A Novel Role for Interleukin-18 in Adhesion Molecule Induction through NFκB and Phosphatidylinositol (PI) 3-Kinase-dependent Signal Transduction Pathways*

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Interleukin-18 (IL-18) is a novel proinflammatory cytokine found in serum and joints of patients with rheumatoid arthritis (RA). We studied a novel role for IL-18 in mediating cell adhesion, a vital component of the inflammation found in RA and other inflammatory diseases. We examined the expression of cellular cell adhesion molecules E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells and RA synovial fibroblasts using flow cytometry. Adhesion of the monocyte-like cell line HL-60 to endothelial cells was determined by immunofluorescence. IL-18 significantly enhanced ICAM-1 and VCAM-1 expression on endothelial cells and RA synovial fibroblasts. In addition, IL-18 induced E-selectin expression on endothelial cells and promoted the adhesion of HL-60 cells to IL-18-stimulated endothelial cells. Neutralizing anti-VCAM-1 and anti-E-selectin could completely inhibit HL-60 adherence to endothelial cells. IL-18-induced adhesion molecule expression appears to be mediated through nuclear factor κB (NFκB) and phosphatidyl-inositol 3 kinase (PI 3-kinase) since addition of inhibitors to either NFκB (pyrrolidine dithiocarbamate and N-acetyl-L-cysteine) or PI 3-kinase (LY294002) inhibited RA synovial fibroblast VCAM-1 expression by 50 to 60%. Addition of both inhibitors resulted in inhibition of VCAM-1 expression by 85%. In conclusion, the ability of IL-18 to induce adhesion molecule expression on endothelial cells and RA synovial fibroblasts indicates that IL-18 may contribute to RA joint inflammation by enhancing the recruitment of leukocytes into the joint. IL-18 requires NFκB as well as PI 3-kinase to induce VCAM-1 on RA synovial fibroblasts, suggesting that there may be two distinct pathways in IL-18-induced adhesion molecule expression.

Adhesion molecules have been classified based on structure into three major groups: selectins, integrins, and the immunoglobulin (Ig) supergene family. The Ig supergene family contains diverse proteins that share the same immunoglobulin amino acid domains including adhesion proteins, such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), as well as proteins not involved in adhesion, such as the T cell receptor or major histocompatibility complex proteins HLA-DR.

Rheumatoid arthritis (RA) is a chronic progressive rheumatic disease characterized by the proliferation of the synovial membrane, which leads to the degradation of articular cartilage and subchondral bone. Another characteristic of RA synovial tissue is the exuberant leukocyte infiltration often present around the newly formed blood vessels. These inflammatory cells in the RA synovium are derived from the peripheral blood, and there is clear evidence that adhesion molecules expressed on endothelial cells mediate the migration of the leukocytes into the synovial membrane. An important example of this process is the localized endothelial expression of VCAM-1 and the selective recruitment of mononuclear leukocytes through its integrin counterreceptor, very late antigen-4 (VLA-4).

Adhesion molecules are also involved in interactions between leukocytes and RA synovial fibroblasts. During this cell/cell contact, adhesion molecules act as costimulators resulting in the activation of transcription factors and the production of cytokines, metalloproteinases, or other effect molecules.

Interleukin-18 (IL-18) is a novel cytokine that has been classified in the IL-1 family in virtue of structural similarity to IL-1β. IL-18 acts as a strong inducer and coinducer of interferon γ (IFNγ) production in T cells and natural killer cells. Recent studies suggest that IL-18 also has some proinflammatory activities independent of IFNγ. IL-18 induces the synthesis of tumor necrosis factor α (TNF-α), granulocyte-macrophage colony stimulating factor, nitric oxide, and chemokines by T cells and natural killer cells. Others and we showed that IL-18 operates through nuclear factor κB (NFκB) in natural killer cells, T lymphocytes, and RA synovial fibroblasts (8–10).

