IFN-γ and STAT1 Arrest Monocyte Migration and Modulate RAC/CDC42 Pathways

Yang Hu,2* Xiaoyu Hu,* Laurence Boumsell,† and Lionel B. Ivashkiv3*‡

Positive regulation of cell migration by chemotactic factors and downstream signaling pathways has been extensively investigated. In contrast, little is known about factors and mechanisms that induce migration arrest, a process important for retention of cells at inflammatory sites and homeostatic regulation of cell trafficking. In this study, we found that IFN-γ directly inhibited monocyte migration by suppressing remodeling of the actin cytoskeleton and cell polarization in response to the chemokine CCL2. Inhibition was dependent on STAT1 and downstream genes, whereas STAT3 promoted migration. IFN-γ migration by suppressing remodeling of the actin cytoskeleton and cell polarization in response to the chemokine CCL2. Inhibition was dependent on STAT1 and downstream genes, whereas STAT3 promoted migration. IFN-γ altered monocyte responses to CCL2 by modulating the activity of Pyk2, JNK, and the GTPases Rac and Cdc42, and inhibiting CCL2-induced activation of the downstream p21-activated kinase that regulates the cytoskeleton and cell polarization. These results identify a new role for IFN-γ in arresting monocyte chemotaxis by a mechanism that involves modulation of cytoskeleton remodeling. Crosstalk between Jak-STAT and Rac/Cdc42 GTPase-mediated signaling pathways provides a molecular mechanism by which cytokines can regulate cell migration. The Journal of Immunology, 2008, 180: 8057–8065.

Monocytes are bone marrow (BM)1-derived cells that circulate in the blood for approximately 1 day and exit into tissues and lymphoid organs where they differentiate into macrophages or specific dendritic cell subsets (1). Monocytes traffic into tissues under homeostatic conditions to maintain tissue macrophage populations and, in response to inflammation, to contribute to immune and inflammatory responses. Migration of monocytes and other immune cells into tissues occurs in response to gradients of chemokines that stimulate chemotaxis by activating G protein-coupled chemokine receptors and downstream signaling pathways. A subset of monocytes that preferentially migrates into inflammatory sites expresses high levels of chemokine receptor CCR2 that responds to its cognate ligand MCP-1/CCL2 that is highly expressed during inflammation (2).

Mechanisms underlying chemotaxis and migration of monocytes into tissues and inflammatory sites have been extensively studied (1, 3). The composition and size of inflammatory infiltrates is determined by the balance between migration into tissues, retention and survival, and egress. The important role of retention of monocytes in determining the extent of inflammation is becoming increasingly apparent (4). One cellular mechanism for retaining cells in a tissue is to generate a “stop” signal that arrests cell migration and positions cells to carry out effector functions or to effectively interact with other cells involved in immune responses. For example, microbial products like LPS arrest migration of monocytes at sites where they are required to exert inflammatory and antimicrobial functions (5), and Ag receptors induce stop signals to facilitate stable conjugate formation between lymphocytes and APCs (6). Excessive retention of monocytes at inflammatory sites can contribute to pathogenesis of inflammatory disorders such as atherosclerosis (7). Compared with the extensive understanding of mechanisms that regulate chemotaxis and migration, very little is known about mechanisms underlying stop signals that induce migration arrest. Although the regulation of chemokine production and establishment of chemokine gradients that position cells in tissues has been extensively studied, the only known cell autonomous mechanism for inhibiting monocyte migration is LPS-induced destabilization of chemokine receptor mRNA (8), which leads to cellular unresponsiveness to chemokines.

IFN-γ is a potent activator of monocytes/macrophages that promotes microbial killing, Ag presentation, and production of inflammatory mediators (9). At the same time, IFN-γ has important homeostatic functions that limit the extent of tissue damage associated with inflammation (10–16). A large body of work has established that IFN-γ inhibits the migration of myeloid cells in vivo (13, 16–24). It is possible that inhibition of migration by IFN-γ traps monocytes at inflammatory sites or facilitates interactions with IFN-γ-secreting Th1 cells (6, 25). However, the preponderance of the evidence suggests that inhibition of myeloid cell migration by IFN-γ in vivo serves a homeostatic function in limiting infiltration of inflamed tissues and controlling associated tissue damage (13, 16–24). For example, IFN-γ suppresses myeloid cell migration into acute and chronic inflammatory sites in models of bacterial infection, wound-associated inflammation, contact hypersensitivity, autoimmune uveitis, experimental allergic encephalomyelitis, and experimental arthritis (17, 19, 20, 22, 24, 26–29).

