Interleukin-18-induced Human Coronary Artery Smooth Muscle Cell Migration Is Dependent on NF-κB- and AP-1-mediated Matrix Metalloproteinase-9 Expression and Is Inhibited by Atorvastatin*

Received for publication, January 9, 2006 Published, JBC Papers in Press, March 22, 2006, DOI 10.1074/jbc.M600200200

Bysani Chandrasekar1,5,1, Srinivasa Mummidi1,5,2, Lenin Mahimainathan1, Devang N. Patel1, Steven R. Bailey1, Syed Z. Imam1, Warner C. Greene1, and Anthony J. Valente1

From the 5Department of Veterans Affairs South Texas Veterans Health Care System, San Antonio, Texas 78229-4404, the 4Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78229-3900, and the 6Gladstone Institute of Virology and Immunology, Department of Microbiology and Immunology, University of California, San Francisco, California 94158-2261

The proliferation and migration of arterial smooth muscle cells (SMCs) are key events in the vascular restenosis that frequently follows angioplasty. Furthermore, SMC migration and neointimal hyperplasia are promoted by degradation of the extracellular matrix by matrix metalloproteinases (MMPs). Because we demonstrated previously that the proinflammatory and proatherogenic cytokine interleukin-18 (IL-18) stimulates SMC proliferation (Chandrasekar, B., Mummidi, S., Valente, A. J., Patel, D. N., Bailey, S. R., Freeman, G. L., Hatano, M., Tokuhisa, T., and Jensen, L. E. (2005) J. Biol. Chem. 280, 26263–26277), we investigated whether IL-18 induces SMC migration in an MMP-dependent manner and whether the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin can inhibit this response. IL-18 treatment increased both mRNA and protein expression of MMP9 in human coronary artery SMCs. Gel shift, enzyme-linked immunosorbent, and chromatin immunoprecipitation assays revealed a strong induction of IL-18-mediated AP-1 and NF-κB (p50 and p65) activation and stimulation of MMP9 promoter-dependent reporter gene activity in an AP-1- and NF-κB-dependent manner. Ectopic expression of p65, c-Fos, c-Jun, and Fra-1 induced MMP9 promoter activity. Specific antisense or small interfering RNA reagents for these transcription factors reduced IL-18-mediated MMP9 transcription. Furthermore, IL-18 stimulated SMC migration in an MMP9-dependent manner. Atorvastatin effectively suppressed IL-18-mediated AP-1 and NF-κB activation, MMP9 expression, and SMC migration. Together, our results indicate for the first time that the proatherogenic cytokine IL-18 induces human coronary artery SMC migration in an MMP9-dependent manner. Atorvastatin inhibits IL-18-mediated aortic SMC migration and has therapeutic potential for attenuating the progression of atherosclerosis and restenosis.

Atherosclerosis is considered to be a chronic inflammatory disease characterized by enhanced expression of proinflammatory cytokines, chemokines, and adhesion molecules (1–3). The cross-talk between cytokines, chemokines, and infiltrating immune cells amplifies the inflammatory cascade in the vessel wall, resulting in atherogenesis (1–3).

Interleukin-18 (IL-18)3 is a proinflammatory and proatherogenic cytokine that induces the expression of other proinflammatory cytokines and adhesion molecules (4). IL-18 has been localized to human atherosclerotic lesions (5, 6), and circulating IL-18, which is increased in acute coronary syndromes (7), has been shown to predict future cardiovascular events (7). A positive correlation between serum IL-18 levels and carotid intima-media thickness has been demonstrated (8). Administration of IL-18 aggravates atherosclerosis in mice (9). Moreover, atherosclerosis is reduced in IL-18-deficient apoE knock-out mice (10), suggesting a causal role for IL-18 in the development and progression of atherosclerosis.

Recently, we demonstrated that IL-18 induces human aortic smooth muscle cell (SMC) proliferation (11). However, it is not known whether IL-18 induces SMC migration. Both migration and proliferation play a role in normal and diseased vessels (1–3). SMC migration contributes to normal angiogenesis. However, SMC migration also plays a causal role in pathological remodeling of the vessel walls during atherosclerosis, arteriosclerosis, and restenosis following angioplasty (1–3).

Vessel wall remodeling is characterized by a disruption in the delicate balance between extracellular matrix (ECM) deposition and degradation, with matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of matrix metalloproteinases [TIMPs]) playing a prominent role. MMPs are zinc-dependent proteases and are classified as collagenases, stromelysins, elastases, and gelatinases based on substrate specificity. Their expression is regulated at both the transcriptional and post-transcriptional levels. They are synthesized as proenzymes and are activated following proteolytic cleavage. SMCs express MMP2 (gelatinase A) and MMP9 (gelatinase B), the two gelatinases described so far (12). Excess activation of MMP2 and MMP9, without alteration of TIMP expression and activation, results in destruction of the ECM and can lead to pathological remodeling and vascular restenosis (12–15). Because increased matrix degradation promotes SMC migration (15), we hypothesized that IL-18 induces SMC migration via induction of MMP9. Our novel findings demonstrate that IL-18 promotes SMC migration.

The abbreviations used are: IL-18, interleukin-18; SMC, smooth muscle cell; ECM, extracellular matrix; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of matrix metalloproteinases; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-18Rα, interleukin-18 receptor-α; FSK, forskolin; 3-isobutyl-1-methylxanthine; 2-deoxyglucose; TNF, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-18Rα; interleukin-18 receptor-α; FSK, phosphatidylinositol 3-kinase; ODNs, oligodeoxynucleotides; siRNA, small interfering RNA; dn, dominant-negative; EMSA, electrophoretic mobility shift assay; CHIP, chromatin immunoprecipitation; GFP, green fluorescent protein.

* This work was supported in part by NHLBI Grant HL68020 from the National Institutes of Health, by the Research Service of the Department of Veterans Affairs, and by a pilot grant from Pfizer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Dept. of Medicine/Cardiology, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900. Tel.: 210-567-4598; Fax: 210-567-6960; E-mail: chandraseka@uthscsa.edu.