The signaling cascade leading to NFκB activation involves several intermediary molecules that finally lead to the activation of inhibitory κB (IκB). Activated IκB releases NFκB, which migrates into the nucleus and induces gene transcription (8, 11). IL-18 has been found in synovial tissue, and enhanced levels of IL-18 were measured in the joint and in the serum of RA patients (12). The role of IL-18 in the pathogenesis of RA

The abbreviations used are: ICAM, intercellular adhesion molecule; VCAM, vascular adhesion molecule; RA, rheumatoid arthritis; IL, interleukin; IFNγ, interferon γ; TNF, tumor necrosis factor; NFκB, nuclear factor κB; IFN, interferon; IκB, inhibitory κB; HMVEC, human dermal microvascular endothelial cells; rhu, recombinant human; FBS, fetal bovine serum; P/S, penicillin/streptomycin; PDTC, pyrrolidine dithiocarbamate; NAC, N-acetyl-L-cysteine; E-selectin, endothelial selectin; HMEC, human dermal endothelial cells; VLA, very late antigen; MAPK, mitogen-activated protein kinase.

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remains poorly understood. However, recent evidence suggests that IL-18 enhances the infiltration of inflammatory cells into the synovial tissue (13, 14). Therefore, we examined the ability of IL-18 to induce endothelial and RA synovial fibroblast adhesion molecule expression and the mechanism of this expression. Here we demonstrate that IL-18 up-regulates ICAM-1 and VCAM-1 either on RA synovial fibroblasts or human dermal microvascular endothelial cells (HMVECs), and we identify IL-18 as a novel inducer of E-selectin on HMVECs. Furthermore, IL-18 promotes endothelial cell-leukocyte adhesion and appears to act in this system via VCAM-1 and E-selectin.

We provide evidence that IL-18 induces RA synovial fibroblast VCAM-1 expression through NFκB. Finally, we report an alternative pathway involving PI 3-kinase, which influences the level of IL-18-induced VCAM-1 in RA synovial fibroblasts.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Recombinant human (rhu) IL-18 (specific activity 9.1 × 10^7 units/mg) was purchased from R&D Systems (Minneapolis, MN), rhu IL-18 (specific activity 4.1 × 10^7 units/mg) from Prepotech (Rocky Hill, NJ) was used for some adhesion assay experiments. rhu TNF-α (specific activity 1.3 × 10^7 units/mg) was obtained from Upjohn Co. (Kalamazoo, MI). rhu IFNγ was obtained from Becton Dickinson (specific activity 2.4 × 10^7 units/mg). Monoclonal mouse anti-human VCAM-1, clones 4B9 and GH12, which respectively recognize domains 1 and 4 of VCAM-1 (15, 16) and monoclonal mouse anti-E-selectin (clone BB11) were a generous gift from Dr. Roy Lobb (Biogen, Cambridge, MA), and monoclonal mouse anti-ICAM-1 (clone BB2) was a generous gift from Drs. Tim Carlos and John Harlan. Isotype-matched antibodies (IgG1, IgG2a, and IgG3) were purchased from Coulter (Miami, FL). Polyclonal rabbit anti-RelA was acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Phycoerythrin-conjugated goat anti-mouse antibody was purchased from Jackson ImmunoResearch (West Grove, PA). RPMI 1640, fetal bovine serum (FBS) and penicillin/streptomycin (P/S) from Sigma. Specific inhibitors LY294002, PD98059, PP2, and SB203580 were obtained from Calbiochem (La Jolla, CA). Endothelial cell basal medium, and the bullet kit containing media supplements were purchased from Life Technologies, Inc. Endothelial cell basal medium, and the bullet kit containing media supplements were purchased from Life Technologies, Inc. Pyrrolidine dithiocarbamate (PDTC) and N-acetyl-l-cysteine (NAC) were acquired from Sigma. Specific inhibitors LY294002, PD98059, PP2, and SB203580 were obtained from Calbiochem (La Jolla, CA).

