Interleukin-17 Stimulates C-reactive Protein Expression in Hepatocytes and Smooth Muscle Cells via p38 MAPK and ERK1/2-dependent NF-κB and C/EBPβ Activation*

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Elevated systemic levels of the acute phase C-reactive protein (CRP) are predictors of future cardiovascular events. There is evidence that CRP may also play a direct role in atherogenesis. Here we determined whether the proinflammatory interleukin (IL)-17 stimulates CRP expression in hepatocytes (Hep3B cell line and primary hepatocytes) and coronary artery smooth muscle cells (CASMBC). Our results demonstrate that IL-17 potently induces CRP expression in Hep3B cells independent of IL-1β and IL-6. IL-17 induced CRP promoter-driven reporter gene activity that could be attenuated by dominant negative IκBα or C/EBPβ knockdown and stimulated both NF-κB and C/EBP DNA binding and reporter gene activities. Targeting NF-κB and C/EBPβ activation by pharmacological inhibitors, small interfering RNA interference and adenoviral transduction of dominant negative expression vectors blocked IL-17-mediated CRP induction. Overexpression of wild type p50, p65, and C/EBPβ stimulated CRP transcription. IL-17 stimulated p38 MAPK and ERK1/2 activation, and SB203580 and PD98059 blunted IL-17-mediated NF-κB and C/EBPβ activation and CRP transcription. These results, confirmed in primary human hepatocytes and CASMC, demonstrate for the first time that IL-17 is a potent inducer of CRP expression via p38 MAPK and ERK1/2-dependent NF-κB and C/EBPβ activation and suggest that IL-17 may mediate chronic inflammation, atherosclerosis, and thrombosis.

C-reactive protein (CRP) is an acute phase reactant that is markedly increased during infection, inflammation, and tissue injury (1–5). It is synthesized and secreted mainly by the liver in response to circulating inflammatory mediators (6, 7). Elevated serum CRP levels serve as a risk marker for cardiovascular disease and predict future cardiovascular events and mortality (8, 9).

Data obtained both in vivo and in vitro indicate that CRP plays a role in vascular inflammation (10–12). CRP can be detected in human atherosclerotic plaques co-localized with modified low density lipoprotein (13, 14). It can also associate with the terminal complex of complement in the arterial wall, inducing its activation in plaques. CRP promotes the uptake of low density lipoprotein by macrophages (15) and exerts a mitogenic effect on vascular smooth muscle cells (16). CRP stimulates chemokine and adhesion molecule expression in vascular endothelial cells and enhances platelet adhesion to endothelial cells (17). These data suggest that CRP is not just a marker of cardiovascular risk but is a risk factor in its own right, and CRP plays a causal role in atherosclerosis and thrombosis. In fact, transgenic overexpression of human CRP has been shown to promote atherosclerosis in apoE−/− mice (18), as does chronic administration (19). These data support an hypothesis that CRP is a proinflammatory and pro-atherogenic factor.

Inflammation is an important component in all stages of atherosclerosis, with proinflammatory cytokines and chemokines playing critical roles. IL-17 is a member of a novel group of proinflammatory cytokines that is composed of six major isoforms, IL-17A, -B, -C, -D, -E (also known as IL-25), and -F (20). These isoforms are encoded by unique genes and share little homology with other interleukins. IL-17 signals via IL-17 receptors, products of unique genes, and includes IL-17RA, -B (also known as IL-25R), -C, -D, and -E (20).

IL-17A is the most widely studied cytokine of the IL-17 family. It signals via IL-17RA and exerts proinflammatory, pro-apoptotic, and pro-mitogenic effects. Unlike IL-17, which is considered a T-cell-specific cytokine (21), many cell types in the body express the receptors and are therefore targets of IL-17 (22). In this study we investigated whether IL-17 stimulates CRP expression in human hepatocytes and CASMC, and we determined the signal transduction pathways involved in