2 Supported by the Merit Review Entry Program of the Department of Veterans Affairs.
migration in an MMP9-dependent manner. IL-18 induced MMP9 expression via activation of AP-1 (activator protein-1) and NF-κB (nuclear factor-κB). We demonstrate that atorvastatin, a potent inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, inhibits IL-18-mediated SMC migration by attenuating AP-1 and NF-κB activation and MMP9 expression.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human IL-18 (catalog no. B001-5), IL-1β (catalog no. 201-LB-005), tumor necrosis factor-α (TNF-α; catalog no. 210-TA-010), and interferon-γ (IFN-γ; catalog no. 285-IF-100); neutralizing goat anti-human IL-1β (catalog no. AB-201-NA), TNF-α (catalog no. AF-210-NA), IFN-γ (catalog no. AF-285-NA), and IL-1 receptor-α (IL-1Rα; catalog no. AF840); antibodies; and normal goat IgG (catalog no. AB-108-C) were purchased from R&D Systems (Minneapolis, MN). Antibodies against NF-κB p65 and IκKβ were purchased from Cell Signaling Technology (Beverly, MA). Normal rabbit IgG (control IgG) was from Jackson ImmunoResearch Laboratories (West Grove, PA). MG-132, wortmannin, SH-5, and (±)-S-nitroso-N-acetylcysteine were purchased from EMD Biosciences. Anti-smooth muscle α-actin antibodies and all other chemicals were purchased from Sigma. Atorvastatin was a kind gift from Pfizer.

Cell Culture—Normal human coronary artery SMCs were obtained from Clonetics Corp. (San Diego CA) and grown in SmGM-2 medium supplied by the manufacturer. At 70–80% confluency, the culture medium was replaced with basal medium containing 0.5% bovine serum albumin (conditioning medium). After a 24-h incubation, recombinant human IL-18 was added and cultured for the indicated time periods. At the end of the experimental period, culture supernatants were collected into slick tubes and snap-frozen. Cells were harvested, snap-frozen, and stored at −80°C. To determine whether IL-18 induces arterial SMC migration directly or is mediated by intermediaries such as IL-1β, TNF-α, and IFN-γ, cells were pretreated with the respective neutralizing antibodies (5 μg/ml for 1 h) prior to IL-18 addition. Normal goat IgG at a similar concentration served as a control. The efficacy of these antibodies was verified in transient transfection assays using SMC transfected with the pNF-κB-Luc or pGAS-Luc vector (Stratagene, La Jolla, CA) (16). pEGFP-Luc served as a control (16). 24 h after transfection, cells were treated with anti-IL-1β, anti-TNF-α, or anti-IFN-γ neutralizing antibody (5 μg/ml for 1 h), followed by the addition of the respective recombinant human protein (100 pg/ml IL-1β, 100 pg/ml TNF-α, or 10 ng/ml IFN-γ) for an additional 7 h. The pRL-TK vector (100 ng; Promega Corp.) served as an internal control. Cell extracts were prepared, and firefly and Renilla luciferase activities were determined using the Biotech Dual-Luciferase reporter assay system (Promega Corp.) and a TD 20/20 luminometer (Turner Designs, Sunnyvale, CA) (11, 16, 17). Data were normalized for transfection efficiency by dividing firefly luciferase activity by the corresponding Renilla luciferase activity and are expressed as mean relative stimulation ± S.E. for a representative experiment from three separate experiments, each performed in triplicate. After transfection, cells were found viable as determined by trypan blue dye exclusion.

Inhibitors, Antisense Oligodeoxynucleotides (ODNs), Small Interfering RNA (siRNA), Expression Vectors, and Adenoviral Transduction—The antisense phosphorothioated ODNs used were as follows: TNF-α, 5′-CAGTGTCTCATGCTTGTC-3′; c-Fos, 5′-GCGTTGAAAGCCCGAGAA-3′; c-Jun, 5′-CGTCTCCCATCCTGGAG-3′; Fra-1 (fos-related antigen-1), 5′-CCCGAAGTCTCAGGAACAT-3′; and scrambled, 5′-CGACTGTCAGGTTCT-3′. ODNs were transfected using Oligofectamine (Invitrogen). MMP2 and MMP9 expression was targeted by siRNA (MMP2, 5′-AGUUG-GCGACUGCAAUCCUGA-3′ (sense); and MMP9, 5′-CAUCACAUACUGGAUCAUUU-3′ (sense)) (18, 19). An siRNA that will not target any genes in the human genome (5′-UUCUCCGAACGUGACACGU-3′ (sense)) (18, 19).

Electrophoretic Mobility Shift Assay (EMSA) and Reporter Assay—NF-κB and AP-1 DNA binding activities were measured by EMSA using gene-specific and consensus ODNs (Table 1). Specificity of DNA-protein complexes was verified using the corresponding mutant ODNs. The subunit composition of NF-κB and AP-1 was determined using an
Atorvastatin Inhibits IL-18-mediated SMC Migration

ELISA-based kit (Active Motif, Carlsbad, CA) (20). The assays were performed according to the manufacturer’s instructions. The assay is based on the immunochromatographic detection of activated transcription factors (TF) in nuclear extracts using subunit-specific antibodies and horseradish peroxidase-conjugated secondary antibody.

IL-18-mediated NF-κB and AP-1 activation was also confirmed in transient transfection assays using transcription-factor–specific reporter vectors (pNF-κB-Luc and pAP-1-Luc; PathDetect® cis-reporting system, Stratagene). Cells were treated with IL-18 24 h post-transfection. pEGFP-Luc served as a control. The pRL-TK vector was used as a transfection efficiency control. Luciferase activities were determined as described above.

Chromatin Immunoprecipitation (ChIP) Assay—SMCs were cultured in complete medium until 70% confluent and then changed to the culture matrix. After electrophoresis, gels were agitated in 2.5% Triton X-100 to partially as described by Mandler et al. (21). Gels were prepared with 1 mg/ml gelatin (catalog no. G2500, Sigma) co-polymerized into the gel matrix. After electrophoresis, gels were agitated in 2.5% Triton X-100 to remove the SDS and to remove enzyme activity and then incubated for 24 h at 37°C in 50 ml of 50 mM Tris-HCl (pH 7.6) containing 0.2 mM NaCl, 5 mM CaCl₂, 0.02% Brij 35, and 0.02% sodium azide. Finally, gels were stained for 1 h in 50% methanol and 1% acetic acid with 0.125% Coomassie Blue G-250 dye and destained in 10% acetic acid. Zones of proteolytic activity were evident as clear bands against a dark blue background.