The role of IFN-γ in limiting inflammatory cell infiltration and significantly attenuating severity of disease in models of human autoimmune diseases (12, 13, 15–17, 19, 20, 24) makes it important to understand mechanisms by which IFN-γ regulates migration and the formation and maintenance of inflammatory infiltrates.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

www.jimmunol.org
Mechanisms described to date are IFN-γ-mediated suppression of chemokine production and inhibition of adhesion molecule expression on endothelial cells (17, 20, 21, 23). IFN-γ can suppress chemokine production by acting directly on tissue or endothelial cells to repress chemokine gene expression, and also indirectly by suppressing Th17 responses (10, 17, 20, 21, 23). In this study, we investigated whether IFN-γ can also suppress migration by acting directly on monocytes to inhibit their migratory properties. IFN-γ had a striking inhibitory effect on monocyte chemotactic responses to the major inflammatory chemokine CCL2. Inhibition of migration was dependent on de novo gene expression and the IFN-γ-activated transcription factor STAT1. IFN-γ worked by suppressing monocyte polarization in response to CCL2 and the underlying mechanism was altered regulation of actin polymerization and Rac/Cdc42-PAK signaling pathways that are important for migration. These results identify a new role for IFN-γ and the Jak-STAT pathway in regulating monocyte chemotaxis, and provide a mechanism by which previously unappreciated crossregulation between Jak-STAT and other pathways.
without chemokine. The plates were incubated at 37°C in 5% CO₂ for 1.5 h and cells that had migrated into the lower chamber were counted using flow cytometry with fluorescent counting beads (Bangs Laboratories) as an internal standard in each tube. A total of 10,000 singlet beads were acquired in each sample.

Flow cytometry, calcium flux, and F-actin polymerization

Cells were analyzed using flow cytometry as described previously (32) using a FACSCalibur flow cytometer with CellQuest software (Becton Dickinson). Ca²⁺ flux was measured using Fluo-3 AM (Molecular Probes). Monocytes were loaded with 5 μM Fluo-3 AM for 30 min at 37°C. Cells were then washed twice with HBSS and diluted in prewarmed medium before FACSCalibur analysis. The fluorescence of resting cells was measured for 30 s to obtain a baseline, and then CCL2 was added and data acquired for another 3 min. The results were analyzed using FCSPress 1.4 (www.FCSPress.com). F-actin polymerization was measured using FITC-phalloidin (Sigma-Aldrich). Monocytes were stimulated with CCL2 (50 ng/ml) for 0, 15, 30, 60, and 120 s and fixed in 4% paraformaldehyde for 10 min at 37°C. Cells were then washed and permeabilized with 0.1% Triton X-100 at room temperature for 4 min before loading with 0.2 μM of FITC-phalloidin on ice for 20 min and were analyzed using flow cytometry. Aliquots of cells were plated on coverslips and visualized by fluorescence microscopy.

Zigmond chamber chemotaxis assay and live imaging of monocyte migration

We followed the procedure described before with minor modification (33). In chemotaxis assays, monocytes were allowed to adhere to coverslips for 15 min at 37°C and were loaded into a Zigmond chamber (Neuro Probe). A chemokine gradient was established by adding CCL2 to one side of the

FIGURE 2. Role of STAT1 and STAT3 in monocyte migration. A, Human monocytes were treated with actinomycin D (5 μg/ml) or cycloheximide (15 μg/ml) for 30 min before addition of IFN-γ, and migration assays were performed as in Fig. 1. Results show the effects of IFN-γ relative to control cells that received each treatment in the absence of IFN-γ and are presented as the mean and SEM obtained from five independent experiments. B, Supernatants from IFN-γ-treated monocytes were incubated with control or neutralizing IFN-γ Abs and were added to freshly isolated monocytes 3 h before migration assays. Results are presented as the mean and SEM obtained from three independent experiments. C, Chemotaxis assays were performed using murine BM-derived macrophages from control and STAT1-deficient mice. n = 3, p < 0.01 as determined by paired Student’s t test. D, Human monocytes were treated with IFN-γ (100 U/ml), IL-10 (100 ng/ml), or IL-6 plus IL-6sR (100 ng/ml) overnight and migration assays were performed. Results are presented as the mean and SEM obtained from three independent experiments. **, p < 0.01 and *, p < 0.05 as determined by paired Student’s t test. E, left panel, Cell lysates from control THP-1 cells or THP-1 cells expressing high STAT3 levels (STAT3-Hi) were analyzed by immunoblotting. Right panel: Control and STAT3-Hi THP-1 cells treated overnight with IL-6 and IL-6sR (100 ng/ml) or IFN-γ were used in Transwell migration assays. Results are presented as the mean and SEM obtained from three independent experiments.
chamber. Live monocyte migration was visualized by time-lapse photography using a Zeiss Axiovert 200 widefield microscope with heating. Differential interference contrast images were captured at 400 magnification every 14 s for 20 min by a charge-coupled device camera connected to the microscope and controlled by Metamorph software (Molecular Devices). The images were converted into movie files using Metamorph and QuickTime player (Apple Computers).

