The Journal *of* Immunology

IL-17 Induces Monocyte Migration in Rheumatoid Arthritis

Shiva Shahrara, Sarah R. Pickens, Andrea Dorfleutner and Richard M. Pope

---

References This article **cites 43 articles**, 11 of which you can access for free at:
http://www.jimmunol.org/content/182/6/3884.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

IL-17 Induces Monocyte Migration in Rheumatoid Arthritis[1]

Shiva Shahrara,[2]* Sarah R. Pickens,* Andrea Dorfleutner,* and Richard M. Pope*[†]

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease which is in part mediated by the migration of monocytes from blood to RA synovial tissue, where they differentiate into macrophages and secrete inflammatory cytokines and chemokines. The T cell cytokine IL-17 is expressed in the RA synovial tissue and synovial fluid. To better understand the mechanism by which IL-17 might promote inflammation, its role in monocyte trafficking was examined. In vivo, IL-17 mediates monocyte migration into sponges implanted into SCID mice. In vitro, IL-17 was chemotactic, not chemokinetic, for monocytes at the concentrations detected in the RA synovial fluid. Further, IL-17-induced monocyte migration was mediated by ligation to IL-17RA and RC expressed on monocytes and was mediated through p38MAPK signaling. Finally, neutralization of IL-17 in RA synovial fluid or its receptors on monocytes significantly reduced monocyte migration mediated by RA synovial fluid. These observations suggest that IL-17 may be important in recruiting monocytes into the joints of patients with RA, supporting IL-17 as a therapeutic target in RA.   *The Journal of Immunology,* 2009, 182: 3884–3891.

I L-17 (also known as IL-17A) is the hallmark cytokine of a novel Th cell population termed TH17, which has altered the TH1/TH2 paradigm in immune biology (1, 2). IL-17 is produced by memory $CD4^+$ T lymphocytes. TGF-β, IL-1β, IL-6, and IL-23 are important for the polarization of TH17 cells from naive human $CD4^+$ T cells, and the absence of TGF-β mediates a shift from a TH17 profile to a TH1-like profile (3, 4). TH17 cell polarization is dependent on the transcription factor RORC2, the human homologue of the murine RORγt (3–5). In addition to IL-17, TH17 cells also produce IL-17F, IL-22, IL-21, IL-26, and the chemokine CCL20 (6–8), all of which may contribute to disease pathogenesis (9).

A number of observations suggest that IL-17 may be important in rheumatoid arthritis (RA).[3] IL-17 is found in RA synovial fluid and in the T cell-rich areas of RA synovial tissue (10, 11). Despite the observations by others (12), we found that TH17 cells were significantly increased in RA synovial fluid compared with RA or normal peripheral blood (13). A two-year prospective study analyzing RA synovial tissues demonstrated that IL-17 and TNF-α mRNA levels were synergistic prognostic factors for worse outcomes (14). The effects of IL-17 may be due to its ability to promote inflammation by inducing cytokines and chemokines (15, 16). Although the direct proinflammatory effects of IL-17 are often small when compared with those of IL-1β and TNF-α, IL-17 may enhance the effects of other cytokines. Using RA synovial tissue fibroblasts, IL-17 enhanced IL-1-mediated IL-6 and CCL20/MIP-3α production (17, 18) and the TNF-α-induced synthesis of IL-1, IL-6, IL-8, and CCL20 (18, 19). Therefore, a major role of IL-17 may be to amplify the effects of macrophage derived cytokines, and it may therefore be the missing link between T cells in RA joint and the effector phase of RA.

The role of IL-17 in chemotaxis has been examined. The ectopic expression of IL-17 intra-articularly enhanced neutrophil migration into the joints of mice (20). In the rat airway, IL-17 mediates neutrophil recruitment via induction of IL-8 (21). Neutrophil chemotaxis caused by conditioned medium from IL-17-stimulated gastric epithelial cells was inhibited by a neutralizing Ab to IL-8 but not to IL-17, suggesting that IL-17 is unable to directly induce neutrophil chemotaxis (22).

In contrast to its effects on neutrophils, little is known about the effect of IL-17 on the recruitment of monocytes. In the current study, therefore, we evaluated the role of IL-17 on monocyte migration. Our results demonstrate that IL-17 recruited monocytes into sponges implanted into SCID mice. In vitro, IL-17 was chemotactic for monocytes at the concentrations detected in the RA synovial fluid. Additionally, neutralizing Abs to IL-17 or to IL-17RA or RC significantly reduced RA synovial fluid-mediated monocyte migration. Taken together, these observations support the importance of IL-17 in recruiting monocytes to the RA synovial tissue. Hence, therapy directed against IL-17 may reduce inflammation by inhibiting monocyte migration into the joint.

Materials and Methods

IL-17 monocyte migration in an in vivo sponge model

Six-week-old female SCID mice (National Cancer Institutes) were anesthetized using an anesthesia machine (IMPACT 6, VetEquip). Thereafter, 8 mm × 2 mm sterile sponges (Ivalon) soaked with PBS (50 μl; $n = 9$), human IL-17 (1 μg/50 μl; $n = 5$) (R&D Systems), human IL-10 (1 μg/50 μl; $n = 4$) (R&D Systems), human IL-8 (1 μg/50 μl; $n = 3$) (R&D Systems), or human MCP-1 (1 μg/50 μl; $n = 7$; positive control) (R&D Systems) were implanted s.c. in the back of the mice. Monocytes isolated from the buffy coats of healthy donors (20, 21) were labeled with PKH26 fluorescent dye (Sigma-Aldrich) according to the manufacturer's instruction (22, 23) and successful labeling was determined using a fluorescence microscope. Labeled cells (5×10^6 cells/mouse) were injected i.v. via the tail vein. Three days later, mice were sacrificed and the sponges were retrieved and cells were eluted by forcing 4 ml 0.03% EDTA in PBS through the sponge using 3-ml syringe and then cytospun on a glass slide.