**Cell Culture**—Fibroblasts were isolated from synovium obtained from patients undergoing the American College of Rheumatology (ACR) defined small joint replacement surgery (17). Fresh synovial tissues were minced and digested in solution of dispase, collagenase, and DNase. Synovial fibroblasts were cultured in RPMI 1640 supplemented with 10% FBS and 1% P/S. The cells were used at passage 5 or older, at which time they were a homogeneous population of fibroblasts. HMVECs were purchased from Endothelial cell growth medium-2 for microvascular cells (Biowhittaker). HMVECs were used between passage 3 and 12. Immortalized human dermal endothelial cells (HMEC-1) were a generous gift of Dr. Edmund W. Ades of the Centers for Disease Control and Dr. Thomas Lawley of Emory University (Atlanta, GA). HMEC-1 were grown in endothelial cell basal medium supplemented with epithelial growth factor (10 ng/ml, Becton Dickenson), hydrocortisone (1 μg/ml, Sigma), and 5% FBS. The promyelocytic leukemia HL-60 cells (HL-60) were cultured in RPMI 1640 supplemented with 10% FBS and 1% P/S. Synovial fibroblasts, HMVECs, and HMEC-1 were grown in 175-mm tissue culture flasks (Falcon) in their respective media at 37 °C in a humidified atmosphere with 5% CO2. Upon reaching confluence, the cells were passaged by brief trypsinization as previously described (18).

**Flow Cytometry**—RA synovial fibroblasts or HMVECs were plated onto 6-cm Petri dishes (Falcon) at 1 × 10^5 cells/ml and then fixed in 1% paraformaldehyde. Samples were assayed using a FACSCalibur (Becton Dickinson) as per the manufacturer’s instructions. Blots were scanned and analyzed for the measurement of the band intensities with the UN-SCAN-IT version 5.1 software (Silk Scientific, Orem, Utah).

**Statistical Analysis**—Data were expressed as the mean ± S.E. Group means were compared with a Student’s t test. p values < 0.05 were considered statistically significant.

**RESULTS**

IL-18 Up-regulates ICAM-1 and VCAM-1 Expression on RA Synovial Fibroblasts—RA synovial fibroblasts obtained from five different donors were cultured in the presence of IL-18 (5 nM) for 8, 16, or 24 h. ICAM-1 and VCAM-1 expression was determined by fluorescence-activated cell sorting analysis. Fig. 1 shows ICAM-1 and VCAM-1 induction on RA synovial fibroblasts by IL-18. ICAM-1 was constitutively expressed on 48.1 ± 4.9% (mean ± S.E.) of RA synovial fibroblasts. Likewise, 54.9 ± 5.4% of RA synovial fibroblasts constitutively expressed VCAM-1. IL-18 stimulation significantly up-regulated the expression of ICAM-1 to 67.7 ± 5.7 at 24 h. IL-18 also significantly increased VCAM-1 expression to 60.0 ± 15 at 8 h, and 41.5 ± 12 at 16 h (p < 0.05).

**IL-18 Induces VCAM-1 and E-selectin on HMVECs—**HMVECs were stimulated with IL-18 (5 nM) for 4, 6, or 24 h before fluorescence-activated cell sorting analysis for VCAM-1, E-selectin, and HLA-DR (Fig. 2). IFNγ (2.5 nM) stimulation was used as positive control for HLA-DR expression. A small percentage of HMVECs expressed VCAM-1 (4.8 ± 1.24) and E-selectin (5.2 ± 1) constitutively. IL-18 significantly induced
between a 5- to 6-fold increase in VCAM-1 and E-selectin expression (p < 0.05), whereas it failed to induce HLA-DR expression.