Immunoblotting

Total cell extracts were obtained as described (32). Cell extracts corresponding to 3.3 x 10^5 cells were fractionated on 10 or 14% SDS-PAGE gels, transferred to polyvinylidene fluoride membranes (Millipore) and incubated with specific Abs; ECL was used for detection.

Rho GTPase activation assay

Pull-down assays were performed to isolate the active GTP-bound form of Cdc42, Rac or RhoA using the Cdc42/Rac and RhoA activation assay kits (Cytoskeleton) following the manufacturer’s instructions. Bound Rho GTPases were analyzed by Western blotting. For normalization, whole cell lysates were run in parallel.

Results

IFN-γ inhibits monocyte migration

Although IFN-γ suppresses trafficking of myeloid cells in vivo, it is not known whether IFN-γ directly inhibits the motility and chemotaxis of monocytes in addition to indirect regulation of chemokine production and endothelial cell function. We tested the direct effects of IFN-γ on monocyte migration using Transwell assays in which primary human monocytes added to the upper chamber migrate in response to chemotactic stimuli added to the lower chamber. We measured migration of freshly isolated primary human monocytes in response to CCL2, a major chemoattractant for monocytes that uses the chemokine receptor CCR2. As expected, CCL2 effectively induced monocyte chemotaxis (Fig. 1A); although there was some variability among blood donors, CCL2 reproducibly increased monocyte migration greater than 7-fold relative to unstimulated controls (p < 0.01, paired t test) and induced migration of greater than 15% of monocytes to the lower Transwell chamber (Fig. 1A). IFN-γ dramatically suppressed CCL2-induced monocyte migration (Fig. 1B); this inhibitory effect was highly reproducible and was observed in 20 independent experiments with different blood donors, was statistically significant (p < 0.0001, paired t test), and was also observed when RANTES/CCL5 was used (data not shown). IFN-γ inhibited CCL2-induced monocyte migration in a dose-dependent manner (Fig. 1C) and inhibition was time-dependent (Fig. 1D). Inhibition of migration was observed when IFN-γ was added simultaneously with CCL2 at the start of a 1.5 h migration assay and increased with the length of IFN-γ preincubation, such that 3 h of preincubation with IFN-γ resulted in nearly complete inhibition of migration (Fig. 1D). The time dependence of the inhibitory effect suggests that inhibition

FIGURE 3. IFN-γ alters p38, JNK, and Pyk2 phosphorylation. A, Control-, IFN-γ (100 U/ml), and PTX- (200 ng/ml) treated human monocytes were loaded with Fluo-3 and stimulated with CCL2 (50 ng/ml), and intracellular calcium levels were measured using flow cytometry. Duplicate samples are shown and one representative experiment out of three is shown. B, Human monocytes were treated with IFN-γ or PTX for 3 h before stimulation with CCL2 and activation of AKT and Erk was measured by immunoblotting. C, Human monocytes treated with IFN-γ, PTX, or IFN-γ + PTX for 3 h were used in Transwell migration assays. Results are presented as the mean and SEM obtained from three independent experiments. D, CCL2-induced activation of p38, JNK, and Pyk2 in human monocytes with or without IFN-γ treatment was measured by immunoblotting. E, Monocytes were pretreated with IFN-γ, DMSO (vehicle control), or inhibitors of JNK (SP600125; 30 μM), MEK-Erk (PD98059; 20 μM), Pyk2 (TyrA9; 2 μM), Syk (piceatannol; 50 μM), Src (PP1; 2 μM), PKC (GF109203X; 10 μM), and CCL2-induced migration was measured using Transwell assays. Results are presented as the mean and SEM obtained from three independent experiments.
required production of negative regulators of migration (further addressed below). We then tested whether decreased migration could be explained by decreased expression of the CCL2 receptor CCR2 or the CCL5 receptors CCR1/CCR3/CCR5. However, IFN-γ had no effect on CCR cell surface expression within the time frame of the migration assays (Fig. 1E) and actually induced CCR2 expression at later time points (data not shown). Thus, inhibition of monocyte chemotaxis by IFN-γ occurs by a fundamentally different mechanism than inhibition by LPS that inhibits CCR expression (8). Collectively, the data show that IFN-γ acts directly on the monocyte to strongly inhibit monocyte migration by a mechanism independent of regulation of chemokine receptor expression.