*Department of Medicine, Feinberg School of Medicine, Northwestern University Chicago, IL 60611; and [†]Jesse Brown VA Chicago Healthcare System, Chicago, IL 60611

[2] Address correspondence and reprint requests to Dr. Shiva Shahrara, Northwestern University Feinberg School of Medicine, Department of Medicine, Division of Rheumatology, McGaw Pavilion, 240 East Huron, Suite M300, Chicago, Illinois 60611. E-mail address: s-shahrara@northwestern.edu

[3] Abbreviations used in this paper: RA, rheumatoid arthritis; p, phospho; HMVEC, human microvascular endothelial cell; HPF, high power field; SF, synovial fluid; OA, osteoarthritis.
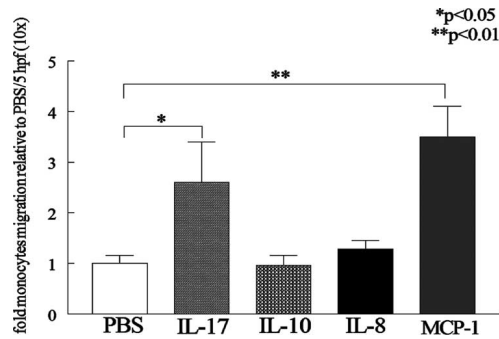
FIGURE 1. IL-17 recruits monocytes into sponge implants in SCID mice. Six-week-old female SCID were anesthetized, and thereafter sterile sponges treated with PBS (50 μl) ($n = 9$), human IL-17 (1 μg/50 μl) ($n = 5$), human IL-10 (1 μg/50 μl) ($n = 4$), human IL-8 (1 μg/50 μl) ($n = 3$), or human MCP-1 (1 μg/50 μl) ($n = 7$; positive control) were implanted s.c. in the back of the mice. Monocytes were tagged with PKH26 fluorescent dye and were injected i.v. via the tail vein. Three days later, mice were sacrificed and the sponges were retrieved and labeled monocytes eluted quantified by counting number of cells/five HPFs for each mouse in different treatment groups. Values are the mean $\pm$ SE. $*$, $p < 0.05$; $**$, $p < 0.01$.

Eluted cells from the sponge were stained with Hoechst (nucleus staining). Monocytes were quantified based on the double PKH26 (red) and Hoechst (blue) staining. Migrating labeled monocytes were quantified by counting the number of cells in five high power field (HPF) per slide prepared from cells eluted from each mouse in different treatment groups. The results were analyzed from 45 HPF in the PBS treatment group, 25 HPF in the IL-17 group, 20 HPF in the IL-10 treatment group, 15 HPF in the IL-8 group, and 35 HPF in the MCP-1 group. Data are shown as fold increase compared with random migration detected in the PBS group (Fig. 1).

Monocyte chemotaxis

Chemotaxis was performed in triplicate for 2 h in Boyden chambers (Neuroprobe) with IL-17 concentrations varying from 0.001 to 100 ng/ml (R&D Systems). FMLP (100 nM) (Sigma-Aldrich) was used as the positive control and PBS as negative control (23). To test specificity of IL-17-induced monocyte migration, monocyte chemotaxis was examined to heat-inactivated IL-17 (1 and 10 ng/ml incubated in 100°C for 15 min) or neutralization of IL-17 by an anti-IL-17 Ab or IgG control (1 or 10 μg/ml in 37°C) (R&D Systems) for 2–3 h. To examine for chemokinesis, a series of checkerboard experiments was performed by placing increasing concentrations of IL-17 (0, 0.01, 0.1, 1, and 10 ng/ml) together with monocytes in the top chamber, as well as in the bottom chamber. To define which signaling pathways mediated IL-17-induced monocyte chemotaxis, monocytes were preincubated with inhibitors to p38 (SB203580; 0.1, 1, and 10 μM), JNK (SP600125; 1, 10, and 20 μM), ERK (PD98059; 1, 20, and 50 μM) and PI3K (LY294002; 10 μM) (Calbiochem) for 1 h. Subsequently, monocyte chemotaxis was performed for 2–3 h.

To determine which IL-17 receptors are important for IL-17 monocyte migration in some experiments, Abs to IL-17 RA (Catalog number: MAB177) and RC (Catalog number: MAB2269; 10 μg/ml) (R&D Systems), TLR2 (10 μg/ml) (Imgenex), or isotype control Ab were incubated with the monocytes for 3 h before adding the cells to the Boyden chambers. Chemotaxis induced by RA synovial fluids was examined following incubation of fluids with control IgG or neutralizing anti-IL-17 Ab (10 μg/ml) for 2–3 h. The fluids were diluted 1/20 before addition to the bottom chambers. To examine whether IL-17 receptors are involved in RA synovial fluid (SF)-induced monocyte migration, monocytes were incubated with Abs to IL-17 RA and RC (10 μg/ml) as well as isotype control for 2–3 h before adding the RA SF to the bottom chambers.

Cytokine quantification

Human IL-17 (R&D Systems) ELISA kit was used according to the manufacturer's instructions.

Characterization of IL-17 signaling pathways in monocytes

Monocytes (2×10^6/ml) were untreated or treated with IL-17 (50 ng/ml) for 15 to 180 min. Cell lysates were examined by Western blot analysis as
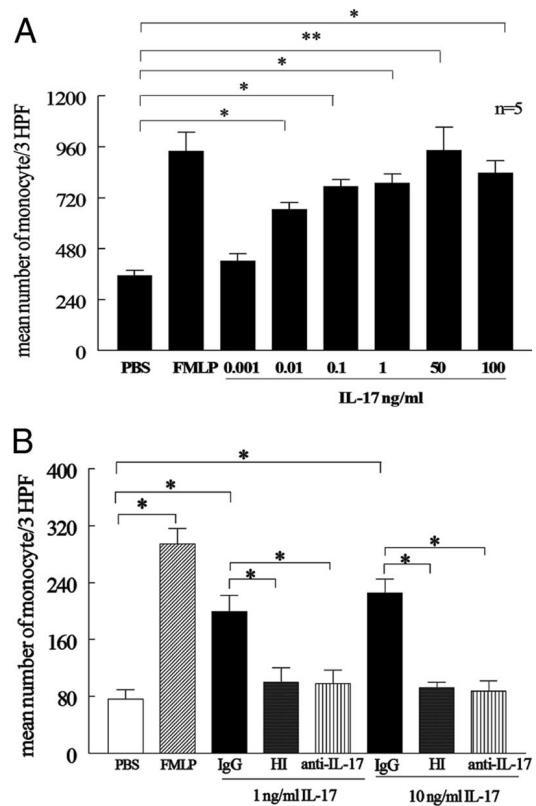

FIGURE 2. IL-17 induces monocyte migration. *A*, Dose-response curve of IL-17-induced monocyte chemotaxis. IL-17 monocyte chemotaxis was performed in a Boyden chemotaxis chamber with varying concentration of IL-17. Values demonstrate mean $\pm$ SE from three independent donors performed in triplicate. *B*, IL-17-induced monocyte chemotaxis was suppressed by heat inactivating IL-17 (both 1 and 10 ng/ml incubated in 100°C for 15 min) or neutralization of IL-17 (1 and 10 ng/ml) by anti-IL-17 Ab or IgG control (10 μg/ml 1h in 37°C) for 2–3 h. Values are the mean $\pm$ SE from three different experiments. $*$, $p < 0.05$; $**$, $p < 0.01$.

previously described (24). Blots were probed with phospho (p)-ERK, pJNK, p-p38 MAPK, and pAKT (Cell Signaling Technology; 1/1000 dilution) overnight and after stripping, were probed with ERK, JNK, p38, or AKT (Cell Signaling Technology; 1/3000 dilution) overnight. Blots were scanned and band intensities determined using UN-SCAN-IT version 5.1 software (Silk Scientific). Band intensities correspond to the sum of all pixel values in the segment selected minus the background pixel value in that segment.