**IL-18 Induces the Adhesion of HL-60 to HMEC-1**—The functional significance of the adhesion molecules induced by IL-18 was evaluated with an adhesion assay in which HMEC-1 cultured in 96-well plates were prestimulated with IL-18 or positive control TNF-α and coincubated with the monocyte precursor cell line HL-60. These cells are known to express high amounts of very late antigen (VLA)-4, lymphocyte function-associated antigen-1, and Lewis X (20, 21), three known ligands for VCAM-1, ICAM-1, and E-selectin, respectively. HMEC-1, which are SV 40-transformed normal HMVECs, were employed in place of HMVECs because they grow rapidly and provide a ready source of endothelial cells. Furthermore, HMEC-1 retain the morphologic, phenotypic and functional characteristics of HMVECs (22). To allow comparison of the different experimental conditions at each incubation time point we used an adhesion index. Following stimulation with IL-18 (5 or 10 nM) for 4 and 6 h, the adhesion index was increased between 1.6- to 2-fold as compared with nonstimulated HMEC-1 (Fig. 3). This effect completely declined after 24 h stimulation with IL-18. Interestingly, we observed similar kinetics when HMEC-1 were stimulated with TNF-α (1.15 nM). Pretreatment of IL-18-stimulated HMEC-1 with anti-VCAM-1 or anti-E-selectin antibodies completely blocked the adhesion of HL-60 to HMEC-1 as assessed by the

**Fig. 1. IL-18 induces adhesion molecule expression in RA synovial fibroblasts.** Surface adhesion molecule expression on RA synovial fibroblasts was detected by flow cytometry after stimulation with IL-18 (5 nM) for 24 h for ICAM-1 (A), for 8 h and 16 h for VCAM-1 (B). Results represent the means ± S.E. n = number of patient samples. p < 0.05 is indicated as *.

**Fig. 2. IL-18 induces adhesion molecule expression in HMVECs.** Surface adhesion molecule expression on HMVECs was detected by flow cytometry after stimulation with IL-18 (5 nM) for 8 h for VCAM-1 (A), 4 h for E-selectin (B), and 24 h for HLA-DR (C). Results represent the means ± S.E. n = number of patient samples. p < 0.05 is indicated as *.

**Adhesion Blockade by Monoclonal Antibodies to VCAM-1 and to E-selectin**—To investigate the role of VCAM-1 and E-selectin expression in IL-18-stimulated HMEC-1 adhesion, HMEC-1 stimulated with IL-18 were incubated with monoclonal anti-VCAM-1 (monoclonal antibodies 4B9 and GH12), anti-E-selectin (monoclonal antibodies BB11) or isotype-matched control (2.5 μg/ml) antibodies (Fig. 4). Although clones 4B9 and GH12 detect two different epitopes of VCAM-1, they both block VLA-4/VCAM-1 binding. Pretreatment of IL-18-stimulated HMEC-1 with anti-VCAM-1 or anti-E-selectin antibodies completely blocked the adhesion of HL-60 to HMEC-1 as assessed by the
adhesion index, which is equal to 1. Interestingly, there was also a significant decrease of the adhesion index when TNF-α-stimulated HMEC-1 were preincubated with the same antibodies (data not shown).

**NFκB Activation Induced by IL-18 Is Inhibited by PDTC**—We previously showed that IL-18 increases the translocation of NFκB p65 to the nucleus in RA synovial fibroblasts by Western blotting (10). This translocation occurred at 1 h and was sustained for at least 4 h. Treatment of RA synovial fibroblasts with PDTC (300 μM) for 1, 2, 4, 6, or 24 h prior to the addition of 5 nM IL-18 inhibited the translocation of NFκB into the nucleus after 2 h of incubation with PDTC (Fig. 5).

**PDTC and NAC Inhibit IL-18-induced VCAM-1 Expression on RA Synovial Fibroblasts**—The effect of NFκB inhibition on VCAM-1 expression was evaluated by flow cytometry. RA synovial fibroblasts were incubated for 12 h with PDTC (300 μM) prior to the addition of IL-18 (5 nM) for 8 h. As shown in Fig. 6A, PDTC inhibited IL-18-induced VCAM-1 expression by 50%. To confirm this result, we tested NAC, an additional antioxidant which is structurally distinct from PDTC. The pH of NAC was adjusted to 7.3 prior to the incubation with RA synovial fibroblasts because the acidity of NAC was toxic to the cells. Prior to IL-18 stimulation, NAC was added at a final concentration of 25 μM for 2 h. As shown in Fig. 6B, NAC effectively inhibited IL-18 activation of VCAM-1 by 60%. The concentration of PDTC and NAC used in all experiments had no cytotoxic effect during 24 h of observation, since the viability of the cell using propidium iodide uptake as an indicator was greater than 95%.