MMP9 Promoter-Reporter Assays—A 726-bp fragment of the 5’-flanking region of the MMP9 gene (GenBank™ accession number D10051.1) was amplified from human genomic DNA (catalog no. G2500, Sigma) co-polymerized into the gel matrix. After electrophoresis, gels were agitated in 2.5% Triton X-100 to remove the SDS and to remove enzyme activity and then incubated for 24 h at 37°C in 50 ml of 50 mM Tris-HCl (pH 7.6) containing 0.2 mM NaCl, 5 mM CaCl₂, 0.02% Brij 35, and 0.02% sodium azide. Finally, gels were stained for 1 h in 50% methanol and 1% acetic acid with 0.125% Coomassie Blue G-250 dye and destained in 10% acetic acid. Zones of proteolytic activity were evident as clear bands against a dark blue background.

Table 1: Primers used in human MMP9 analysis

| Parameter | Primer sequence |
|-----------|-----------------|
| cDNA (GenBank™ accession no. NM_004994; 552-nt product size) | 5'-ATCCAGTTGTTGTTGGGAG-3' (sense), 5'-GAAGGGAGACCAGCCACGACT-3' (antisense) |
| DNA-binding activity | 5'-CTGCGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense), 5'-CTGCGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense) |
| Site-directed mutagenesis | 5'-AGCTCCTGGTAGTcaGCACCTT-3' (sense), 5'-CTGACCCCTGGAGCGAGGAAGTA-3' (sense) |
| MMP9-726 | 5'-aagctcATGGTGAGGGCAGAGGTG-3' |
| MMP9-608 | 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense), 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense) |
| MMP9-66 | 5'-aagctcATGGTGAGGGCAGAGGTG-3' |
| NP-κB | 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense), 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense) |
| AP-1 | 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense), 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense) |
| ORF (294-nt product size) | 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense), 5'-CTGCGGAGAACAGGAGGCTTGGCCATGGGAATTCCTCC-3' (sense) |
Atorvastatin Inhibits IL-18-mediated SMC Migration

FIGURE 1. IL-18 stimulates human coronary artery SMC migration. A, cultured SMCs were trypsinized, suspended in Dulbecco’s modified Eagle’s medium and 0.5% bovine serum albumin, and layered on Matrigel basement membrane matrix-coated filters. Cells were stimulated with IL-18 (10 ng/ml). The lower chamber had medium containing IL-18 at the same concentration. Plates were incubated at 37 °C for 24 h to allow cell migration. Cells migrating to the other side of the membrane were quantified using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Specificity of IL-18 was verified by preincubating IL-18 with anti-IL-18R neutralizing antibody (5 μg/ml) for 1 h prior to the addition of the respective recombinant proteins. 7 h later, luciferase activities were determined. PBS, phosphate-buffered saline. A: * p < 0.001 versus the control; † p < 0.01 versus IL-18. B–F, * p < 0.001 versus untreated and the respective pEGFP-Luc-transfected cells; † p < 0.01 versus the respective cytokine-treated pNF-xB-Luc-transfected (IL-1β, TNF-α, and IL-18) and pGAS-Luc-transfected (IFN-γ) cells.

Statistical Analysis—Comparisons between controls and various treatments were performed by analysis of variance with Dunnett’s post hoc t tests. All assays were performed at least three times, and the error bars in the figures indicate the S.E.

RESULTS

IL-18 Stimulates Human Coronary Artery SMC Migration—To determine whether IL-18 induces SMC migration, we used Matrigel™ invasion chambers. A significant increase in cell migration was detected 24 h following IL-18 (10 ng/ml) treatment (Fig. 1). Because IL-18 is a potent inducer of IL-1β, TNF-α, and IFN-γ production (4), and as these cytokines play a role in atherogenesis (1–3), we investigated whether IL-18-induced SMC migration is mediated by these cytokines. Pretreatment with the respective neutralizing antibodies for 1 h prior to IL-18 treatment failed to modulate cell migration. In contrast, incubation of IL-18 with anti-IL-18R neutralizing antibody for 1 h blocked IL-18-induced cell migration. Specificity of the neutralizing antibodies was determined in transient transfection assays using NF-κB and γ-interferon activation sequence reporter gene vectors. The results in Fig. 1 (B–E) demonstrate that anti-IL-1β, anti-TNF-α, anti-IFN-γ, and anti-IL-18R neutralizing antibodies blocked the respective cytokine-induced reporter gene activities (NF-κB for IL-1β, TNF-α, and IL-18 (panels B, C, and E) and γ-interferon activation sequence for IFN-γ (panel D)). However, anti-IL-1β, anti-TNF-α, and anti-IFN-γ neutralizing antibodies failed to block IL-18-mediated NF-κB reporter gene activity (Fig. 1F). These results indicate that IL-18 is a potent inducer of SMC migration and mediates its effect independently of the proinflammatory cytokines IL-1β, TNF-α, and IFN-γ (Fig. 1).

IL-18 Stimulates MMP2 and MMP9 Secretion—Because migration follows matrix degradation by MMPs, we screened for the expression of MMPs and TIMPs in the SMC culture supernatants using an antibody array that detects seven MMPs and four TIMPs simultaneously (Fig. 2A). Quiescent SMCs were treated with saline or IL-18 (10 ng/ml) for 24 h, and pooled culture supernatants from three independent experiments were used to quantitate the extracellular matrix proteins (Fig. 2B). The signal intensities for each of the ECM proteins were normalized to the saline-treated samples (Fig. 2C). The results indicate that, although IL-18 stimulated secretion of the gelatinases MMP2 and MMP9, it had no significant effect on other MMPs or on TIMPs, suggesting that IL-18 induces matrix degradation by up-regulating MMP2 and MMP9 (Fig. 2).