		0 ng/ml IL-17	0.01 ng/ml IL-17	0.1 ng/ml IL-17	1 ng/ml IL-17	10 ng/ml IL-17
	0 ng/ml IL-17	162±25	135±30	150±35	132±38	207±42
Lower Chamber	**0.01 ng/ml IL-17**	435±52	210±55	158±21	185±37	164±28
	1 ng/ml IL-17	490±68	519±51	280±38	196±41	212±32
	10 ng/ml IL-17	526±52	580±41	435±61	380±35	216±25

(Header spanning: **Upper chamber**)

FIGURE 3. IL-17 does not induce chemokinesis. A series of checkerboard experiments were performed by placing increasing doses of IL-17 together with 2×10^6/ml monocytes in the top chamber, in addition to placing different concentrations of IL-17 in the bottom wells of the chemotaxis chamber. The data presented are representative of three independent experiments.
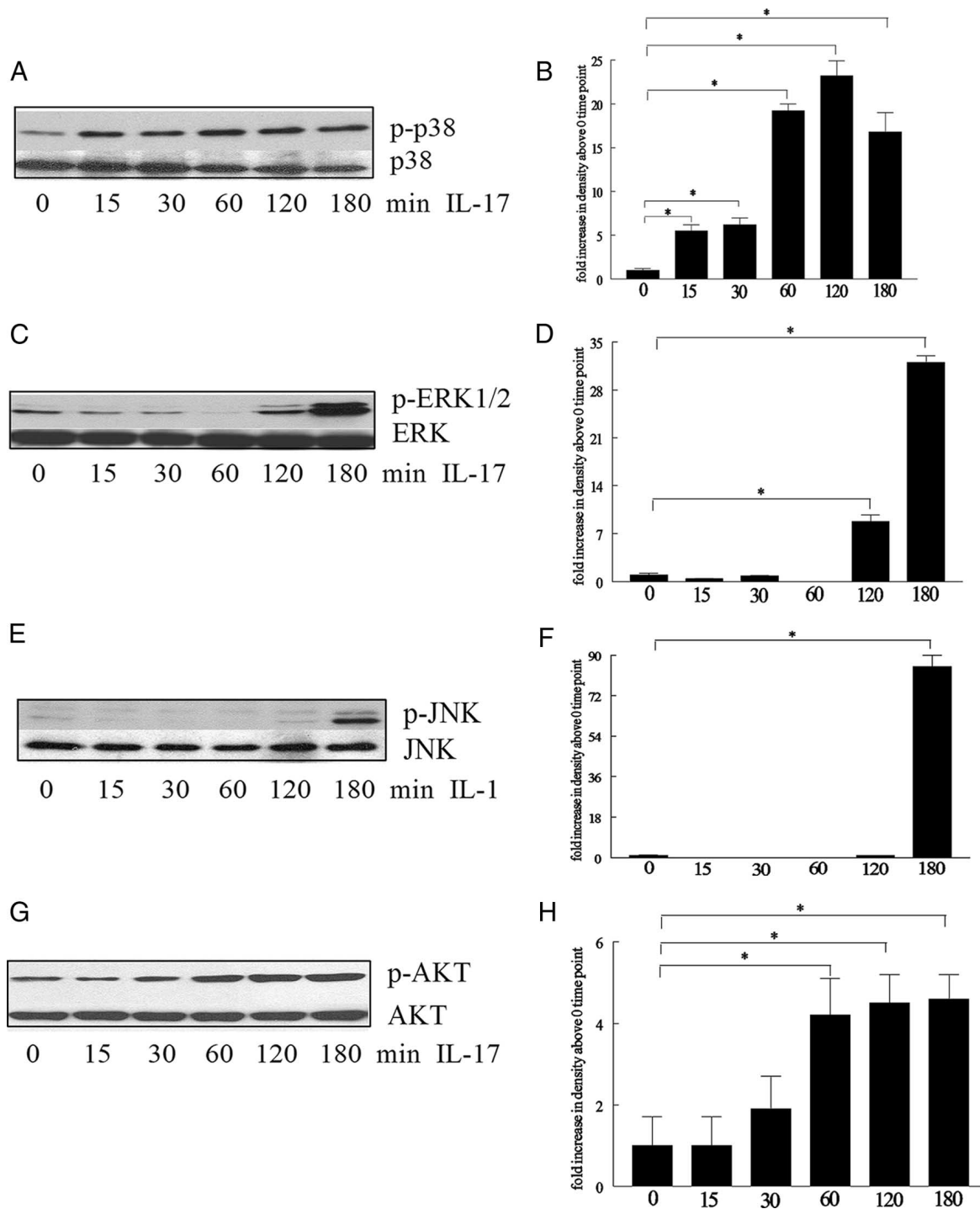
FIGURE 4. IL-17-induced monocyte migration is suppressed by p38 MAPK inhibition. To determine the mechanism of IL-17 in monocytes, cells were stimulated with IL-17 (50 ng/ml) for 0–180 min, and the cell lysates were probed for p-p38 (*A* and *B*), pERK (*C* and *D*), pJNK (*E* and *F*), and pAKT (*G* and *H*). These results are representative of three experiments. Blots were scanned and band intensities determined using UN-SCAN-IT version 5.1 software (Silk Scientific). Band intensities correspond to the sum of all pixel values in the segment selected minus the background pixel value in that segment (*B*, *D*, *F*, and *H*). Values demonstrate mean $\pm$ SE of three experiments in triplicate. $*$, $p < 0.05$.

To examine whether inhibition of p38 may effect IL-17 activation of PI3K, monocytes were treated with SB203580 10 μM or DMSO an hour before IL-17 (50 ng/ml) activation for 0 to 120 min. These cell lysates were then probed for p-p38 MAPK and p-AKT as well as p38 or AKT (Cell Signaling). To verify that 20 μM of PD98059 could effectively inhibit IL-17-induced ERK phosphorylation, monocytes were treated with PD98059 20 μM or DMSO an hour before IL-17 (50 ng/ml) activation for 0 or 180 min. To determine signaling pathways associated with IL-17 monocyte migration, monocytes were preincubated with the identified chemical inhibitors for p38 (SB203580; 0.1, 1 and 10 μM) or PI3K

(LY294002; 10 μM) as well as JNK (SP600125; 1, 10, and 20 μM) or ERK (PD98059; 1, 20, and 50 μM) and for 1 h. Subsequently, monocyte chemotaxis was performed for 2–3 h.