Antioxidant PDTC and NAC partially inhibit VCAM-1 expression, suggesting that there are probably other intracellular pathways that mediate VCAM-1 induction.

**Inhibition of PI 3-Kinase Down-regulates IL-18-induced VCAM-1 Expression**—To determine whether PI 3-kinase is involved in IL-18 signaling since LY294002 significantly down-regulates VCAM-1 expression on IL-18-stimulated RA synovial fibroblasts. Data presented in Fig. 7 indicate that PI 3-kinase is involved in IL-18 signaling since LY294002 significantly down-regulates VCAM-1 expression on IL-18-stimulated RA synovial fibroblasts. This effect was not a toxic effect of LY294002 since the viability of the cells evaluated by trypan blue exclusion was greater than 90%.

**Inhibition of PI 3-Kinase and NFκB Almost Completely Inhibits VCAM-1 Expression**—To determine whether PI 3-kinase and NFκB act sequentially in the same pathway or through separate pathways, we studied the effect of simultaneous addition of the PI 3-kinase inhibitor (LY294002) with the NFκB inhibitor (PDTC). RA synovial fibroblasts were pretreated with PDTC (300 μM) for 14 h and LY294002 (10 μM) for 1 h and then stimulated for 8 h with IL-18 (5 nM). VCAM-1 expression on RA synovial fibroblasts was detected by flow cytometry. The viability of the cells evaluated by trypan blue was greater than 80%. When applied together, PDTC and LY294002 decreased VCAM-1 expression on IL-18-stimulated RA synovial fibroblasts to the level of that found on nonstimulated cells (Fig. 8).

**DISCUSSION**

Adhesion molecules have been extensively studied in RA. Immuno-staining of RA synovial tissue revealed that ICAM-1 and VCAM-1 are expressed on synovial fibroblasts and endothelial cells of the microvasculature. RA synovial fibroblasts express and secrete a variety of molecules that mediate adhesion molecule expression and subsequent cell binding. The effect of PDTC and NAC on VCAM-1 expression was investigated to determine whether PI 3-kinase and NFκB are involved in IL-18 signaling.

**FIG. 3.** IL-18 promotes adhesion of HL-60 to HMEC-1. Adhesion of 25 × 10⁴ HL-60/100 μl to confluent monolayer of HMEC-1 that were pretreated with media, IL-18 (5 and 10 nM) or TNF-α (1.15 nM), for 4, 6, and 24 h. The adhesion index expresses the ratio of adhesion of HL-60 to stimulated HMEC-1 (measured in relative fluorescence units) to the adhesion of HL-60 to untreated HMEC-1. Results are expressed as means of three different experiments and error bars indicate S.E.

**FIG. 4.** Anti-VCAM-1 and anti-E-selectin antibodies block IL-18-induced adhesion of HL-60 to HMEC-1. Adhesion of 25 × 10⁴ HL-60/100 μl to confluent monolayer of HMEC-1 that were pretreated with media or IL-18 (5 nM), for 6 h. HMEC-1 were further preincubated with monoclonal antibody (mAb) to VCAM-1, E-selectin, or control isotype-matched antibody (10 μg/ml). n = number of experiments. Results are expressed as means, and error bars indicate S.E. p < 0.05 is indicated as *.
thelial cells, whereas E-selectin is expressed only on endothelial cells. Further up-regulation of these adhesion molecules occurs in vitro following exposure to inflammatory cytokines TNF-α and IL-1β (23). The novel proinflammatory cytokine IL-18 has been demonstrated in synovial fluid, synovial tissue, and serum of patients with RA, but its role in the pathogenesis of the disease remains unclear. Here, we report that IL-18 up-regulates ICAM-1 and VCAM-1 expression on endothelial cells and RA synovial fibroblasts, and we extend the known effects of IL-18 to include its capacity to induce endothelial cell E-selectin expression. Indeed, the ability of IL-18 to induce E-selectin had never been described to date in any cell type. In fact, very few studies exist on the role of IL-18 in relation to cellular adhesion molecules. In myeloid KG-1 cells, IL-18 increases expression of ICAM-1 and mediates heterotypic aggregation between the KG-1 myeloid cell line and the Peer T cell line (24). This interaction was blocked by anti-ICAM-1. In melanoma cells (B16M), IL-18 up-regulates VCAM-1 expression as well as the adhesion of B16M to the hepatic sinusoidal endothelium (25). B16M adherence to hepatic sinusoidal endothelium was blocked by anti-VCAM-1 antibody. In this report, we show that IL-18 stimulates endothelial cells to increase binding to promyelocytic leukemia HL-60. The adhesion of HL-60 to IL-18-stimulated endothelial cells.