IL-18 Stimulates SMC Migration via MMP2 and MMP9—Because IL-18 stimulated MMP2 and MMP9 secretion, we next investigated whether inhibiting their activity attenuates IL-18-mediated SMC

primers listed in Table 1 and was confirmed by complete nucleotide sequencing.
migration. MMP2/MMP9 Inhibitor I inhibits activation of both MMP2 and MMP9. Inhibition of MMP2 and MMP9 significantly attenuated IL-18-mediated SMC migration (Fig. 3A). In contrast, MMP3- and MMP8-specific inhibitors and the solvent control Me2SO failed to modulate IL-18-mediated cell migration, further indicating the likely roles of MMP2 and MMP9 in IL-18-mediated SMC migration. To discriminate between the MMP2 and MMP9 isoforms, we targeted MMP2 and MMP9 expression using specific siRNAs. The efficacy of these siRNAs has been demonstrated previously (18, 19). MMP2, MMP9, or control siRNA did not induce cell death (data not shown). The siRNA-mediated knockdown of both MMP2 and MMP9 attenuated IL-18-mediated SMC migration (Fig. 3B). (The selective knockdown of target mRNA is shown Fig. 3B (right panels).) However, knockdown of MMP9 was more effective than that of MMP2. On the other hand, treatment with control siRNA had no effect on SMC migration. Together, these results indicate that, although both MMP2 and MMP9 mediate SMC migration generally, MMP9 plays a predominant role in IL-18-mediated SMC migration (Fig. 3).

IL-18 Induces MMP9 Expression in SMCs—Because MMP9 expression may be regulated at the levels of gene transcription and proenzyme activation and secretion, we analyzed whether IL-18 stimulates MMP9 mRNA expression and enzyme activation and secretion in SMCs. IL-18 up-regulated MMP9 mRNA expression in a dose-dependent manner (Fig. 4A). The increase in MMP9 mRNA expression was detectable at 1 ng/ml, and maximal levels were attained with 10 ng/ml. Therefore, in subsequent studies, IL-18 was used at 10 ng/ml. In time course studies, a robust increase in MMP9 mRNA expression was observed at 6 h and persisted for up to 24 h (Fig. 4B).

MMP9 in a latent or proenzyme form is secreted by a variety of cell types. The latent form is converted to active MMP9 by proteolytic cleavage at the 10-kDa prodomain. Therefore, we quantified the latent and active forms of MMP9 in SMC culture supernatants by gelatin zymography. Under basal conditions, SMCs predominantly secrete the latent form of MMP9 (Fig. 4C). Treatment with IL-18 (but not neutralized IL-18 Ra) induced the activation of MMP9 as seen by increased levels of the active form. These results were confirmed by Western blotting using an antibody that detects both the active and latent forms of MMP9 (Fig. 4C), showing increased levels of the active form of MMP9 following IL-18 treatment (Fig. 4D). Together, these results demonstrate that IL-18 increases MMP9 in SMCs both by increased mRNA expression and by activation of the protein (Fig. 4).

IL-18 Induces MMP9 Promoter-Reporter Activity in an AP-1- and NF-κB-dependent Manner—Because IL-18 induced MMP9 mRNA expression, we investigated whether IL-18 regulates MMP9 expression at the transcriptional level. In particular, we investigated whether IL-18 induces MMP9 gene transcription via NF-κB and AP-1, both of which have been implicated previously in MMP9 gene regulation (25). SMCs...
Atorvastatin Inhibits IL-18-mediated SMC Migration

FIGURE 4. IL-18 induces MMP9 expression in SMCs. A, IL-18 stimulated MMP9 mRNA expression in dose-dependent manner. Quiescent SMCs were treated with IL-18 at various concentrations for 24 h. Total RNA was extracted and analyzed (20 μg) by Northern blotting. 28 S rRNA served as a control. A representative of three independent experiments is shown. B, IL-18 stimulated MMP9 mRNA expression in time-dependent manner. Quiescent SMCs were treated with IL-18 (10 ng/ml). At the indicated intervals, cells were harvested, and total RNA was extracted and analyzed (20 μg) for MMP9 mRNA expression by Northern blotting. Expression of 28 S rRNA served as an internal control. C, control. D, IL-18 stimulated MMP9 enzyme activity. 24 h following IL-18 treatment, culture supernatants were collected and analyzed for MMP9 enzyme activity by gelatin zymography. Actin levels in cell extracts demonstrated a similar number of cells plated in each treatment. Ab, antibody. D, activation of MMP9 was also confirmed by Western blotting. Quiescent SMCs were treated with IL-18 for 24 h as described above. Cells were harvested, and protein was extracted and analyzed for MMP9 levels by Western blotting using antibodies that detect both latent and mature MMP9. Actin served as an internal control. A representative of three or four independent experiments is shown.