Detection of IL-17 receptors

Total cellular RNA for IL-17 RA and RC (Applied Biosystems) were extracted from human microvascular endothelial cells (HMVECs) and monocytes using TRIzol, and reverse transcription and Real-time RT-PCR were
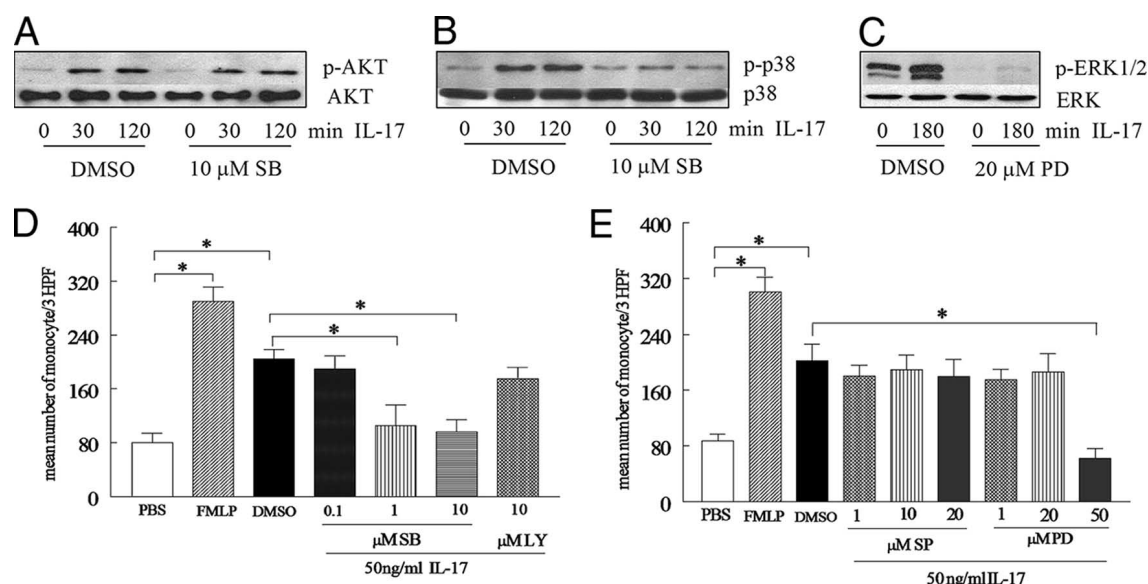
FIGURE 5. IL-17 mediates monocyte migration through p38 MAPK activation. To examine whether inhibition of p38 may affect IL-17 activation of PI3K, monocytes were also treated with SB203580 10 μM or DMSO an hour before IL-17 (50 ng/ml) activation for 0 to 120 min. Thereafter, cells were probed for p-p38 MAPK (*A*) and pAKT (1/1000 dilution) (*B*) as well as p38 or AKT (1/3000 dilution). *C*, To ensure that 20 μM of PD98059 could effectively inhibit IL-17-induced ERK phosphorylation, monocytes were treated with PD98059 20 μM or DMSO an hour before IL-17 (50 ng/ml) activation for 0 or 180 min (experiments were done in duplicates). To determine signaling pathways associated with IL-17 monocyte migration, monocytes were preincubated with the identified chemical inhibitors for p38 (SB203580; 0.1, 1, and 10 μM) or PI3K (LY294002; 10 μM) (*D*) as well as JNK (SP600125; 1, 10, and 20 μM) or ERK (PD98059; 1, 20, and 50 μM) (*E*) and for 1 h. Subsequently, monocyte chemotaxis was performed for 2–3 h. Values demonstrate mean $\pm$ SE of three experiments in triplicate. *, $p < 0.05$.

performed as previously described (13, 25). Relative gene expression was determined by the $\Delta\Delta C_t$ method.

Using mAbs to human IL-17 RA and RC (R&D Systems; 1 μg/ml) the protein expression of these receptors was determined in untreated HMVECs and monocytes from three donors. Blots were stripped and probed with actin (Sigma-Aldrich; 1/3000 dilution).

Statistical analysis

The data were analyzed using the 2-tailed Student's t tests for paired and unpaired samples. The p values <0.05 were considered significant.

Results

IL-17 induces monocyte migration in vivo

In preliminary studies, we observed that IL-17 in vivo resulted in the accumulation of macrophages in the joints of experimental animals (data not shown). Therefore, experiments were performed to determine whether IL-17 might promote the recruitment of monocytes in vivo using sponges placed s.c. After 3 days, the number of murine cells that had migrated into the sponges soaked with PBS, MCP-1, IL-8, IL-10, and IL-17 was similar. In contrast, the number of labeled human monocytes that migrated into the sponges was significantly ($p < 0.05$) increased by IL-17 compared with PBS (Fig. 1). The number of monocytes attracted to the positive control, MCP-1, was increased compared with PBS ($p < 0.05$). In contrast, neither IL-8 nor IL-10 induced monocyte migration into the sponges. Therefore, these observations suggest that IL-17 may be chemotactic for monocytes while IL-8 and IL-10 are not.

IL-17 is chemotactic for monocytes

Next, experiments were performed to determine whether IL-17 was directly chemotactic for monocytes. Using Boyden chambers, IL-17 was chemotactic for monocytes at concentrations ranging from 0.01 ng/ml ($p < 0.05$) to 100 ng/ml ($p < 0.01$) (Fig. 2*A*). Heat inactivation of IL-17, or incubation of IL-17 with neutralizing

Abs to IL-17, suppressed monocyte migration (Fig. 2*B*). Consistent with these data, in Fig. 2*B*, 10 μg/ml anti-IL-17 neutralized 10 ng/ml rIL-17, a concentration that was greater than that observed in the synovial fluids. These observations suggest that IL-17 is capable of mediating monocyte migration.

Next, experiments were performed to determine whether the effects of IL-17 were mediated through chemokinesis. The presence of higher concentrations of IL-17 in the upper chamber did not enhance migration of monocytes (Fig. 3). Likewise, when the concentrations of IL-17 in the upper and lower chamber were the same, little or no enhancement of migration was observed (Fig. 3). Taken together, our results suggest that IL-17 mediates monocyte chemotaxis.

p38 MAPK blockade inhibits IL-17-induced monocyte migration

Experiments were performed to determine the monocyte signaling pathway(s) responsible for monocyte chemotaxis induced by IL-17. Because the monocyte chemotaxis assays were performed for 2 h, IL-17-activated signaling pathways were analyzed between 0 and 180 min. The ability of IL-17 to activate the pathways examined was determined by phosphorylation of MAPK mediators and AKT. The MAPK p38 pathway was activated as early as 15 min (Fig. 4, *A* and *B*), followed by AKT at 60 min (Fig. 4, *C* and *D*). However, ERK and JNK were not activated until 120 and 180 min, respectively (Fig. 4, *E* and *F* and Fig. 4, *G* and *H*).