Role of STAT1 and STAT3 in monocyte migration

The time dependence of the IFN-γ-mediated inhibitory effect on migration suggested a requirement for de novo synthesis of inhibitory molecules. This notion was further investigated using actinomycin D and cycloheximide to inhibit, respectively, mRNA and protein synthesis. Both actinomycin D and cycloheximide abrogated the inhibitory effect of IFN-γ on monocyte migration (Fig. 2A) under conditions where these treatments do not affect proximal IFN-γ signaling or STAT1 activation (34). These results support the notion that IFN-γ induces inhibitors of migration and suggest that these inhibitors are proteins. Such inhibitors could either be secreted proteins that antagonize CCL2 function, or intracellular proteins that suppress cellular responses to CCL2 or inhibit cellular motility. We first tested whether supernatants of IFN-γ-treated monocytes contained secreted factors that suppress migration. The suppressive activity of these supernatants was completely blocked by anti-IFN-γ neutralizing Abs (Fig. 2B), indicating that IFN-γ did not induce production of secreted inhibitors of migration.

STAT1 is the major mediator of IFN-γ-induced gene expression (9), and we next tested the role of STAT1 in IFN-γ-mediated inhibition of migration. IFN-γ readily inhibited migration of control genetically matched murine BM-derived macrophages, but this inhibitory effect was completely abrogated in STAT1-deficient macrophages (Fig. 2C). These results suggest that STAT1, and STAT1-induced gene products, mediate the inhibitory effects of IFN-γ on migration. STAT1 and STAT3 have opposing functions in the regulation of cell survival/proliferation and inflammation (35) and thus we tested the role of STAT3 in myeloid cell migration. In contrast to IFN-γ that activates STAT1, IL-6, and IL-10 (which activate STAT3) increased monocyte migration (Fig. 2D). Moreover, increased expression of STAT3 that leads to increased STAT3 activation in monocytic THP-1 cells (31) resulted in increased migration (Fig. 2E). However, IFN-γ still suppressed THP-1 cell migration, even in cells that expressed high STAT3 levels (Fig. 2F). Thus, although STAT1 and STAT3 play different roles in cytokine-mediated regulation of monocyte migration, STAT3 does not appear to oppose the effects of IFN-γ. Collectively, the results suggest that IFN-γ induces expression of a STAT1-dependent gene product that suppresses CCL2-induced monocyte migration.

IFN-γ effects on CCL2-induced signal transduction

IFN-γ could inhibit monocyte migration by inhibiting CCR2 signaling, or by altering cellular responses to these signals. First, we investigated the effects of IFN-γ on CCL2-induced, CCR2-mediated signal transduction in primary human monocytes. The seven transmembrane chemokine receptor CCR2 signals via the receptor-coupled G protein Gαi to activate a rapid and transient calcium flux and downstream signaling cascades that are important for cell motility (3). As expected, CCL2 induced a rapid and transient calcium flux in monocytes that was completely abrogated by PTX, a Gαi inhibitor (Fig. 3A). In contrast to PTX, IFN-γ did not inhibit the CCL2-induced calcium flux (Fig. 3A). In addition, IFN-γ did not suppress CCL2-induced phosphorylation of Akt and ERKs that was completely blocked by PTX and thus was Gαi-dependent (Fig. 3B). Addition of IFN-γ together with PTX resulted in an additive inhibitory effect that essentially completely blocked monocyte migration (Fig. 3C). These results show that a subset of Gαi-dependent signals remained intact in IFN-γ-treated cells, and inhibition of these signals using PTX further suppressed migration. Thus, IFN-γ does not inhibit a proximal step in signaling that results in a global block of CCR2 responses. We next considered the possibility that IFN-γ may regulate CCR2 signals that are not dependent on Gαi. Pyk2 and the MAPks JNK and p38 are important for cell motility and are activated by chemokine receptors at least in part independently of Gαi (36, 37), and the effect of IFN-γ on these signaling molecules was investigated. Interestingly, preincubation with IFN-γ resulted in increased basal phosphorylation of these molecules, an effect that was most prominent for Pyk2, and altered the kinetics of CCL2-induced p38, JNK, and Pyk2 activation (Fig. 3D). Inhibition of JNK (using SP600125), Pyk2 (using

FIGURE 4. IFN-γ suppresses CCL2-induced F-actin polymerization and monocyte polarization. A. CCL2 was added at time = 0 and F-actin polymerization was measured in human monocytes treated with IFN-γ and PTX by staining with FITC-phalloidin and flow cytometry. F-actin levels relative to pre-CCL2 controls for each treatment group are shown. Results are presented as the mean and SEM obtained from three independent experiments. B. Human monocytes were stained with FITC-phalloidin and imaged using fluorescence microscopy. One representative experiment of three is shown.
TyrA9), or the Syk kinase upstream of Pyk2 (using piceatannol) suppressed CCL2-induced migration (Fig. 3E), supporting the notion that dysregulation of the activity of these signaling molecules by IFN-γ contributes to IFN-γ-mediated suppression of migration. In contrast, inhibitors of MEK, Src, PKC, and CaMK had minimal effects on monocyte migration (Fig. 3E). The results show that IFN-γ alters the activation state of the cell, with increased and sustained basal activity of molecules important for migration. Transient signaling is important for the rapid turnover of the cytoskeleton and cell adhesion structures that is important for migration, and increased and sustained activation of signaling pathways important for migration has been associated with diminished cell polarization and directional migration (3, 38, 39). Therefore, we next investigated the effects of IFN-γ on cytoskeletal remodeling and cell polarization.