FIGURE 5. IL-18 induces MMP9 promoter-reporter activity in an AP-1- and NF-κB-dependent manner. A, neutralization of IL-18Rα attenuated MMP9 promoter-reporter activity. Quiescent SMC transfected with MMP9-726 and pRL-TK were treated with anti-18Rα antibody for 1 h followed by IL-18 for 7 h. Normal goat IgG served as a control. B, IL-18-induced MMP9 promoter-reporter activity was not mediated by IL-18, TNF-α, or IFN-γ. Quiescent arterial SMCs transfected with MMP9-726 and pRL-TK were incubated with anti-IL-1β, anti-TNF-α, or anti-IFN-γ neutralizing antibody for 1 h prior to the addition of IL-18 for an additional 7 h. TNF-α expression was also targeted by antiapoptotic ODNs. 24 h after antisense ODN treatment, arterial SMCs transfected with MMP9-726 and pRL-TK were treated with IL-18 for an additional 7 h. C, IL-18 stimulated MMP9 promoter activity via AP-1 and NF-κB. Quiescent SMCs were transfected with deletion constructs derived from MMP9-726 and cotransfected with the pRL-TK vector. 24 h later, cells were treated with IL-18 (10 ng/ml) for an additional 7 h, and luciferase activities were determined. nt, nucleotides; Luc, luciferase. D, IL-18-induced AP-1- and NF-κB-dependent MMP9 promoter activity was also confirmed using mutant AP-1 and NF-κB constructs. Quiescent SMCs were transfected with the MMP9 promoter construct (MMP9-726) with mutations in putative NF-κB and AP-1. Cells were cotransfected with the pRL-TK vector. Firefly and Renilla luciferase activities were determined as described above. A–D, *p < 0.001 versus IL-18-treated pGL3-Basic; †, p < 0.01 versus IL-18-treated MMP9-726. B, *, p < 0.001 versus untreated cells. Were transiently transfected with an MMP9 promoter-reporter construct (MMP9-726) containing both the NF-κB- and AP-1-binding sites. Following treatment with IL-18 (but not neutralized IL-18 Rα), a robust increase in reporter gene activity was observed (Fig. 5A). Furthermore, pretreatment with anti-IL-1β, anti-TNF-α, or anti-IFN-γ neutralizing antibody or with TNF-α antisense ODNs failed to modulate either the low basal (data not shown) or IL-18-induced increase in MMP9 promoter-reporter activity, suggesting a direct effect by the cytokine. To investigate whether MMP9 transcription is NF-κB- and AP-1-dependent, we transfected cells with MMP9 deletion constructs lacking either or both of the NF-κB and AP-1 sites. Our results show that, although the deletion construct lacking NF-κB (MMP9-660) was less responsive (p < 0.05), the construct lacking both NF-κB and AP-1 (MMP9-66) had activity close to that of the promoterless pGL3-Basic vector (p < 0.001) (Fig. 5C), suggesting that both NF-κB and AP-1 play a role in IL-18-mediated MMP9 transcription. To further confirm these observations, SMCs were transfected with MMP9-726 constructs containing mutations in the NF-κB or AP-1 core-binding site. A significant decrease (p < 0.01) in the reporter activity was observed when either the NF-κB or AP-1 site was mutated (Fig. 5D). However, the effect was more pronounced when both mutations were present (p < 0.001). These data demonstrate that IL-18 induces MMP9 transcription in SMCs via NF-κB and AP-1 transcription factors (Fig. 5).

IL-18 Stimulates NF-κB Activation—Because IL-18-induced MMP9 promoter activity was significantly reduced following deletion (Fig. 5C) or mutation (Fig. 5D) of the potential NF-κB or AP-1 binding in the MMP9-726 construct, we determined whether these sites in fact bind NF-κB and AP-1 in vitro and in vivo following IL-18 stimulation. We
performed EMSA with the NF-κB-binding site and its mutant sequence from the MMP9 promoter using nuclear extracts from SMCs that were either untreated or treated with IL-18 for 2 h. IL-18 induced a robust increase in specific NF-κB binding activity in the SMCs (Fig. 6A, compare lanes 4 and 6). No specific binding was detected when the labeled mutant sequence was used in EMSA (lanes 5 and 7). In a separate set of experiments, we also confirmed that IL-18 induced NF-κB activity in SMCs using a consensus NF-κB gel shift ODN probe (data not shown). Because the subunit composition of NF-κB determines its transactivation potential, we determined the induction of main components of NF-κB by IL-18 using ELISA. Fig. 6B shows that IL-18 significantly increased p65 and p50 in the SMC nuclear protein extracts. However, c-Rel, p52, and RelB levels were not affected by IL-18 treatment. Because IL-18 induced NF-κB DNA binding activity, we investigated whether NF-κB complexes interact in vivo with the MMP9 promoter. We therefore performed ChIP assays on arterial SMCs that were either untreated or treated with IL-18. This analysis demonstrated that IL-18 treatment increased p65 binding to the NF-κB site in vivo (Fig. 6C). We next investigated the direct role of NF-κB in MMP9 transcription. The results in Fig. 6D show that knockdown of IκB kinase-β or p65 or transfection with dnIκBα significantly attenuated IL-18-induced MMP9 transcription. ( Knockdown of p65 and IκB kinase-β in whole cell homogenates was confirmed by Western blotting (Fig. 6D, lower panels). In contrast, ectopic expression of wild-type p50 or p65 induced MMP9.
transcription (Fig. 6E). Together, these results demonstrate that IL-18-mediated MMP9 transcription is NF-κB-dependent (Fig. 6).

**IL-18 Stimulates AP-1 Activation**—Because IL-18-mediated MMP9 promoter-reporter activity was significantly reduced when a deletion construct lacking AP-1 was used (Fig. 5C) or when the AP-1-binding site was mutated (Fig. 5D), we investigated whether IL-18 stimulates AP-1 activation in SMCs. EMSA was performed using gene-specific ODNs. Low levels of AP-1 DNA binding activity were detected following IL-18 treatment (lane 6). When the labeled double-stranded mutant ODN was used in EMSA, no specific complexes were detected (lanes S and 7). Similar results were obtained when consensus ODNs were used (data not shown). ELISA of nuclear proteins revealed a significant increase in c-Fos, Fra-1, and c-Jun and, to a smaller extent, in FosB and JunB. Fra-2 and JunD levels were not modulated by IL-18 (Fig. 7B). Because IL-18 induced AP-1 DNA binding activity, we investigated whether AP-1 complexes interact in vivo with the MMP9 promoter. We therefore performed ChIP assays on arterial SMCs that were either untreated or treated with IL-18. This analysis demonstrated that IL-18 treatment increased Fra-1 binding to the AP-1 site in vivo (Fig. 7C). We next investigated whether targeting AP-1 will modulate MMP9 transcription. The results in Fig. 7D show that antisense ODN targeting of c-Fos, c-Jun, and Fra-1 significantly attenuated MMP9 transcription. In contrast, ectopic expression of c-Fos, c-Jun, and Fra-1 induced MMP9 transcription (Fig. 7E). It appears that Fra-1 is more potent than c-Fos and c-Jun in inducing MMP9 promoter activity.

Together, these results demonstrate that IL-18-mediated MMP9 transcription is AP-1-dependent (Fig. 7).