qPCR, quantitative PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT, reverse transcription; m.o.i., multiplicity of infection; PHH, primary human hepatocytes.
IL-17-mediated CRP expression. Our data show for the first time that IL-17 stimulates CRP expression in hepatocytes and coronary artery smooth muscle cells, independently of IL-1β and IL-6, and mediates CRP induction via p38 MAPK and ERK1/2-dependent NF-κB and C/EBPβ activation. These results suggest that IL-17-CRP signaling may play a role in chronic inflammatory conditions such as atherosclerosis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Recombinant human (rh) IL-1ra (280-RA/CF), IL-6 (206-IL-010), rhIL-17 (317-IL-050), IL-17R-Fc chimera (177-1R), Fc (110-HG), anti-IL-6 neutralizing antibodies (AB-206-NA), IL-6 ELISA kit (D6050), and normal goat IgG (AB-108-C) were purchased from R & D Systems. rhIL-1β (200-01B) was purchased from PeproTech, Inc. (Rocky Hill, NJ). Functional grade purified anti-human IL-17 antibodies (16-1718) and normal mouse IgG antibodies were obtained from eBioscience (San Diego, CA). Antibodies against C/EBPα (sc-61X), C/EBPβ (sc-150X), TRAF2 (sc-877), TRAF6 (sc-7221), and actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-p38, phospho-p38 (PhosphoPlus® p38 MAPK (Thr-180/Tyr-182) antibody kit), ERK1/2 (9102), phospho-ERK1/2 (9101S), and anti-phospho-C/EBPβ (3084S) antibodies were from Cell Signaling Technology, Inc. (Beverly, MA). SN-50 (cell-permeable peptide inhibitor of NF-κB, 50 μg/ml in phosphate-buffered saline), SN-50M (SN-50 mutant, 50 μg/ml in phosphate-buffered saline), MG-132 (a proteasomal inhibitor, 5 μM in Me2SO for 1 h), SB203580 (p38 MAPK inhibitor, 1 μM in Me2SO for 30 min), PD98059 (ERK inhibitor, 10 μM in Me2SO for 1 h), and genistein (induces ER stress and mitochondrial insult, 100 μM in Me2SO for 48 h) and Me2SO were purchased from EMD Biosciences (San Diego). All other chemicals were purchased from Sigma.

**Cell Culture**—Human hepatoma Hep3B cells (HB-8064; ATCC, Manassas, VA) were grown in Dulbecco’s modified Eagle’s medium supplemented with fetal bovine serum at 10% (complete media). At ~70% confluency, the complete medium was replaced with media containing 0.5% bovine serum albumin. Because IL-17 stimulates IL-6 expression (24), and IL-6 is a potent inducer of CRP (25), we investigated whether IL-17-stimulates IL-6 expression in hepatocytes and whether IL-17-mediated CRP expression is dependent on IL-6. Therefore, hepatocytes were treated with IL-17, IL-6 (10 ng/ml), or IL-17 + IL-6. IL-6 expression was targeted by siRNA (sense, 5'-CUCACCUCUUCAGAACGAAAT-3', 100 nm (26)) or anti-IL-6 neutralizing antibodies (10 μg/ml for 1 h) prior to IL-17 addition. Normal goat/mouse IgG served as a control. Knockdown of IL-6 was confirmed by RT-qPCR (IL-6 qPCR was performed using a Cytoxpress kit, BIOSOURCE). IL-17 is also known to induce IL-1β expression (27). However, it has been reported previously that IL-1β fails to stimulate CRP expression in Hep3B cells but potentiates IL-6-mediated CRP expression (25). Therefore, we investigated whether IL-1α blocks IL-1β, IL-6, IL-1β+IL-6, or IL-17-mediated CRP secretion. Quiescent Hep3B cells were treated with IL-1α simultaneously with IL-1β (10 ng/ml), IL-6 (10 ng/ml), IL-1β+IL-6 (10 ng each/ml), or IL-17 (100 ng/ml) for 24 h. Hep3B cells were not pretreated with IL-1α, and at these concentrations these cytokines did not affect cell viability (data not shown). CRP levels in culture supernatants were quantified by ELISA.

**Adenoviral Vectors, Propagation, and Infection**—Recombinant, replication-deficient adenoviral vectors encoding green fluorescent protein (Ad-CMV-GFP), dominant negative (dn) IκKB, and dnIkB-α (S32A/S36A) have been described (28). Cells were infected at 100 m.o.i. as described previously (28).