To demonstrate that inhibition of p38 specifically blocks p38 but not pAKT, monocytes were treated with p38 inhibitor (SB203580 10 μM) or control an hour before IL-17 activation. Results from these studies demonstrate that inhibition of p38 MAPK in monocytes had no effect on activation of AKT by IL-17, indicating that p38 MAPK is not upstream PI3K signaling pathway (Fig. 5, *A* and *B*).
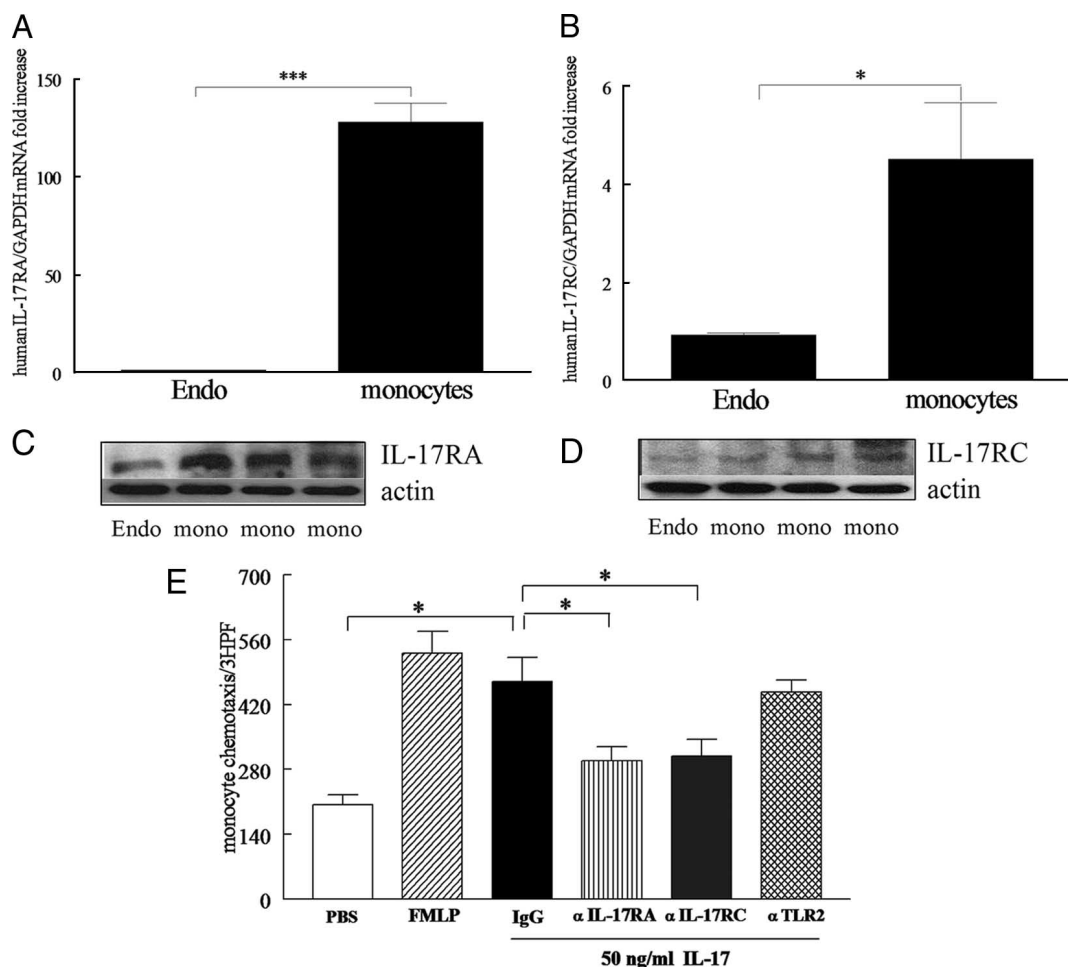
FIGURE 6. Human peripheral blood monocytes express IL-17 RA and RC, which are important for IL-17 mediated monocyte migration. Real-time RT-PCR (*A* and *B*) and Western blot analysis (*C* and *D*) were used to quantify IL-17 RA and RC expression levels in monocytes compared with HMVECs (Endo). mRNA and protein values were normalized to GAPDH or actin content, respectively. IL-17-induced monocyte chemotaxis was suppressed by neutralizing anti-IL-17 receptor Abs. Monocytes were incubated with mouse anti-human IL-17 RA or RC Ab (10 μg/ml), control IgG (10 μg/ml), and anti-TLR2 Ab (10 μg/ml) for 3 h. Thereafter, monocyte chemotaxis was performed in response to IL-17 (50 ng/ml) for 2–3 h. Values are the mean $\pm$ SE from three different experiments. *, $p < 0.05$.

To determine which of these pathways may contribute to IL-17-mediated chemotaxis, monocytes were then preincubated with inhibitors of the ERK, JNK, p38, and PI3K pathways before performing the chemotaxis. Only inhibition of the MAPK p38 (1 and 10 μM) pathway significantly reduced IL-17-induced monocyte migration (Fig. 5*D*). Different concentrations of inhibitors of the PI3K (10 μM), ERK (1, 20, and 50 μM), and JNK (1, 10, and 20 μM) pathways were unable to inhibit IL-17-mediated monocyte migration except at the highest concentration of the ERK inhibitor (50 μM), which was toxic for monocytes, as determined by trypan blue staining (Fig. 5, *D* and *E*). None of the other inhibitors were toxic at the concentrations used. Because 50 μM of ERK inhibitor reduced IL-17 induced monocyte chemotaxis due to its toxic effect on monocytes, and monocyte chemotaxis was not affected by 20 μM of PD98059, experiments were performed to ensure that 20 μM of PD98059 was efficient in blocking IL-17 induced ERK phosphorylation in monocytes (Fig. 5*C*). These results suggest that IL-17 can directly mediate monocyte migration through activating the p38 MAPK pathway.

Monocytes express IL-17 RA and RC, which are involved in IL-17 mediated monocyte chemotaxis

Experiments were performed to determine which IL-17R were involved with monocyte migration. Using real-time RT-PCR,

we demonstrated that the expression levels of IL-17 RA and RC (Fig. 6, *A* and *B*) on monocytes are significantly higher than that of HMVECs, with IL-17 RA being more the prominent receptor in monocytes compared with IL-17 RC. The Western blot data also confirmed that monocytes express both receptors (Fig. 6, *C* and *D*). We found that both anti-IL-17 RA and RC Abs are efficient in reducing IL-17-induced IL-6 production in RA synovial tissue fibroblasts (data not shown). Neutralization of monocyte IL-17 receptors by adding anti-IL-17 RA and RC Abs to monocytes before their addition to the Boyden chamber suppressed IL-17-mediated chemotaxis (Fig. 6*E*). Control IgG or Ab to TLR2 did not suppress IL-17-mediated chemotaxis (Fig. 6*E*). These results indicate that both IL-17 receptors contribute to monocyte migration.