The ability of IL-18 to promote cell adhesion is particularly interesting since binding of leukocytes to endothelial cells is the first step in the emigration of leukocytes into perivascular space, an important early development of chronic inflammation. This, in combination with the coordinated generation of chemokines by IL-1-stimulated RA synovial fibroblasts, as we previously described (10), provides an important mechanism promoting the migration of leukocytes into and within the synovium. Data obtained from animal models tend to support this hypothesis. Indeed, mice immunized with type II collagen in incomplete Freund’s adjuvant and treated with IL-18 exhibit an extensive inflammatory infiltrate consisting of mononuclear cells (13). Furthermore, IL-18-defective mice develop a less severe collagen-induced arthritis than wild-type mice with a lower histological cellular infiltration in the joints (14). As compared with wild-type mice, cells isolated from arthritic IL-18-deficient mice also produce significantly lower amounts of TNF-α and IFNγ. Since IL-18 has the ability to induce TNF-α and IFNγ in vitro, it is possible that IL-18 exerts its proinflammatory effects, including adhesion molecule expression, via these cytokines. However, in vitro, the neutralization of TNF-α and IFNγ did not block the IL-18 induced up-regula-
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Figure 7. The PI 3-kinase inhibitor (LY294002) down-regulates IL-18-induced VCAM-1 expression. Surface VCAM-1 expression as detected by flow cytometric analysis on RA synovial fibroblasts pretreated with specific inhibitors of PI 3-kinase (LY294002), src kinase (PP2), p38 MAPK (SB203580), Erk1/Erk2 (PD98059), or Me2SO (DMSO) vehicle control for 1 h before IL-18 (5 nM) stimulation for 8 h. Results represent the means ± S.E. of two different experiments with three different patient samples (n = 2 × 3 = 6). p < 0.05 is indicated as *.

Figure 8. The PI 3-kinase inhibitor (LY294002) and NFκB (PDTC) inhibitors in combination block IL-18-induced VCAM-1 expression. Surface VCAM-1 expression was detected by flow cytometric analysis. RA synovial fibroblasts were pretreated with PDTC (300 μM) or phosphate-buffered saline vehicle control for 14 h and with LY294002 or Me2SO vehicle control for 1 h before IL-18 (5 nM) stimulation for 8 h. Results represent the means ± S.E. of four different patient samples. p < 0.05 is indicated as *.