**Transient Cell Transfections and Reporter Assays**—A DNA fragment containing human CRP promoter (−300/19) was amplified by PCR from human genomic DNA (Promega) using the primers sense, 5'-aga tct AGAGCTACCTCCTCTCGC-CTGG-3', and antisense, 5'-acgcgtACCCAGATGGCCACTC-3'. Mutation of the C/EBP site was accomplished by converting −72 AAATT to −72 TTAAT using the primers 5'-GGCC-CCACTATGTAATATTATTAACCAATTTGTTGGG-GCC-3' and 5'-GCCCAACAAGCAATGTGGTTTAAATATA-TTTACATAGTGCGC-3'. Mutation of the C/EBP site was performed using the primers 5'-GGAAAAATTTACATA-GTGTAGCCTACTCCCCCTACTGTTGG-3' and 5'-CCAGAAGCAGTAAGGGAGTACGATCTATGTTAATTATTTTCG-3'. All constructs were verified by restriction mapping and bidirectional sequencing.

**Cell Transfection and Reporter Assays**—Cells were transfected with 3 μg of the CRP reporter constructs and 100 ng of the control Renilla luciferase vector pRL-TK (Promega) using Lipofectamine®, Luciferase activity was determined using the Promega Biotech™ dual-luciferase reporter assay system (23). Firefly luciferase data were normalized with the corresponding Renilla luciferase and expressed as mean relative stimulation ± S.E. for a representative experiment from three to six separate experiments, each performed in triplicate. Transfection efficiency of hepatocytes was determined using pEGFP-N1 vector (Clontech) and was found to be 34.3%.

To investigate pathways involved in IL-17-mediated CRP expression, hepatocytes were transiently transfected with wild type or dominant negative expression vectors using Lipofectamine 2000 (Invitrogen). Wild type (CMV-C/EBPβ) and dnC/EBP-β (CMV-dnC/EBP-β) were generous gifts from Richard M. Pope (Northwestern University Medical School, Chicago). dnTRAF6 (pRK5-TRAF6-(289–522)-FLAG), dnTRAF2 (pRK5-TRAF2-(87–501)-FLAG), kdNIK (pRK7-NIK(K429A/K430A)-FLAG) were described previously (30, 31). pRK5 and pRK7 served as controls. To compensate for variations in transfection efficiency, cells were co-transfected with pRL-Renilla luciferase vector (pRL-TK vector; Promega, Madison, WI).
C/EBPβ expression was also targeted by C/EBPβ siRNA duplex (sense, 5’-GAAGACCCGGCAAGCAGTCTT-3’; 100 nm). TRAF2 expression was targeted by two siRNA duplexes (sense, 5’-AUACGAGACGUCGCCAGAATCAGTCTT-3’; and sense, 5’-AGAGGCGAGUCAACGACAUCAGTCTT-3’; 50 nm each) and TRAF6 by a siRNA duplex (5’-CUUGCUCCAGAUCUAGGCC-AdTdT-3’) as described previously (32). As negative control, siRNA that does not target any genes in the human genome (5’-UUCUCCGAAGGUGUCGAGUdTdT-3’; catalog number 1022076, Qiagen Inc.; 100 nm) was used.

Gel Shift, Supershift, ELISA, and Reporter Assays—NF-κB and C/EBP DNA binding activities were assessed by EMSA. Double-stranded consensus wild type (NF-κB, 5’-AGGTGGAAGGTTCGTGGCGG-3’; C/EBP, 5’-TGCAGAGTCGGAC-3’), and mutant (NF-κB, 5’-AGGTGGAAGGTTCGTGGCGG-3’; C/EBP, 5’-TGCAGAGTCGGAC-3’) oligonucleotides (Santa Cruz Biotechnology, Inc.) were used as before (23, 28, 30, 31). Activation and subunit composition were determined by supershift (C/EBP) and TransAM™ NF-κB (catalog number 43296) and C/EBP α/β (catalog number 44196) transcription factor ELISA (Active version 2.02). Real time quantitative PCR was performed as described previously by Ivashchenko et al. (34) using Quantitect SYBR-Green Probe RT-PCR Kit (Qiagen). Each sample was assayed in triplicate. For relative quantification, the Ct method (ratio = 2 – (Ct(CRP) – Ct(GAPDH))) was used with GAPDH as a control. For copy number determination, a calibration curve was obtained using serial dilutions of linearized GAPDH cDNA as template and the GAPDH primers 5’-GAAGGTGGAAGGTTCGTGGCGG-3’ and 5’-GAAGATGGTGATGGGATTTC-3’; human CRP primer pair 1 (product size 133 bp), forward, 5’-ACCCTCTTAATGTAATCCCTC-3’; and reverse, 5’-TCTATGGTGGTGATGGGATTTC-3’, human CRP primer pair 2 (product size 440 bp), forward, 5’-TGCTTGACGCCTGTACAATGGA-3’; and reverse, 5’-AACACCTTGCCCCTGACCCTC-3’; Primer pair 3 distinguishes between mRNA and genomic DNA (expected product size 196 bp for mRNA and 481 bp for genomic DNA): forward, 5’-TCTATGGTGGTGATGGGATTTC-3’; and reverse, 5’-TCTATGGTGGTGATGGGATTTC-3’.