IL-17 and its receptors significantly contribute to RA SF-induced monocyte chemotaxis

Studies were performed to determine whether the IL-17 identified in RA synovial fluid was chemotactic for monocytes. The mean concentration of IL-17 in the 30 RA synovial fluids analyzed was 233 $\pm$ 64 pg/ml (Fig. 7*A*), a concentration that was highly chemotactic (Fig. 2*A*). The concentration of IL-17 in the RA synovial fluids was significantly greater than observed in osteoarthritis synovial fluid or RA or normal serum. Confirming our results others
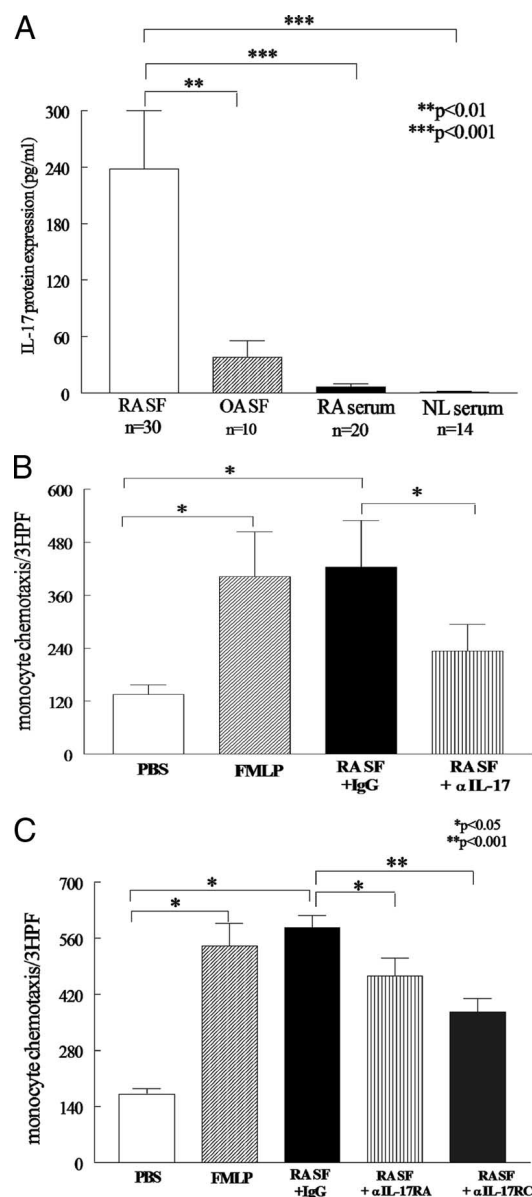
FIGURE 7. RA synovial fluid-induced monocyte chemotaxis is mediated by IL-17. *A*, The levels of IL-17 were quantified in RA and osteoarthritis (OA) synovial fluid and RA and normal (NL) peripheral blood (PB) by ELISA. To investigate the role of IL-17 in RA SFs (*B*), anti-IL-17 (10 μg/ml) or control IgG was added to RA synovial fluids from nine patients (1/20 dilution) (2–3 h) before performing monocyte chemotaxis in response to the RA SFs. *C*, Monocytes were incubated with Abs to IL-17 RA and RC (10 μg/ml), as well as isotype control for 2–3 h before performing monocyte chemotaxis in response to six RA SFs. The values presented represent the mean $\pm$ SE. *, $p < 0.05$; **, $p < 0.01$; and ***, denotes $p < 0.001$.

have demonstrated that IL-17 levels are markedly increased in RA synovial fluid compared with osteoarthritis (OA) synovial fluid (26, 27). The basal levels of IL-17 were not different in RA pheripheral blood compared with that of OA patients. However, peripheral blood cells activated with PHA resulted in higher production of IL-17 in RA compared with OA patients (28).

RA synovial fluid was chemotactic for monocytes, similar to the positive control FMLP (Fig. 7*B*). Next, experiments were performed to determine whether the chemotaxis mediated by RA synovial fluid was mediated by IL-17. Neutralization of IL-17 using a mAb to IL-17 significantly reduced (40%; $p < 0.05$) monocyte

chemotaxis compared with control IgG-treated RA synovial fluids (Fig. 7*B*). Using the current data, set there was no significant correlation (r = 0.03) between the levels of IL-17 in the RA synovial fluid (up to 500 pg/ml) and the percent chemotaxis reduction achieved by using anti-IL-17 at 10 μg/ml. Consistent with these data, in Fig. 2*B*, 10 μg/ml anti-IL-17 neutralized 10 ng/ml rIL-17, a concentration that was greater than that observed in the synovial fluids.

Additionally, neutralization of IL-17 RA and RC on monocytes was effective in suppressing RA SF-mediated monocyte migration (Fig. 7*C*). These results suggest that IL-17 and its receptors IL-17 RA and RC may play an important role in migration of monocytes into the joints of patients with RA.

Discussion

In this study, we demonstrate that IL-17 promotes monocyte recruitment using an in vivo sponge model, supporting the novel role of IL-17 in monocyte migration. Therefore, studies were performed to determine whether IL-17 might directly mediate monocyte recruitment in vitro and whether a pathogenic role of IL-17 in RA may in part be due to its ability to promote monocyte migration. In the present study, in vitro data demonstrates that monocyte migration is mediated by a direct effect of IL-17 on monocytes, because heat inactivation, neutralization of IL-17, and antagonist Abs to IL-17 RA and RC, abrogate IL-17-mediated monocyte chemotaxis. Further, IL-17 is chemotactic for monocytes at concentrations detected in RA synovial fluid, and neutralization of IL-17 suppressed the monocyte chemotaxis induced with RA synovial fluid.

Previous studies demonstrated that intratracheal administration of IL-17-mediated neutrophil migration by inducing the expression of MIP-2 (rat analog of IL-8) (21). Neutralization of MIP-2 abrogated IL-17-induced neutrophil migration into the rat airways, suggesting an indirect effect of IL-17 via chemokine production (21). In contrast to our results with monocytes, IL-17 did not directly mediate neutrophil migration in vitro (22).

Next, experiments were performed to define the pathway(s) mediating IL-17 monocyte chemotaxis. IL-17 activates the p38, ERK, JNK, and AKT pathways. The phosphorylation of p38 and AKT occurred within 1 h, while activation of ERK and JNK was not observed until 2–3 h. It is possible that IL-17 activation of ERK and JNK is downstream of the PI3K pathway, and therefore phosphorylation of these specific signaling molecules occurs subsequent to activation of PI3K as shown in other cell types (29, 30). Inhibition of each of these signaling pathways demonstrated that only p38 MAPK mediates IL-17-induced monocyte migration. Efforts to use a more specific means to suppress chemotaxis, using p38 siRNA, were not successful, because incubation of monocytes with nonspecific siRNA interfered with monocyte chemotaxis. Similar to our results with IL-17, CCL2/MCP-1, CCL5/RANTES, and CCL3/MIP-1α mediated monocyte chemotaxis was dependent on p38 (31), while the MCP-1-induced endothelial migration was through activation of ERK and PI3K (32). Also similar to the results with IL-17, monocyte migration induced by CCL2/MCP-1, CCL5/RANTES, CCL3/MIP-1α, and FMLP was not mediated through PI3K or ERK signaling (33). The effects of p38 activation may be mediated through actin filament reorganization, which is essential for monocyte recruitment (34). HSP27 and lymphocyte specific protein are two substrates activated by p38, and are associated with regulation of actin filament dynamics (35–37). Taken together, p38 MAPK seems to be an important signaling

pathway for monocyte migration that is used by IL-17, as well as other monocyte chemokines.