Therefore, there should be no migration of NFκB into the nucleus. This is exactly what we observed in RA synovial fibroblasts, since the level of NFκB present in the nucleus of IL-18 stimulated fibroblasts following 2 h of preincubation with PDTC is similar to the level of NFκB present in nonstimulated cells. On the basis of this finding, we assessed the inhibitory effect of PDTC on IL-18-induced VCAM-1 expression. PDTC reduced the level of VCAM-1 detected on RA synovial fibroblasts by 50% following IL-18 or IL-1β stimulation. This result was confirmed using NAC. Inhibition of VCAM-1 expression is partial, whereas the concentrations of PDTC and NAC are optimal to block NFκB activation during exposure to IL-18. Interestingly, in a previous study we showed that treatment of cells with antisense NFκB p65 reduced IL-18-induced IL-8 production by about 50% (10). These results are consistent with Thomas et al. (33) who demonstrated that splenocytes extracted from mice deficient in IL-1R-associated kinase (IRAK), a serine-threonine kinase involved in NFκB activation following stimulation with IL-18 (8), secrete one-half the amount of IFNγ protein produced by wild-type cells. Hence, blocking NFκB seems to partially inhibit the effect of IL-18. These observations strongly suggest that there are alternative pathways, which can compensate for the inactivation of NFκB. This hypothesis is supported by Kalina et al. who demonstrated that IL-18 activates the transcription factor STAT3. The same authors reported that IL-18 may operate through the MAPK p38 since an inhibitor of this MAPK partially blocks the production of IFNγ in the natural killer 92 cell line (34).

To explore alternative pathways, we tested different inhibitors of intracellular signaling intermediates on VCAM-1 expression. In RA synovial fibroblasts, surprisingly, MAPK inhibitors (SB203580 and PD98059) and the src kinase inhibitor (PP2) did not have any effect on VCAM-1 expression, whereas the PI 3-kinase inhibitor (LY294002) reduced VCAM-1 expression by 50%. PI 3-kinase is a serine threonine kinase, which can become activated by several pathways (35). The mechanism of activation of PI 3-kinase by IL-18 could be either direct or indirect. The indirect mechanism could involve a cytokine produced by IL-18-stimulated RA synovial fibroblasts, which may act in an autocrine fashion to induce VCAM-1 expression through PI 3-kinase activation. For example, growth factors are known to act through PI 3-kinase (35) and can modulate adhesion molecule expression in endothelial cells (36). Alternatively, IL-18 could directly activate PI 3-kinase following binding to its receptor. PI 3-kinase relays the signaling of G protein-coupled receptors (37). Hence, it is possible, although not described to date, that a G protein-coupled IL-18 receptor could exist. A similar hypothesis has been discussed for IL-1 to explain the modulating activity of G protein inhibitors and activators on IL-1β-induced ICAM-1 expression (38). Another possibility might be that IL-18 could activate PI 3-kinase through a receptor tyrosine kinase pathway. Mechanistically, this pathway could involve the small GTPase Ras, or it might rely on a direct binding of PI 3-kinase to receptor tyrosine kinases (35). To activate transcription factors, PI 3-kinase has been demonstrated to operate through Akt (39) or small GTPases proteins such as Cdc42, Rac, and Ral-G (37). Min and Pober demonstrated that small GTPases initiate E-selectin transcription in human endothelial cells through a parallel pathway to NFκB involving JNK-c-Jun/ATF2 (40). Likewise, we hypothesized that IL-18 might induce adhesion molecule expression through two parallel pathways. Experiments using PI 3-kinase and NFκB inhibitors together strongly support this hypothesis since LY294002 in combination with PDTC virtually inhibited IL-18-induced VCAM-1 expression. These data suggest that IL-18 mediates VCAM-1 synthesis.
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through two distinct pathways, one involving NFκB and the other involving PI 3-kinase. To induce VCAM-1 expression, PI 3-kinase may use transcription factors other than NFκB such as API and STAT3 that are already known to be activated by PI 3-kinase (41, 42). However, we cannot exclude that PI 3-kinase may use NFκB, at least in part, to induce VCAM-1 transcription. Indeed, it is possible that PI 3-kinase may initiate an activation of NFκB that is not inhibited by PDC. This theory is supported by Sizemore et al. who showed that PI 3-kinase could regulate NFκB-dependent promoter expression independently of the IκB degradation-NFκB liberation pathway (43).

In conclusion, IL-18 induces the expression of functional adhesion molecules in two different cell types, endothelial cells and fibroblasts. IL-18-induced RA synovial fibroblast VCAM-1 expression is regulated by a NFκB and PI 3-kinase way (43).

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