**IL-18 Stimulates AP-1 and NF-κB DNA Binding Activities via PI3K and Akt Activation**—We demonstrated that IL-18 activates NF-κB and AP-1. We have shown previously that IL-18 induces PI3K and Akt activation (11, 26). We next investigated whether IL-18-mediated NF-κB and AP-1 activation is dependent on PI3K and Akt. PI3K activation was targeted by the pharmacological inhibitors wortmannin as well as by overexpression of dnPI3K in the pcDNA3 expression vector. Akt was targeted by SH-5 and adenoviral transduction of hemagglutinin-tagged dnAkt1. Our results indicate that, although pretreatment with MeSO4, the pcDNA3 vector, or control green fluorescent protein (GFP) failed to modulate, treatment with wortmannin, and SH-5 and overexpression of dnPI3K or dnAkt1 attenuated IL-18-mediated NF-κB and AP-1 activation (Fig. 8A) and AP-1 (Fig. 8B) DNA binding activities, indicating that IL-18 induces NF-κB and AP-1 activation via PI3K and Akt (Fig. 8).

**Atorvastatin Inhibits SMC Migration**—Statins are potent inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (27, 28). Atorvastatin lowers serum cholesterol levels and has been used successfully in clinical practice to treat hypercholesterolemia and to inhibit atherogenesis in human coronary arteries (29–31). To investigate whether atorvastatin can inhibit SMC migration, a critical component of atherogenesis and restenosis, SMCs were pretreated with atorvastatin before assay for IL-18-induced migration. Significant inhibition of SMC migration was detected at 2.5 μM, but the inhibitory effect was more pronounced at 5 and 10 μM (Fig. 9A). Also, neither IL-18 alone nor the combination of
IL-18 and atorvastatin induced cell death (Fig. 9B). However, the nitric oxide donor (±)-S-nitroso-N-acetylpenicillamine (used as a positive control) induced significant cell death. To determine the molecular mechanisms involved in its inhibitory effects, the effect of atorvastatin on IL-18-induced transcription factor activation was investigated. Pre-treatment with atorvastatin significantly inhibited IL-18-mediated AP-1-dependent (Fig. 9C) and NF-κB-dependent (Fig. 9D) reporter gene activities. Furthermore, atorvastatin inhibited IL-18-mediated MMP9 transcription (Fig. 9E), mRNA expression (Fig. 9F), and enzyme activity (Fig. 9G). Together, these results indicate that atorvastatin is a potent inhibitor of IL-18-mediated MMP9 expression and SMC migration (Fig. 9F).

**DISCUSSION**

Our novel data demonstrate that the proinflammatory and proatherogenic cytokine IL-18 can induce human coronary artery SMC migration independently of other proinflammatory cytokines. IL-18 induced the transcription and miRNA expression of MMP9 through a PI3K- and Akt-dependent AP-1 and NF-κB-mediated mechanism and stimulated the production of active MMP9. Atorvastatin significantly inhibited IL-18-mediated NF-κB- and AP-1-dependent MMP9 expression and SMC migration (Fig. 9F). These results suggest a causal role for IL-18 in atherogenesis and the possible therapeutic potential of atorvastatin for the inhibition of IL-18-mediated and MMP9-dependent SMC migration.

SMC migration and proliferation are important features of both normal physiological vessel growth and vascular pathology (1–3). In post-angioplastic restenosis, intimal thickening and hyperplasia are characterized by the proliferation of SMCs in the tunica media and their migration toward the luminal surface. In normal vessels, SMCs are largely confined to the tunica media, where they interact directly with components of the ECM. In addition to this physical interaction with the SMCs, ECM proteins regulate the expression, secretion, activation, and breakdown of various cytokines, chemokines, growth factors, and adhesion molecules by the adjacent cells (12–15). ECM expression, deposition, and degradation are all tightly regulated by a balance between MMP and TIMP activities. The disruption of this delicate balance in favor of MMP activation results in ECM degradation and induction of SMC motility and migration (12–15).

MMPs are zinc-dependent proteases that are classified as collagens, stromelysins, elastases, and gelatinases based on substrate specificity. Their expression is regulated at both the transcriptional and post-transcriptional levels. They are synthesized as proenzymes and are activated following proteolytic cleavage. SMCs express MMP2 (gelatine A) and MMP9 (gelatine B), the two MMPs with gelatine activity described so far. The excess activation of MMP2 and MMP9 results in destruction of the ECM and leads to pathological remodeling and vascular restenosis (13–15, 29).

IL-18 is a pleiotropic cytokine with proinflammatory and proatherogenic properties. In patients with systemic atherosclerotic vascular disease, an elevated plasma level of IL-18 is a strong predictor of mortality (7) and is associated with greater carotid intima-media thickness (8), suggesting a causal role for IL-18 in the development and progression of atherosclerosis. Although these studies demonstrate an association between increased IL-18 levels and atherosclerosis, it is unknown whether IL-18 plays a direct role in SMC migration and proliferation. Recently, we demonstrated that IL-18 exerts a mitogenic effect on SMCs, inducing proliferation via AP-1-mediated CXCL16 expression (11). In the present study, we have shown that IL-18 induces SMC migration through the induction of AP-1- and NF-κB-mediated MMP9 expression, which is in turn mediated through PI3K and Akt activation. Because PI3K-dependent Akt activation transmits survival signals, our results further confirm that IL-18 is promitogenic to SMCs, inducing their proliferation (11) and migration.

In addition to MMP9 expression, IL-18 also induced MMP2 expression. Using an antibody array that detects 11 extracellular matrix proteins, we observed that IL-18 stimulated MMP2 and MMP9 expression without modulating TIMP levels. Similar results have been reported in peripheral blood mononuclear cells, in which IL-18 stimulated MMP9 release without modulating TIMP1 levels (30), suggesting that IL-18 alters the MMP/TIMP balance in favor of MMP expression and induces ECM degradation. Our results also show that, although the knockdown of MMP2 and MMP9 significantly inhibits IL-18-mediated SMC migration, the effects are more pronounced when MMP9 expression is targeted, indicating that MMP9 might play a more dominant role in IL-18-mediated SMC migration. These results corroborate and extend the observations of Zhang et al. (31), who showed that IL-18 stimulates HL-60 myeloid leukemia cell migration in an MMP9-dependent manner. However, in this study, the mechanisms responsible for IL-18-mediated MMP9 expression were not investigated.