ELISA—CRP levels in culture supernatants were quantified by an ELISA (IMUCLONE® High Sensitivity CRP ELISA test kit,
**RESULTS**

**IL-17 Stimulates CRP Expression in Hep3B Cells**—IL-17 functions as a proinflammatory cytokine in various models of inflammation (20, 21). Because CRP exerts proinflammatory effects in atherosclerosis (16–19), we investigated whether IL-17 stimulates CRP expression using a hepatic cell line. Quiescent Hep3B cells were treated with rhIL-17 for 24 h, and CRP mRNA expression was quantified by RT-qPCR. IL-17 stimulated CRP mRNA expression was quantified by RT-qPCR. IL-17 stimulated CRP expression dose-dependently with significant stimulation in CRP expression detectable at 10 ng/ml and peak levels at 100 ng/ml. Time course studies revealed that IL-17 at 100 ng/ml increased CRP expression at 24 h, with no further increases detected at 48 h (Fig. 1B). Therefore, in all subsequent experiments IL-17 was used at 100 ng/ml. IL-17 at this dose stimulated CRP secretion (6-fold, \( p < 0.001 \); Fig. 1C), and treatment with anti-IL-17 neutralizing antibodies or IL-17Fc chimera blocked this expression (Fig. 1D). Although IL-17 is known to induce pro-apoptotic gene expression (24), results in Fig. 1E show that IL-17 failed to induce cell death. However, genistein, a known inducer of ER stress and mitochondrial insult in hepatocytes (36), induced significant cell death. These results indicate that IL-17 is a potent inducer of CRP expression in Hep3B cells (Fig. 1).

**IL-17-mediated CRP Expression Is Independent of IL-1β and IL-6 in Hep3B Cells**—IL-6 is a proinflammatory cytokine whose expression is increased in both subclinical and overt inflammatory models of inflammation (20, 21). Because CRP exerts proinflammatory effects in atherosclerosis (16–19), we investigated whether IL-17 stimulates CRP expression using a hepatic cell line. Quiescent Hep3B cells were treated with rhIL-17 for 24 h, and CRP mRNA expression was quantified by RT-qPCR. IL-17 stimulated CRP mRNA expression was quantified by RT-qPCR. IL-17 stimulated CRP expression dose-dependently with significant stimulation in CRP expression detectable at 10 ng/ml and peak levels at 100 ng/ml. Time course studies revealed that IL-17 at 100 ng/ml increased CRP expression at 24 h, with no further increases detected at 48 h (Fig. 1B). Therefore, in all subsequent experiments IL-17 was used at 100 ng/ml. IL-17 at this dose stimulated CRP secretion (6-fold, \( p < 0.001 \); Fig. 1C), and treatment with anti-IL-17 neutralizing antibodies or IL-17Fc chimera blocked this expression (Fig. 1D). Although IL-17 is known to induce pro-apoptotic gene expression (24), results in Fig. 1E show that IL-17 failed to induce cell death. However, genistein, a known inducer of ER stress and mitochondrial insult in hepatocytes (36), induced significant cell death. These results indicate that IL-17 is a potent inducer of CRP expression in Hep3B cells (Fig. 1).
significant stimulatory effects on CRP secretion. However, IL-1β potentiated IL-6-induced CRP secretion. Importantly, simultaneous treatment with IL-1ra blocked IL-1β + IL-6-mediated but not IL-17-stimulated CRP secretion. IL-1ra did not affect basal CRP secretion. These results demonstrate that IL-17 stimulates CRP expression in Hep3B cells independent of IL-1β and IL-6 (Fig. 2).

**IL-17 Stimulates CRP Transcription via NF-κB and C/EBP in Hep3B Cells**—Because IL-17 induced CRP expression, we next investigated whether IL-17-mediated CRP expression is regulated at transcriptional level. IL-17 induced CRP transcription (Fig. 3A) and potently stimulated CRP promoter (−300/−1) reporter activity in Hep3B cells (Fig. 3B), and mutation of the NF-κB or C/EBP sites blunted this response, indicating that IL-17 induces CRP transcription via NF-κB and C/EBP. Furthermore, ectopic expression of NF-κB p50, p65, or C/EBPβ all significantly stimulated CRP transcription in Hep3B (Fig. 3C), indicating that IL-17 stimulates CRP transcription in hepatic cells through NF-κB and C/EBPβ (Fig. 3).