**IFN-γ suppresses monocyte polarization and alters Rac and Cdc42 activation**

Cell polarization in response to chemokines is regulated by the small GTPases Rac, Cdc42, and Rho and downstream polymerization of F-actin (3). We first analyzed the effects of IFN-γ on F-actin polymerization. IFN-γ-treated monocytes showed a trend toward higher baseline F-actin that did not reach statistical significance (data not shown). However, IFN-γ effectively suppressed CCL2-induced actin polymerization as assessed by flow cytometry using FITC-phalloidin staining (Fig. 4A). Next, fluorescence microscopy was used to analyze monocyte polarization. Addition of CCL2 induced polymerization of a subset of monocytes that clearly showed an F-actin-containing leading edge and a lagging tail or uropod (Fig. 4B, left panels). In contrast, IFN-γ completely blocked monocyte polarization (Fig. 4B, right panels). It is important to consider that in these experiments there is no chemokine gradient, and thus only a small fraction of control monocytes polarized, consistent with previous reports (33, 40).

Monocyte polarization and migration in response to a chemokine gradient were next studied using a Zigmond chamber. Control monocytes clearly polarized in response to a CCL2 gradient (toward left direction in Fig. 5A) and vigorously migrated toward increasing concentrations of CCL2 (Movie 1a, available as part of Supplementary data). In contrast, polarization toward a CCL2 gradient was completely blocked in IFN-γ treated monocytes (Fig. 5A), and IFN-γ treated monocytes exhibited minimal migration toward a CCL2 gradient (Movie 1b). Collectively, the data show that IFN-γ blocks cellular F-actin polymerization and polarization in response to CCL2, with an attendant block in chemotaxis.

We next analyzed the effects of IFN-γ on activation of the Rac, Cdc42, and Rho GTPases. Rac and Cdc42 regulate actin polymerization at the leading edge and thus polarization toward a chemokine gradient, whereas Rho controls actin assembly/disassembly in the uropod (3). As expected, CCL2 induced increased levels of active, GTP-bound Rac, Cdc42, and Rho in control monocytes (Fig. 5B). In contrast, IFN-γ-treated monocytes showed increased baseline levels of GTP-bound Rac and Cdc42, and these were not further increased in response to CCL2 (Fig. 5B). Baseline and CCL2-induced levels of GTP-Rho did not differ between control and IFN-γ treated cells. The PAK kinase is a key downstream effector of Rac and Cdc42 that regulates migration (41). Consistent with increased basal Rac and Cdc42 activity, baseline levels of phosphorylated PAK were elevated in IFN-γ treated monocytes.

---

**The online version of this article contains supplemental information.**
and its receptor Plexin C1 were measured using real time PCR. Cdc42-PAK pathway. Because the inhibitory effect of IFN-α/β alters cellular responses to CCL2 by dysregulating the cycling of Rac and Cdc42 between active and inactive forms and activation of the downstream PAK kinase, which is required for maximal CCL2-induced PAK activation, and, as expected (45), IFN-γ suppressed TGF-β signaling (Fig. 6E). However, IFN-γ did not induce Smad7 expression in human monocytes (data not shown), suggesting that IFN-γ inhibits TGF-β signaling in monocytes by an alternative mechanism. Collectively, the data suggest that IFN-γ induces the expression of proteins that modulate the Rac/Cdc42-PAK pathway, and that at least one of these proteins targets TGF-β-mediated inputs into PAK activity.

Discussion

Negative regulation of cell migration is not well understood. In this study, we found that IFN-γ directly inhibits chemokine-induced monocyte migration by mechanisms that involve decreased remodeling of the actin cytoskeleton and an associated block in monocyte polarization toward a chemotactic gradient. Actin cytoskeleton remodeling that is required for cell polarization and migration is regulated by the small GTPases Rac and Cdc42 and the downstream PAK kinase, and IFN-γ modulated the activity of these types via induction of Smad7 expression (43–46). First, we tested whether TGF-β played a role in monocyte migration in our system. Neutralization of endogenous TGF-β using its specific physiological inhibitor latency associated peptide suppressed monocyte migration in response to CCL2 (Fig. 6C). This result suggests that endogenous TGF-β serves as a cofactor for maximal CCL2-induced migration, and we next tested whether TGF-β was required for CCL2-induced PAK activation. Strikingly, inhibition of endogenous TGF-β abolished CCL2-induced PAK activation (Fig. 6D), similar to the inhibition that was observed with IFN-γ (Fig. 5B). These results suggested that IFN-γ regulates PAK activation at least in part by inhibiting a signaling input from TGF-β that is required for maximal CCL2-induced PAK activation, and, as expected (45), IFN-γ suppressed TGF-β signaling (Fig. 6E).