Although IL-17 is secreted from TH-17 memory T cells, IL-17 receptor is widely expressed on many cell types including RA synovial tissue fibroblasts and peripheral blood monocytes (10, 11). Using real-time RT-PCR and Western blot analysis, we demonstrated that both IL-17RA and IL-17RC are expressed by monocytes. Because the monocyte migration induced by the cytokine macrophage migration inhibitory factor is mediated through binding to G coupled protein receptor CXCR2 (38), we asked whether monocyte trafficking induced by IL-17 was due to binding to IL-17 RA or RC. IL-17-mediated monocyte chemotaxis was suppressed by neutralization of either IL-17 RA or RC, suggesting that that the effects of IL-17 were not mediated by the promiscuous binding of IL-17 to a classical, the seven trans-membrane domain, chemotactic receptor.

IL-17 contributed to the chemotaxis induced by RA synovial fluid. Neutralizing Abs to IL-17 or antagonist Abs to IL-17 RA and RC reduced the RA synovial fluid-induced monocyte migration, suggesting that IL-17 may play an important role in the recruitment of monocytes into RA synovial tissue. Other factors in RA synovial fluid capable of inducing monocyte migration include: CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, CXCL16, CCL20/MIP-3α, and CX3CL1/fractalkine (23, 39–42), which are predominately produced by RA synovial tissue fibroblasts and macrophages. In contrast to these chemokines, IL-17 is secreted by RA synovial tissue memory T cells, suggesting a distinct role of T cells in the pathogenesis of RA. In addition to the direct effects on monocyte chemotaxis, IL-17 may also mediate monocyte chemotaxis through induction of monocyte chemokines from RA synovial fibroblasts (CCL7 and CCL20) (43).

In conclusion, we demonstrate that IL-17 induces monocyte migration in vitro and in vivo. IL-17-mediated monocyte migration was dependent on p38 activation and was associated with binding to IL-17 RA and RC on monocytes. Neutralization of IL-17 and its receptors significantly reduced monocyte chemotaxis induced by RA synovial fluid, suggesting that IL-17 may play an important role in monocyte ingress into inflamed RA synovial tissue.

Disclosures

The authors have no financial conflict of interest.

References

1. Harrington, L. E., R. D. Hatton, P. R. Mangan, H. Turner, T. L. Murphy, K. M. Murphy, and C. T. Weaver. 2005. Interleukin 17-producing CD4$^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6: 1123–1132.
2. Dong, C. 2006. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. *Nat. Rev. Immunol.* 6: 329–333.
3. Manel, N., D. Unutmaz, and D. R. Littman. 2008. The differentiation of human TH-17 cells requires transforming growth factor-β and induction of the nuclear receptor RORγt. *Nat. Immunol.* 9: 641–649.
4. Volpe, E., N. Servant, R. Zollinger, S. I. Bogiatzi, P. Hupe, E. Barillot, and V. Soumelis. 2008. A critical function for transforming growth factor-β, interleukin 23 and proinflammatory cytokines in driving and modulating human TH-17 responses. *Nat. Immunol.* 9: 650–657.
5. Yang, L., D. E. Anderson, C. Baecher-Allan, W. D. Hastings, E. Bettelli, M. Oukka, V. K. Kuchroo, and D. A. Hafler. 2008. IL-21 and TGF-β are required for differentiation of human TH17 cells. *Nature* 454: 350–352.
6. Chen, Z., C. M. Tato, L. Muul, A. Laurence, and J. O'Shea. 2007. Distinct regulation of interleukin-17 in human T helper lymphocytes. *Arthritis Rheum.* 56: 2936–2946.
7. Wilson, N. J., K. Boniface, J. R. Chan, B. S. McKenzie, W. M. Blumenschein, J. D. Mattson, B. Basham, K. Smith, T. Chen, F. Morel, et al. 2007. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat. Immunol.* 8: 950–957.
8. Acosta-Rodriguez, E. V., G. Napolitani, A. Lanzavecchia, and F. Sallusto. 2007. Interleukins 1β and 6 but not transforming growth factor-β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol.* 8: 942–949.
9. Ouyang, W., J. K. Kolls, and Y. Zheng. 2008. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 28: 454–467.
10. Stamp, L. K., M. J. James, and L. G. Cleland. 2004. Interleukin-17: the missing link between T-cell accumulation and effector cell actions in rheumatoid arthritis? *Immunol. Cell Biol.* 82: 1–9.
11. Lubberts, E., M. I. Koenders, and W. B. van den Berg. 2005. The role of T-cell interleukin-17 in conducting destructive arthritis: lessons from animal models. *Arthritis Res. Ther.* 7: 29–37.
12. Yamada, H., Y. Nakashima, K. Okazaki, T. Mawatari, J. I. Fukushi, N. Kaibara, A. Hori, Y. Iwamoto, and Y. Yoshikai. 2008. Th1 but not Th17 cells predominate in the joints of patients with rheumatoid arthritis. *Ann. Rheum. Dis.* 67: 1299–1304.
13. Shahrara, S., Q. Huang, A. M. Mandelin, 2nd, and R. M. Pope. 2008. TH-17 cells in rheumatoid arthritis. *Arthritis Res. Ther.* 10: R93.
14. Kirkham, B. W., M. N. Lassere, J. P. Edmonds, K. M. Juhasz, P. A. Bird, C. S. Lee, R. Shnier, and I. J. Portek. 2006. Synovial membrane cytokine expression is predictive of joint damage progression in rheumatoid arthritis: a two-year prospective study (the DAMAGE study cohort). *Arthritis Rheum.* 54: 1122–1131.
15. Lundy, S. K., S. Sarkar, L. A. Tesmer, and D. A. Fox. 2007. Cells of the synovium in rheumatoid arthritis. T lymphocytes. *Arthritis Res. Ther.* 9: 202.
16. Gaffen, S. L. 2004. Biology of recently discovered cytokines: interleukin-17: a unique inflammatory cytokine with roles in bone biology and arthritis. *Arthritis Res. Ther.* 6: 240–247.
17. Chabaud, M., F. Fossiez, J. L. Taupin, and P. Miossec. 1998. Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines. *J. Immunol.* 161: 409–414.
18. Chabaud, M., G. Page, and P. Miossec. 2001. Enhancing effect of IL-1, IL-17, and TNF-α on macrophage inflammatory protein-3α production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. *J. Immunol.* 167: 6015–6020.
19. Katz, Y., O. Nadiv, and Y. Beer. 2001. Interleukin-17 enhances tumor necrosis factor α-induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible role as a "fine-tuning cytokine" in inflammation processes. *Arthritis Rheum.* 44: 2176–2184.
20. Lubberts, E., L. A. Joosten, B. Oppers, L. van den Bersselaar, C. J. Coenen-de Roo, J. K. Kolls, P. Schwarzenberger, F. A. van de Loo, and W. B. van den Berg. 2001. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. *J. Immunol.* 167: 1004–1013.
21. Laan, M., Z. H. Cui, H. Hoshino, J. Lotvall, M. Sjostrand, D. C. Gruenert, B. E. Skoogh, and A. Linden. 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J. Immunol.* 162: 2347–2352.
22. Luzza, F., T. Parrello, G. Monteleone, L. Sebkova, M. Romano, R. Zarrilli, M. Imeneo, and F. Pallone. 2000. Up-regulation of IL-17 is associated with bioactive IL-8 expression in *Helicobacter pylori*-infected human gastric mucosa. *J. Immunol.* 165: 5332–5337.
23. Ruth, J. H., S. Shahrara, C. C. Park, J. C. Morel, P. Kumar, S. Qin, and A. E. Koch. 2003. Role of macrophage inflammatory protein-3α and its ligand CCR6 in rheumatoid arthritis. *Lab. Invest.* 83: 579–588.
24. Pagliari, L. J., H. Perlman, H. Liu, and R. M. Pope. 2000. Macrophages require constitutive NF-κB activation to maintain A1 expression and mitochondrial homeostasis. *Mol. Cell Biol.* 20: 8855–8865.
25. Huang, Q., Y. Ma, A. Adebayo, and R. M. Pope. 2007. Increased macrophage activation mediated through toll-like receptors in rheumatoid arthritis. *Arthritis Rheum.* 56: 2192–2201.
26. Kotake, S., N. Udagawa, N. Takahashi, K. Matsuzaki, K. Itoh, S. Ishiyama, S. Saito, K. Inoue, N. Kamatani, M. T. Gillespie, et al. 1999. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest.* 103: 1345–1352.
27. Kageyama, Y., T. Ichikawa, T. Nagafusa, E. Torikai, M. Shimazu, and A. Nagano. 2007. Etanercept reduces the serum levels of interleukin-23 and macrophage inflammatory protein-3 α in patients with rheumatoid arthritis. *Rheumatol. Int.* 28: 137–143.
28. Kim, K. W., M. L. Cho, M. K. Park, C. H. Yoon, S. H. Park, S. H. Lee, and H. Y. Kim. 2005. Increased interleukin-17 production via a phosphoinositide 3-kinase/Akt and nuclear factor κB-dependent pathway in patients with rheumatoid arthritis. *Arthritis Res. Ther.* 7: R139–148.
29. Chang, L. C., and J. P. Wang. 1999. Examination of the signal transduction pathways leading to activation of extracellular signal-regulated kinase by formyl-methionyl-leucyl-phenylalanine in rat neutrophils. *FEBS Lett.* 454: 165–168.
30. Arndt, P. G., N. Suzuki, N. J. Avdi, K. C. Malcolm, and G. S. Worthen. 2004. Lipopolysaccharide-induced c-Jun NH$_2$-terminal kinase activation in human neutrophils: role of phosphatidylinositol 3-Kinase and Syk-mediated pathways. *J. Biol. Chem.* 279: 10883–10891.
31. Ayala, J. M., S. Goyal, N. J. Liverton, D. A. Claremon, S. J. O'Keefe, and W. A. Hanlon. 2000. Serum-induced monocyte differentiation and monocyte chemotaxis are regulated by the p38 MAP kinase signal transduction pathway. *J. Leukocyte Biol.* 67: 869–875.
32. Arefieva, T. I., N. B. Kukhtina, O. A. Antonova, and T. L. Krasnikova. 2005. MCP-1-stimulated chemotaxis of monocytic and endothelial cells is dependent on activation of different signaling cascades. *Cytokine* 31: 439–446.