**IL-17 Stimulates NF-κB Activation in Hep3B Cells**—We have demonstrated that IL-17-induced CRP promoter-driven reporter gene activity is attenuated when NF-κB core DNA-binding sequence is mutated (Fig. 3B). Conversely, ectopic expression of wild type NF-κB p50 or p65 stimulated CRP promoter-reporter activity (Fig. 3C). Therefore, we investigated whether IL-17 induces NF-κB activation in Hep3B cells. Our results show that IL-17 potently stimulated NF-κB DNA binding activity within 1 h (Fig. 3D, lane 6) and was attenuated by preincubation with anti-IL-17 neutralizing antibodies (Fig. 3E, lane 8). Furthermore, IL-17 increased the levels of p65, p50, and c-Rel proteins in the nucleus (Fig. 3F) and stimulated NF-κB-driven luciferase activity (Fig. 3G). Together, these results indicate that IL-17 is a potent inducer of NF-κB activation in Hep3B cells (Fig. 3).

**IL-17 Induces NF-κB Activation and CRP Expression via TRAF6, NIK, IKK, and IκB-α**—We next investigated the pathway involved in IL-17-mediated NF-κB activation and CRP gene expression. IL-17-mediated NF-κB activation was inhibited by adenoviral transduction of dnIKKβ (Fig. 4A, lane 10) and dnIκB-α (lane 11). Furthermore, treatment with SN-50, a peptide inhibitor of NF-κB activation, blunted IL-17-mediated NF-κB activation (Fig. 4B, lane 10), as did the proteasome inhibitor MG-132 (Fig. 4B, lane 12). Similarly, transient overexpression of dnTRAF6 (Fig. 4C, lane 12), but not dnTRAF2 (lane 13), and kinase-deficient NIK (lane 14) attenuated IL-17-mediated NF-κB activation. Importantly, both overexpression of dnTRAF6 and TRAF6 knockdown (Fig. 4D, right-hand panels) blunted IL-17-mediated CRP mRNA expression. IL-17-me-
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*A* competition assay with CRP promoter-driven reporter gene activity was attenuated when C/EBP core DNA binding sequence is mutated (Fig. 3C). Therefore, we next investigated whether IL-17 stimulates C/EBP activation in Hep3B cells. Our results show that IL-17 stimulated C/EBP DNA binding activity with high levels at 2 h (Fig. 5A, lane 6), and supershift assays revealed predominantly C/EBPβ in the protein-DNA complexes (Fig. 5A, lane 8). Our results also show that anti-IL-17 antibodies blunt IL-17-mediated C/EBP activation (Fig. 5B). Confirming the EMSA results (Fig. 5A), ELISA of nuclear protein extracts revealed a significant increase in C/EBPβ and a small, but statistically insignificant, increase in C/EBPα levels following IL-17 treatment (Fig. 5C). IL-17 also induced C/EBP-driven luciferase activity (Fig. 5D) and C/EBPβ phosphorylation (Fig. 5E).

**Activation**—Because IL-17-mediated NF-κB and C/EBPβ activation is TRAF6-dependent, and IL-17 is known to activate MAPKs (38, 39), we investigated the role of MAPKs in TRAF6-dependent transcription factor activation following IL-17 treatment. IL-17 induced SB203580-inhibitable p38 MAPK activation (Fig. 6A). Immune complex kinase assays confirmed IL-17-mediated p38 MAPK activity (Fig. 6B). Similarly, IL-17 induced PD98059-inhibitable ERK1/2 phosphorylation (Fig. 6C) and activity (Fig. 6D). Importantly, TRAF6, but not TRAF2, knockdown abrogated IL-17-mediated p38 MAPK (Fig. 6E) and ERK1/2 (Fig. 6F) activities. Finally, inhibition of p38 MAPK and ERK1/2 attenuated IL-17-mediated NF-κB p65 (Fig. 6G) and C/EBPβ (Fig. 6H) activation and CRP mRNA expression (Fig. 6I). Together, these results indicate that IL-17 induces CRP mRNA expression via TRAF6-dependent p38 MAPK and ERK1/2 activation.