FIGURE 6. Regulation of Semaphorin 4D by IFN-γ and role of TGF-β in monocyte migration. A and B, IFN-γ-induced expression of semaphorin 4D does not mediate inhibition of monocyte migration. A, Human monocytes were treated with IFN-γ for 3 h and mRNA levels of Semaphorin 4D (Sema 4D) and its receptor Plexin C1 were measured using real time PCR. B, Monocytes were stimulated with IFN-γ in the presence of Sema 4D blocking Ab or control Ab. C–E, IFN-γ and TGF-β regulation of monocyte migration. C, CCL2-induced migration of human monocytes treated with or without latency associated peptide was measured using Transwell assays. D, Lysates from control and latency associated peptide-treated human monocytes were analyzed by immunoblotting. One representative experiment of three is shown. E, TGF-β-induced p38 activation was measured by immunoblot. One representative experiment of four is shown.
proteins and altered their pattern of activation by the chemokine CCL2. These findings identify crosstalk between Jak-STAT and GTPase-mediated signaling pathways, provide cellular and molecular mechanisms by which cytokines can suppress chemotaxis and help explain how IFN-γ controls myeloid cell migration and cellular infiltration during inflammatory processes.

Although IFN-γ suppressed polarization and migration of monocytes in response to CCL2, IFN-γ did not suppress CCL2-induced signal transduction. Instead, IFN-γ changed the state of the cell such that it did not respond to the CCL2 signal by remodeling the actin cytoskeleton that is required for polarization. This provides an example of how myeloid cells can use one set of receptors to sense the general environment and regulate cell responses to heterologous receptors to ensure that these responses are appropriate (47). One molecular change induced by IFN-γ was to fix Rac and Cdc42 in an active state, thereby preventing the rapid and transient cycling between active and inactive forms that is required for dynamic actin remodeling and migration. Increased activity of Rac and Cdc42 also likely explains increased basal activation of downstream signaling molecules such as MAPKs that was observed in IFN-γ-treated monocytes. Rac and Cdc42 signal to PAK, a key downstream effector that regulates cytoskeletal remodeling that is necessary for migration (41). IFN-γ suppressed CCL2-induced activation of PAK, thus providing a mechanism by which IFN-γ inhibits CCL2-induced migration. Interestingly, IFN-γ did not appear to directly inhibit CCL2-signaling, but instead suppressed a signal from autocrine-acting TGF-β that was required for PAK activation. Such a requirement for costimulation for effective responses to chemokines has been previously described, including in work from our laboratory (32, 48, 49).

It is interesting to consider the implications of our results for the effects of IFN-γ on monocyte migration in vivo during immune responses and in an autoimmune disease setting. Our results suggest that exposure of circulating monocytes to systemic IFN-γ, during either acute or chronic inflammation, will serve a homeostatic function by limiting monocyte migration into sites of inflammation. Indeed, such a homeostatic function for IFN-γ in limiting myeloid cell infiltration has been extensively documented in the literature, including in contact hypersensitivity, experimental allergic encephalitis, and inflammatory arthritis (13, 16–24, 26–29). It is possible that IFN-γ-induced migration arrest may also promote inflammation by trapping monocytes at inflammatory sites in vivo. We attempted to address this possibility but were not able to resolve this question for technical reasons. Thus, the role of IFN-γ in retaining monocytes at inflammatory sites will need to be addressed in future work.

Trafficking of cells during the course of immune and inflammatory responses is directly regulated by chemokines. However, cytokines such as IFN-γ coordinate the overall response and thereby regulate inflammatory and immune cell infiltration of tissues and migration into secondary lymphoid organs. The current paradigms about how cytokines regulate cell trafficking focus on indirect mechanisms such as regulation of chemokine production or chemokine receptor expression (3). Indeed, IFN-γ can indirectly promote migration by increasing IL-6 expression (50, 51). Our results highlight that direct inhibition of monocyte migration by IFN-γ can counterbalance the effects of increased chemokine expression by attenuating monocyte chemotaxis in response to chemokines. IFN-γ inhibited migration by a mechanism that depends on STAT1 and de novo gene expression. A role for STAT3 in invasive cell migration, possibly mediated by STAT3-dependent matrix metalloproteinase expression has been previously suggested (52–54), but the role of STATs and their target genes in regulating cell migration is mostly unexplored. This study opens a new area of investigation of the direct regulation of cell migration by cytokines and the Jak-STAT pathway, and the functions of cytokine-induced STAT-dependent genes in regulating the actin cytoskeleton and cell polarization. STAT1 and STAT3 often oppose each other’s function, for example in the regulation of cell growth and inflammatory mediator production. Interestingly, cytokines that activate STAT3 increased monocyte migration, extending the concept of opposing actions of STAT1 and STAT3 to the regulation of cell migration.