Our results also show that IL-18 stimulates MMP9 expression at both the transcriptional and post-transcriptional levels. IL-18 stimulated MMP9 promoter-reporter activity, mRNA expression, enzyme activity, and secretion. Investigation into the possible signal transduction pathways involved in IL-18-mediated MMP9 expression indicated that IL-18-induced AP-1 and NF-κB activation was responsible for these responses. ELISA of nuclear proteins revealed that IL-18 induced AP-1 complexes containing c-Fos, c-Jun, and Fra-1 and an NF-κB complex containing p50 and p65. Although ectopic expression of c-Fos, c-Jun, and Fra-1 stimulated MMP9 transcription, antisense ODN-mediated suppression of c-Fos, c-Jun, and Fra-1 inhibited MMP9 transcription. Similarly, overexpression of wild-type p50 and p65 stimulated MMP9 expression, and siRNA-mediated knockdown attenuated MMP9 expression. These results suggest that both AP-1 and NF-κB are critical mediators of IL-18-mediated MMP9 gene transcription. Interestingly, compared with c-Fos and c-Jun, Fra-1 levels were much higher in...
Atorvastatin Inhibits IL-18-mediated SMC Migration

**FIGURE 9.** Atorvastatin inhibits IL-18-mediated SMC migration. A, atorvastatin inhibits IL-18-mediated SMC migration. Quiescent SMCs were layered on Matrigel matrix-coated membrane and treated with atorvastatin in phosphate-buffered saline for 1 h, followed by the addition of IL-18 (10 ng/ml) for 24 h. Cells migration was determined as described under “Experimental Procedures.” B, atorvastatin does not induce cell death. Quiescent SMCs were treated with atorvastatin (Atorvas) for 24 h. Cell death was assessed by quantifying mono- and oligonucleosomal fragmented DNA in the cytoplasmic extracts by ELISA. The nitric oxide donor (L)-S-nitroso-N-acetylpenicillamine (SNAP) served as a positive control. The results are the means ± S.E. of six experiments.

C, atorvastatin inhibits AP-1-dependent reporter gene activity. Quiescent SMCs cotransfected with the pAP-1-Luc and pRL-TK transfection control vectors were pretreated with atorvastatin (5 μM) for 1 h prior to IL-18 addition for 7 h. pEGFP-Luc served as a control. Reporter gene activity was assayed as described above. The results are the means ± S.E. of three independent experiments.

D, atorvastatin inhibits NF-κB-dependent reporter gene activity. Quiescent SMCs cotransfected with pNF-κB-Luc and the pRL-TK transfection control vector were treated as described for A. The results are the means ± S.E. of three independent experiments.

E, atorvastatin inhibits IL-18-induced MMP9 promoter-reporter activity. Quiescent SMCs cotransfected with MMP9-726 and the pRL-TK transfection control vector were treated with atorvastatin (5 μM) for 1 h, followed by IL-18 for 7 h. Reporter gene activity was assayed as described above. The results are the means ± S.E. of three independent experiments.

F, atorvastatin inhibits IL-18-induced MMP9 mRNA expression. Quiescent SMCs were treated with atorvastatin for 1 h prior to IL-18 for 24 h. Culture supernatants were assayed for MMP9 enzyme activity by gelatin zymography as described under “Experimental Procedures.” Actin levels in cell homogenates were analyzed by Western blotting to demonstrate similar number of cells plated in each treatment. A representative of three independent experiments is shown.

H, schematic showing possible signal transduction pathways involved in IL-18-mediated SMC migration and that targeted by atorvastatin. TRAF6, TNF receptor-associated factor-6.
nuclear extracts from IL-18-treated cells. Furthermore, Fra-1 antisense ODNs were more potent than c-Fos and c-Jun antisense ODNs in suppressing IL-18-mediated MMP9 induction. These observations are of particular interest because Fra-1 has been shown to confer invasiveness and motility in various cancer cell lines (32). It is possible that Fra-1 may function in a similar fashion in mediating IL-18-stimulated SMC migration.

Statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) are extensively used in clinical practice to lower cholesterol and to improve the outcome in patients with coronary artery diseases (27, 28, 33–36). Statins also exert beneficial effects that are independent of their lipid-lowering properties (37–39). Statins improve endothelial function; stabilize atherosclerotic plaques; and inhibit platelet aggregation, vascular SMC proliferation, and vessel wall inflammation (27, 28, 33–36, 40–42). In this study, we identified an additional beneficial effect of atorvastatin, i.e. the dose-dependent inhibition of SMC migration. Atorvastatin inhibited IL-18-mediated AP-1 and NF-κB activation and MMP9 transcription, mRNA expression, and enzyme activity, indicating that it exerts anti-proliferative effects by attenuating ECM degradation. In addition to AP-1 and NF-κB, atorvastatin has also been shown to target activation of other transcription factors involved in inflammation. In human vascular endothelial cells and SMCs, atorvastatin attenuates TNF-α-induced hypoxia-inducible factor-1α activation; hypoxia-inducible factor-1α plays a role in the induction of various inflammatory mediators (43), indicating that atorvastatin also exerts anti-inflammatory and antioxidant properties.

The results of our studies have implications for potential clinical intervention in cardiovascular disease. (i) Targeting expression of IL-18 or its specific downstream mediators may reduce atherosclerosis and restenosis. (ii) Statin therapy may reduce progression of the restenotic intervention in cardiovascular disease. (i) Targeting expression of IL-18 inhibits MMP activation and ECM degra-

Acknowledgments—We thank Dr. G. L. Freeman for helpful discussions and critical reading of the manuscript. We gratefully acknowledge the excellent technical assistance of Jie Li.