**IL-17 Stimulates CRP Expression via p38 MAPK and ERK1/2-dependent NF-κB and C/EBP Activation in Primary Human Hepatocytes and Coronary Artery Smooth Muscle Cells**—We have demonstrated that IL-17 stimulates CRP expression in Hep3B cells via TRAF6-dependent p38 MAPK and ERK1/2-dependent NF-κB and C/EBP activation.
mediated NF-κB and C/EBP activation. Because Hep3B cells are derived from human hepatoma, we investigated whether IL-17 exerts similar effects in PHH. Our results show that IL-17 is a potent inducer of CRP mRNA expression in PHH (Fig. 7A), and knockdown of TRAF6, C/EBPβ, and adenoviral transduction of dnIκB-α or pretreatment with SB203580 or PD98059 attenuate IL-17-mediated CRP mRNA expression (Fig. 7A). Furthermore, pretreatment with MG-132, SN-50, SB203580, or PD98059 significantly attenuated IL-17-mediated CRP secretion in PHH. However, SN-50M and Me2SO had no modulatory effects. Similar to its effects on PHH, IL-17 induced CRP mRNA expression in CASMC via similar signaling pathways (Fig. 7C). Together, these results indicate that IL-17 is a potent inducer of CRP expression in primary hepatocytes and CASMC, and IL-17-mediated CRP induction is dependent on TRAF6, p38 MAPK, ERK1/2, NF-κB, and C/EBPβ (Fig. 7).

**DISCUSSION**

The major novel finding of this study is that the proinflammatory cytokine interleukin-17 stimulates CRP expression in a human hepatoma cell line, primary human hepatocytes, and human coronary artery smooth muscle cells. In all of these cells, IL-17 stimulates CRP expression via p38 MAPK and ERK1/2-dependent NF-κB and C/EBPβ activation (Fig. 7D). These results suggest that IL-17-CRP signaling may play a role in chronic inflammatory conditions.

The IL-17 family of proinflammatory cytokine contains six members (A–F) that share little to no homology with other interleukins (21, 22). IL-17 has been shown to play a role in various models of inflammation and autoimmune diseases, including rheumatoid arthritis (21, 22, 40, 41). IL-17 is reported to stimulate a variety of genes, including chemokines, cytokines, and transcription factors (42), and the stimulatory effects of IL-17 are enhanced when combined with suboptimal doses of tumor necrosis factor-α (43). In co-cultures of mouse bone marrow cells and osteoblasts, IL-17 is reported to increase osteoclast formation in a dose-dependent manner (40). Here we demonstrate that IL-17 stimulates CRP and IL-6 expression in hepatocytes. However targeting of IL-6 expression by neutralizing antibodies, antisense oligonucleotides, and siRNA-mediated knockdown all failed to block IL-17-mediated CRP induction. Therefore IL-6 is not required for IL-17-mediated CRP expression. IL-6 however, potentiated the IL-17 effects. Potentiating effects of IL-6 have also been reported previously on CRP induction in hepatocytes treated with IL-1 (29). In that study, although IL-1 failed to stimulate CRP expression, a significant induction of CRP was observed when IL-1 was combined with IL-6 (29). Because inflammation is characterized by the up-regu-
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A