Acknowledgments

We thank Dr. William Muller and Dr. Theresa Lu for helpful discussion and critical review of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

1. Tacke, F., and G. J. Randolph. 2006. Migratory fate and differentiation of blood monocyte subsets. *Immunobiology* 211: 699–618.
2. Goeppmann, F., S. Jung, R. J. Littman. 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19: 71–82.
3. Rot, A., and U. H. von Andrian. 2004. Chemokines in innate and adaptive host defense: basic chemokine grammar for immune cells. *Annu. Rev. Immunol.* 22: 619–648.
4. Ludewig, B., and J. D. Laman. 2004. The in and out of monocytes in atherosclerotic plaques: balancing inflammation through migration. *Proc. Natl. Acad. Sci. USA* 101: 11529–11530.
5. Rotta, G., E. W. Edwards, S. Sanguetti, C. Bennett, S. Ronzoni, M. P. Colombo, R. M. Steinman, G. J. Randolph, and M. Rescigno. 2003. Lipopolysaccharide or whole bacteria block the conversion of inflammatory monocytes to dendritic cells in vivo. *J. Exp. Med.* 198: 1253–1263.
6. Schneider, H., J. Downey, A. Smith, B. H. Zinselmeyer, C. Rush, J. M. Brewer, B. Wei, N. Hogg, P. Garbrecht, and C. E. Rudd. 2006. Reversal of the TCR stop signal by CTLA-4. *Science* 313: 1972–1975.
7. Lloreda, J., V. Angelii, J. Liu, E. Trogan, E. A. Fisher, and G. J. Randolph. 2004. Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. *Proc. Natl. Acad. Sci. USA* 101: 11779–11784.
8. Sica, A., A. Saccani, A. Borsatti, C. A. Power, T. N. Wells, W. Luini, N. Polentarutti, S. Sozzani, and A. Mantovani. 1997. Bacterial lipopolysaccharide rapidly inhibits expression of C-C chemokine receptors in human monocytes. *J. Exp. Med.* 185: 969–974.
9. Schroder, K., P. J. Hertzog, T. Ravasi, and D. A. Hume. 2004. Interferon-γ: an overview of signals, mechanisms and functions. *J. Leukocyte Biol.* 75: 163–189.
10. Bettelli, E., M. Ouakka, and V. K. Kuchroo. 2007. T(H)-17 cells in the circle of T cell priming. *J. Immunol.* 178: 5501–5506.
11. Vermeire, K., H. Heremans, M. Vandeputte, S. Huang, A. Billiau, and P. Matthys. 1997. Accelerated collagen-induced arthritis in IFN-γ receptor-deficient mice. *J. Immunol.* 158: 5507–5513.
12. Guedez, Y. B., K. B. Whittington, J. L. Clayton, L. A. Joosten, F. A. van de Loo, W. B. van den Berg, and E. F. Rosloniec. 2001. Genetic ablation of interferon-γ up-regulates interleukin-1β expression and enables the elicitation of collagen-induced arthritis in a nonsusceptible mouse strain. *Arthritis Rheum.* 44: 2443–2444.
13. Krakowski, M., and T. Owens. 1996. Interferon-γ confers resistance to experimental allergic encephalomyelitis. *Eur. J. Immunol.* 26: 1641–1646.
14. Willenborg, D. O., S. Fordham, C. C. Bernardi, W. B. Cowden, and I. A. Ramshaw. 1996. IFN-γ plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J. Immunol.* 157: 3223–3227.
15. Kelchtermans, H., S. Sreyf, B. De Klecker, T. Mitera, M. Atlan, L. Geboes, M. Van Balen, C. Dilleen, W. Put, C. Gyselmes, et al. 2007. Protective role of IFN-γ in collagen-induced arthritis conferred by inhibition of mycobacteria-induced granulocyte chemotactic protein-2 production. *J. Leukocyte Biol.* 81: 1031–1035.
16. Bettelli, E., B. Sullivan, S. J. Szabo, R. A. Sobel, L. H. Glimcher, and V. K. Kuchroo. 2004. Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 200: 79–87.
17. Wu, X., W. Hou, S. Sun, E. Bi, Y. Wang, M. Shi, J. Zhang, C. Dong, and B. Sun. 2006. Novel function of IFN-γ: negative regulation of dendritic cell migration and T cell priming. *J. Immunol.* 177: 934–943.
20. Robson, R. L., R. M. McLaughlin, J. Witowski, P. Loetscher, T. S. Wilkinson, S. A. Jones, and N. Topley. 2001. Differential regulation of chemokine production in human peritoneal mesothelial cells: IFN-γ controls neutrophil migration across the mesothelium in vitro and in vivo. J. Immunol. 167: 1028–1038.