33. Fine, J. S., H. D. Byrnes, P. J. Zavodny, and R. W. Hipkin. 2001. Evaluation of signal transduction pathways in chemoattractant-induced human monocyte chemotaxis. *Inflammation* 25: 61–67.

34. Mairesse, N., S. Horman, R. Mosselmans, and P. Galand. 1996. Antisense inhibition of the 27 kDa heat shock protein production affects growth rate and cytoskeletal organization in MCF-7 cells. *Cell Biol. Int.* 20: 205–212.

35. Guay, J., H. Lambert, G. Gingras-Breton, J. N. Lavoie, J. Huot, and J. Landry. 1997. Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. *J. Cell Sci.* 110: 357–368.

36. Landry, J., and J. Huot. 1995. Modulation of actin dynamics during stress and physiological stimulation by a signaling pathway involving p38 MAP kinase and heat-shock protein 27. *Biochem. Cell Biol.* 73: 703–707.

37. Huang, C. K., L. Zhan, Y. Ai, and J. Jongstra. 1997. LSP1 is the major substrate for mitogen-activated protein kinase-activated protein kinase 2 in human neutrophils. *J. Biol. Chem.* 272: 17–19.

38. Bernhagen, J., R. Krohn, H. Lue, J. L. Gregory, A. Zernecke, R. R. Koenen, M. Dewor, I. Georgiev, A. Schober, L. Leng, et al. 2007. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med.* 13: 587–596.

39. Tokuhira, M., S. Hosaka, M. V. Volin, G. K. Haines, 3rd, K. J. Katschke, Jr., S. Kim, and A. E. Koch. 2000. Soluble vascular cell adhesion molecule 1 mediation of monocyte chemotaxis in rheumatoid arthritis. *Arthritis Rheum.* 43: 1122–1133.

40. Volin, M. V., M. R. Shah, M. Tokuhira, G. K. Haines, J. M. Woods, and A. E. Koch. 1998. RANTES expression and contribution to monocyte chemotaxis in arthritis. *Clin. Immunol. Immunopathol.* 89: 44–53.

41. Ruth, J. H., C. S. Haas, C. C. Park, M. A. Amin, R. J. Martinez, G. K. Haines, 3rd, S. Shahrara, P. L. Campbell, and A. E. Koch. 2006. CXCL16-mediated cell recruitment to rheumatoid arthritis synovial tissue and murine lymph nodes is dependent upon the MAPK pathway. *Arthritis Rheum.* 54: 765–778.

42. Ruth, J. H., M. V. Volin, G. K. Haines III, D. C. Woodruff, K. J. Katschke, Jr., J. M. Woods, C. C. Park, J. C. M. Morel, and A. E. Koch. 2001. Fractalkine, a novel chemokine in rheumatoid arthritis and in rat adjuvant-induced arthritis. *Arthritis Rheum.* 44: 1568–1581.

43. Zrioual, S., M. L. Toh, A. Tournadre, Y. Zhou, M. A. Cazalis, A. Pachot, V. Miossec, and P. Miossec. 2008. IL-17RA and IL-17RC receptors are essential for IL-17A-induced ELR$^+$ CXC chemokine expression in synoviocytes and are overexpressed in rheumatoid blood. *J. Immunol.* 180: 655–663.