B

C

D

E

F

G

H

I

FIGURE 6. IL-17 stimulates CRP expression via p38 MAPK and ERK1/2 in Hep3B cells. A, IL-17 induces p38 MAPK activation. Quiescent Hep3B cells were treated with SB203580 (SB; 1 μM in Me2SO (DMSO) for 1 h) prior to IL-17 treatment. Total and phospho-p38 MAPK levels at 30 min were analyzed by Western blotting using activation-specific antibodies. B, IL-17 stimulates p38 MAPK activity. Quiescent Hep3B cells treated as in A were analyzed for p38 MAPK activity by immune complex kinase assay using ATF-2 as a substrate. C, IL-17 induces ERK1/2 activation. Quiescent Hep3B cells were treated with PD98059 (PD; 10 μM for 1 h) prior to IL-17 addition. Total and phospho-ERK1/2 levels were analyzed at 30 min by Western blotting. D, IL-17 stimulates ERK1/2 activity. Quiescent Hep3B cells treated as in C were analyzed for ERK1/2 activity by immune complex kinase assay using Elk as a substrate. E, TRAF6 knockdown blocks IL-17-mediated p38 MAPK activation. Hep3B cells were treated with TRAF6, TRAF2, or control siRNA for 48 h and then treated with IL-17 for 30 min. p38 MAPK activity was determined as in A. F, Hep3B cells treated as in E were analyzed for ERK1/2 activity as described in D. G, inhibition of p38 MAPK and ERK1/2 attenuates IL-17-mediated NF-κB activation. Quiescent Hep3B cells were treated with SB203580 (1 μM) or PD98059 (10 μM) for 1 h prior to IL-17 addition. Nuclear protein was extracted at 2 h and analyzed for NF-κB activity by gel shift assay. H, inhibition of p38 MAPK and ERK1/2 attenuate IL-17-mediated C/EBPβ expression. Quiescent Hep3B cells were treated with SB203580 or PD98059 (10 μM for 1 h) prior to IL-17 addition. Nuclear protein was extracted at 2 h and analyzed for C/EBPβ levels by ELISA. I, inhibition of p38 MAPK and ERK1/2 attenuates IL-17-mediated CRP mRNA expression. Quiescent Hep3B cells were treated with SB203580 (1 μM) or PD98059 (10 μM) for 1 h prior to IL-17 addition. CRP mRNA expression was analyzed at 24 h by RT-qPCR.

lulation of various cytokines that stimulate CRP induction, it is possible that IL-17 may act in synergy with other proinflammatory cytokines in stimulating CRP expression in vivo in liver and other tissues.

Our results also show that IL-17-mediated CRP induction is dependent on p38 MAPK and ERK1/2-dependent C/EBPβ and NF-κB activation. While targeting C/EBPβ or NF-κB each reduced CRP activity, their combined significantly attenuated, but not abrogated, CRP transcription. These results suggest that both C/EBPβ and NF-κB play critical roles in IL-17-CRP signaling but that other transcriptional elements are also involved in CRP induction. In a series of well executed studies, Voleti and Agrawal (29) have demonstrated that IL-6 stimulates CRP expression in hepatocytes via synergistic activation of C/EBPβ and NF-κB. These authors also demonstrated that Oct-1 and Stat3 also contribute to basal and induced CRP expression (44), suggesting that multiple transcriptional regulatory elements contribute to CRP induction in agonist and cell type-dependent manner. Studies are in progress to investigate if similar interactions occur in hepatocytes treated with IL-17. Because CRP is also known to stimulate NF-κB activation (42), IL-17-CRP signaling may play a role in vascular inflammation via activation of signal transduction pathways that converge at NF-κB.

Our results also show that IL-17 stimulates CRP expression in human coronary artery smooth muscle cells through NF-κB and C/EBP activation. Although hepatocytes are reported to be the major source of circulating CRP (6, 7), CRP expression has also been detected in human atherosclerotic lesions and is associated with calcification and plaque rupture (13, 14). In coronary vessels, CRP is localized to macrophages and smooth muscle cells (17) and mediates SMC proliferation (45, 46). CRP also promotes endothelial dysfunction (11), a hallmark of atherosclerosis. In endothelial progenitor cells, CRP stimulates reactive oxygen species generation, inhibits antioxidative enzyme levels, inactivates telomerase, and promotes cell death (47). These reports indicate that CRP may differentially affect various cell types in a vessel wall, resulting in the development and progression of atherosclerosis.

Recently CRP has been shown to be involved in the pathogenesis of obesity and its metabolic complications. CRP binds leptin and prevents its effects on food intake, body weight, blood glucose, and lipid metabolism (48). Because obesity plays a significant role in coronary artery and cardiovascular diseases, it appears that CRP acts on several cellular targets (endothelial cells, smooth muscle cells, hepatocytes, and adipocytes) to regulate energy metabolism and promote atherosclerosis. CRP is
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Our studies have several important implications as follows: (i) IL-17 can mediate chronic inflammation and increase CRP expression in hepatocytes and smooth muscle cells, and by doing so enhance atherosclerosis; (ii) IL-17 may enhance myocardial inflammation and injury via up-regulation of IL-6 and other proinflammatory and pro-apoptotic cytokines; (iii) IL-17 may enhance atherogenesis and plaque rupture by stimulating the expression of pro-atherogenic cytokines (e.g. tumor necrosis factor), chemokines, and extracellular matrix-degrading matrix metalloproteinases through NF-κB activation. Thus the IL-17-CRP signaling pathway may be a significant inflammatory component in atherogenesis and cardiovascular diseases.

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