21. Melrose, J. N., T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.

22. van der Pouw Kraan, T. C., F. A. van Gaalen, P. V. Kasperkovitz, N. L. Verbeet, T. K. Tarrant, C. C. Chan, B. Wiggert, W. H. J. Wu, H. J. Sawaya, B. Binstadt, M. Brickelmaier, A. Blasius, L. Gorelik, and R. R. Caspi. 1997. IFN-γ-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. J. Immunol. 158: 5997–6005.

23. Robson, R. L., R. M. McLoughlin, J. Witowski, P. Loetscher, T. S. Wilkinson, and P. P. Tak. 2003. Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. Arthritis Rheum. 48: 2132–2145.

24. Kopydlowski, K. M., C. A. Salkowski, M. J. Cody, N. van Roojen, M. T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.

25. Granstein, R. D., M. R. Deak, S. L. Jacques, R. J. Margolis, T. J. Flotte, T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.

26. Jones, L. S., L. V. Rizzo, R. K. Agarwal, T. K. Tarrant, C. C. Chan, B. Wiggert, W. H. J. Wu, H. J. Sawaya, B. Binstadt, M. Brickelmaier, A. Blasius, L. Gorelik, and R. R. Caspi. 1997. IFN-γ-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. J. Immunol. 158: 5997–6005.

27. Oliveira, I. C., P. J. Sciacovilino, T. H. Lee, and J. Vilcek. 1992. Downregulation of interleukin 8 gene expression in human fibroblasts: unique mechanism of transcriptional inhibition by interferon. Proc. Natl. Acad. Sci. USA 89: 9049–9053.

28. Kopydlowski, K. M., C. A. Salkowski, M. J. Cody, N. van Roojen, M. T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.

29. Wu, H. J., H. Sawaya, B. Binstadt, M. Brickelmaier, A. Blasius, L. Gorelik, U. Mahmood, R. Weissleder, J. Carulli, C. Benoist, and D. Mathis. 2007. Inflammation can be reined in by CpG-induced DC NK cell cross talk. J. Exp. Med. 204: 1911–1922.

30. Williams, A. S., P. J. Richards, E. Thomas, S. Carty, M. A. Nowell, T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.

31. Levy, D. E., and J. E. Darnell, Jr. 2002. Stats: transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol. 3: 651–662.

32. Mellado, M., J. M. Rodriguez-Frade, S. Manes, and A. C. Martinez. 2001. Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. Annu. Rev. Immunol. 19: 397–421.

33. Melrose, J. N., T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.

34. Uitto, I., J. Doody, and J. Massague. 1999. Inhibition of transforming growth factor β signaling via PIR-B. Immunity 22: 235–246.

35. Aizawa, S., C. Miyake, N. Kagara, Y. S. Che, and T. Hirano. 2004. Transforming growth factor β signaling via Ras in mesenchymal cells requires p21-activated kinase 2 for extracellular signal-regulated kinase-dependent transcriptional responses. Cancer Res. 64: 3673–3682.

36. Klesney-Tait, J., I . R. Turnbull, and M. Colonna. 2006. The TREM receptor family and signal integration. Nat. Immunol. 7: 1266–1273.

37. Raptis, Z. S., D. Shapiro, P. M. Simmons, A. M. Cheng, and C. T. Pham. 2005. Serine protease cathepsin G regulates adhesion-dependent neutrophil effector functions by modulating integrin clustering. Immunity 22: 697–699.

38. Zhang, H., F. Meng, C. L. Chu, T. Takai, and C. A. Lowell. 2005. The Src family kinases Hck and Fgr negatively regulate neutrophil and dendritic cell chemokine signaling via PIR-B. Immunity 22: 235–246.

39. Suzuki, K., M. C. Wilkes, N. Garamszegi, M. Edens, and E. B. Leof. 2007. Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizers. Nature 452: 298–302.

40. Hu, Y., and L. B. Iavshik. 2006. Costimulation of chemokine receptor signaling by matrix metalloproteinase-9 mediates enhanced migration of IFN-α dendritic cells. J. Immunol. 176: 6022–6033.

41. Suzuki, K., M. C. Wilkes, N. Garamszegi, M. Edens, and E. B. Leof. 2007. Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizers. Nature 452: 298–302.

42. Melrose, J. N., T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.

43. Melrose, J. N., T. A. Hamilton, and S. N. Vogel. 1999. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J. Immunol. 163: 1537–1544.