769.

87. Rendon BE, Roger T, Teneng I, et al. Regulation of human lung adenocarcinoma cell migration and invasion by macrophage migration inhibitory factor. *J Biol Chem*. 2007;282:29910-29918.

88. Swant JD, Rendon BE, Symons M, Mitchell RA. Rho GTPase-dependent signaling is required for macrophage migration inhibitory factor-mediated expression of cyclin D1. *J Biol Chem*. 2005;280:23066-23072.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.

---

**How to cite this article:** Ives A, Le Roy D, Théroude C, Bernhagen J, Roger T, Calandra T. Macrophage migration inhibitory factor promotes the migration of dendritic cells through CD74 and the activation of the Src/PI3K/myosin II pathway. *The FASEB Journal*. 2021;35:e21418. [https://doi.org/10.1096/fj.202001605R](https://doi.org/10.1096/fj.202001605R)