REFERENCES
1. Ross, R. (1999) Am. Heart J. 138, 5419–5420
2. Ross, R. (1999) N. Engl. J. Med. 340, 115–126
3. Libby, P. (2002) Nature 420, 868–874
4. Dinarello, C. A. (2000) Eur. J. Immunol. 31, 483–486
5. Mallat, Z., Corbaz, A., Schoaeez, A., Besnard, L., Leseche, G., Chvatchko, Y., and Tedgui, A. (2001) Circulation 104, 1598–1603
6. Gerdes, N., Sukhova, G. K., Libby, P., Reynolds, R. S., Young, J. L., and Schonbeck, U. (2002) J. Exp. Med. 195, 245–257
7. Mallat, Z., Henry, P., Fressonnet, R., Alounai, S., Schoaeez, A., Beaufils, P., Chvatchko, Y., and Tedgui, A. (2002) Heart 88, 467–469
8. Yamagarni, H., Kitazawa, K., Hohi, T., Furukado, S., Hougaku, H., Nagai, Y., and Hori, M. (2005) Arterioscler. Thromb. Vasc. Biol. 25, 1458–1462
9. Whitman, S. C., Ravisankar, P., and Daugherty, A. (2002) J. Biol. Chem. 277, 20221–20233
10. Chandrasekar, B., Murrula, K., Surahbi, R. M., Li-Weber, M., Owen-Schaub, L. B., Jensen, E. L., and Mummidi, S. (2004) J. Biol. Chem. 279, 3188–3196
11. Auge, N., Maupas-Schwalm, F., Elbaz, M., Thiess, C. J., Wasyhbor, A., Ihothara, S., Krell, H. W., Salvayre, R., and Negre-Salvayre, A. (2004) Circulation 110, 571–578
12. Sanceau, J., Truchet, S., and Bauvois, B. (2003) J. Biol. Chem. 278, 36537–36546
13. Chandrasekar, B., Valante, A. J., Freeman, G. L., Mahimainathan, L., and Mummidi, S. (2006) Biochem. Biophys. Res. Commun. 339, 956–963
14. Mandler, R. N., Dencoff, J. D., Midani, F., Ford, C. C., Ahmed, W., and Rosenberg, G. A. (2001) Brain 124, 493–498
15. Cho, A., and Reidy, M. A. (2002) Circ. Res. 91, 845–851
16. Newby, A. C., and Zaltsman, A. B. (2000) J. Pathol. 190, 300–309
17. Arnaud, C., Braunersreuther, V., and Mach, F. (2005) J. Biol. Chem. 280, 4553–4567
18. Weil, I. H., Lai, K. P., Chen, C. A., Chung, C. H., Huang, Y. J., Chou, C. H., Kuo, M. L., and Hsieh, C. Y. (2005) Oncogene 24, 390–398
19. Sato, H., and Seiki, M. (1993) Oncogene 8, 395–405
20. Chandrasekar, B., Mummidi, S., Claycomb, W. C., Mestril, R., and Nemer, M. (2005) Circulation 112, 2712–2717
21. Vaughan, C. I., Murphy, M. B., and Buckley, B. M. (1996) Lancet 348, 1079–1082
22. Arnaud, C., Braunerreuther, V., and Mach, F. (2005) Trends Cardiovasc. Med. 15, 202–206
23. Galia, Z. S., Johnson, C., Godin, D., Magid, R., Shipley, J. M., Senior, R. M., and Ivan, E. (2002) Circ. Res. 91, 852–859
24. Nold, M., Goede, A., Eberhardt, W., Pfeilschifter, J., and Mahl, H. (2003) Naunyn-Schmiedeberg’s Arch. Pharmacol. 367, 68–75
25. Zhang, H., Wu, K. F., Cao, Z. Y., Rao, Q., Ma, X. T., Zheng, G. G., and Li, G. (2004) Leuk. Res. 28, 91–95
26. Belskies, G., Kersaurol, N., Galtier, F., and Chalbos, D. (2005) J. Biol. Chem. 280, 1434–1444
27. LaRosa, J. C., Grundy, S. M., Waters, D. D., Shear, C., Barter, P., Fruchart, J. C., Gotto, A. M., Greten, H., Kastelein, J. J., Shepherd, J., and Wenger, N. K. (2005) N. Engl. J. Med. 352, 1425–1435
28. Sever, P. S., Dahlof, B., Poulter, N. R., Wedel, H., Beevers, G., Caulfield, M., Collins, R., Kjeldsen, S. E., Kristinsson, A., McInnes, G. T., Mehlisen, J., Nieminen, M., O’Brien, E., and Ostergren, J. (2003) Lancet 361, 1119–1158
29. Sever, P. S., Poulter, N. R., Dahlof, B., Wedel, H., Collins, R., Beevers, G., Caulfield, M., Kjeldsen, S. E., Kristinsson, A., McInnes, G. T., Mehlisen, J., Nieminen, M., O’Brien, E., and Ostergren, I. (2005) Diabetes Care 28, 1151–1157
30. Schwartz, G. G., Olsson, A. G., Ezekowitz, M. D., Ganz, P., Oliver, M. F., Waters, D., Zeiher, A., and Chaitman, B. R. (2001) J. Am. Med. Assoc. 285, 1711–1718
31. Lete, A. M., Scalia, R., and Lete, D. J. (2001) Cardiovasc. Res. 49, 281–287
32. Weber, C., Erb, W., Weber, K. S., and Weber, P. C. (1997) J. Am. Coll. Cardiol. 30, 1212–1217
33. Sumi, D., Hayashi, T., Thakur, N. K., Jayachandran, M., Asai, Y., Kano, H., Matsu, H., and Iguchi, A. (2000) Atherosclerosis 155, 347–357
34. Riikler, P. M., Rifi, N., Clearfield, M., Downs, J. R., Weis, S. E., Miles, J. S., and Gotto, A. M., Jr. (2001) N. Engl. J. Med. 344, 1959–1965
35. Nissen, S. E., Tuzcu, E. M., Schoenhagen, P., Crowe, T., Sasiela, W. J., Tsai, J., Orazen, J., Magorien, R. D., O’Sullivan, S., and Ganz, P. (2005) N. Engl. J. Med. 352, 29–38
36. Dichl, W., Dalk, J., Frick, M., Alber, H. F., Schwarzacher, S. P., Ares, M. F., Nilsson, J., Pachinger, O., and Weidinger, F. (2003) Arterioscler. Thromb. Vasc. Biol. 23, 58–63
37. Hellwig-Burgel, T., Stiehl, D. P., Wagner, A. E., Metzen, E., and Jelkmann, W. (2005) J. Interferon Cytokine Res. 25, 297–310

JUNE 2, 2006•VOLUME 281•NUMBER 22
JOURNAL OF BIOLOGICAL CHEMISTRY 15109