Interleukin-17 and Interferon-γ Are Produced Concomitantly by Human Coronary Artery–Infiltrating T Cells and Act Synergistically on Vascular Smooth Muscle Cells

Raymond E. Eid, MD; Deepak A. Rao, MS; Jing Zhou, MD, PhD; Sheng-fu L. Lo, BA; Hooman Ranjbaran, MD; Amy Gallo, MD; Seth I. Sokol, MD; Steven Pfau, MD; Jordan S. Pober, MD, PhD; George Tellides, MD, PhD

Background—Atherosclerosis is an inflammatory disease in which interferon (IFN)-γ, the signature cytokine of Th1 cells, plays a central role. We investigated whether interleukin (IL)-17, the signature cytokine of Th17 cells, is also associated with human coronary atherosclerosis.

Methods and Results—Circulating IL-17 and IFN-γ were detected in a subset of patients with coronary atherosclerosis and in referent outpatients of similar age without cardiac disease but not in young healthy individuals. IL-17 plasma levels correlated closely with those of the IL-12/IFN-γ/CXCL10 cytokine axis but not with known Th17 inducers such as IL-1β, IL-6, and IL-23. Both IL-17 and IFN-γ were produced at higher levels by T cells within cultured atherosclerotic coronary arteries after polyclonal activation than within nondiseased vessels. Combinations of proinflammatory cytokines induced IFN-γ but not IL-17 secretion. Blockade of IFN-γ signaling increased IL-17 synthesis, whereas neutralization of IL-17 responses decreased IFN-γ synthesis; production of both cytokines was inhibited by transforming growth factor-β1. Approximately 10-fold fewer coronary artery–infiltrating T helper cells were IL-17 producers than IFN-γ producers, and unexpectedly, IL-17/IFN-γ double producers were readily detectable within the artery wall. Although IL-17 did not modulate the growth or survival of cultured vascular smooth muscle cells, IL-17 interacted cooperatively with IFN-γ to enhance IL-6, CXCL8, and CXCL10 secretion.

Conclusions—Our findings demonstrate that IL-17 is produced concomitantly with IFN-γ by coronary artery–infiltrating T cells and that these cytokines act synergistically to induce proinflammatory responses in vascular smooth muscle cells. (Circulation. 2009;119:1424-1432.)

Key Words: coronary disease • inflammation • interleukins • lymphocytes • muscle, smooth
cytokine IFN-γ and the Th1-inducing factor IL-12 suppress the differentiation of Th17 cells. Evidence that the transcription factor ROR-γt specifically controls Th17 cell differentiation supports the concept of a distinct T-cell lineage. From studies in mice, the differentiation of Th17 and Th1 cells is generally thought to be mutually exclusive. The paradigm is more complex in humans, in whom single effector T helper cells may produce both IL-17 and IFN-γ. Even in mice, double producers of IL-17 and IFN-γ have been noted under certain circumstances such as infection with extracellular bacteria.

Clinical Perspective p 1432

Th17 cells have an antimicrobial role through the production of proinflammatory factors from diverse cell types by IL-17, including the cytokine IL-6 and the chemokine CXCL8. Defining features of IL-17–dependent immune responses include the recruitment of neutrophils and protection against extracellular bacterial and fungal infection. Beyond their function in host defense, Th17 cells have been responsible for pathological outcomes in experimental models of arthritis, autoimmune encephalitis, and colitis. In humans, several studies have described a correlation between plasma or tissue concentrations of IL-17 and the severity of rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease. However, the roles of Th17 cells and IL-17 in atherosclerotic arterial disease remain undefined. Here, we report the concomitant presence of IL-17 and IFN-γ in patients and clinical specimens of coronary atherosclerosis, the presence of IL-17/IFN-γ dual-producing T cells within coronary plaques, and a synergistic effect of IL-17 and IFN-γ on elicitation of proinflammatory cytokine and chemokine production by cultured human vascular smooth muscle cells (VSMCs).

Methods

Patients and Artery Donors

Research protocols were approved by the institutional review boards of the West Haven VA Hospital, Yale University, and the New England Organ Bank. Clinical characteristics are detailed in the Methods section and Table I of the online Data Supplement. Briefly, there were 108 patients with symptomatic coronary atherosclerosis documented by cardiac catheterization (63.7±10.5 years of age; 99.1% male; and 88.0% white), 39 referent outpatients treated for diverse medical conditions but without a diagnosis of coronary atherosclerosis (66.4±9.7 years of age; 94.9% male; and 89.8% white), and 18 healthy laboratory personnel and students (32.4±4.8 years of age; 66.1% male; and 44.4% white). The 2 patient cohorts with and without a diagnosis of coronary atherosclerosis had similar demographics, whereas the healthy control group was younger (P<0.0001) and had fewer men (P<0.001) and fewer whites (P<0.001). Human coronary arteries and aortas were obtained from explanted hearts of transplant recipients or cadaver organ donors.

Artery and Cell Culture

Epicardial coronary arteries were divided into 3-mm rings and cultured in M-199 medium supplemented with 20% FBS (Invitrogen, Carlsbad, Calif). The medium was changed after 6 hours before treatment with agonistic antibodies to CD3 (eBioscience, San Diego, Calif) and CD28 (BD Biosciences, San Jose, Calif), with PMA and ionomycin (Sigma-Aldrich, St Louis, Mo), and/or with human cytokines. Human aortic and coronary artery VSMCs were isolated by explant outgrowth, serially cultured in M199 medium supplemented with 20% FBS, and used at passage 3 to 4. VSMCs were serum deprived in 0.5% FBS for 48 hours before cytokine treatment.

Circulating CD4+ T cells were isolated by positive selection with magnetic beads (Dynal Biotech, Oslo, Norway). Human coronary artery-infiltrating leukocytes were isolated from enzyme-digested, minced artery segments as described in the supplemental Methods section, followed by magnetic bead selection, yielding a uniformly positive cell population with >90% CD4 expression.

Cytokines and Antibodies

Human coronary arteries and VSMCs were treated with recombinant human IFN-γ, IL-1α, IL-6, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, and TGF-β1, as well as neutralizing antibodies to IL-17RA, IFN-γR1, or irrelevant, isotype-matched antibody (R&D Systems, Minneapolis, Minn).

Cytokine Array and ELISA

Cytokine arrays were performed with soluble biotinylated detection antibodies and immobilized capture antibodies on nitrocellulose membranes, and bound cytokine- or chemokine-antibody complexes were detected by chemiluminescent reagents according to the manufacturer’s instructions (R&D Systems). The spot pixel density was measured by image analysis software in arbitrary units.

Flow Cytometry and Immunoblotting

Details of the techniques are provided in the supplemental Methods. In brief, for flow cytometric analysis, the cells were labeled with mouse anti-human CD4 (Beckman, Miami, Fla), IL-17, and IFN-γ (eBioscience) monoclonal antibodies or isotype-matched, irrelevant IgG and analyzed with a FACSort (BD Biosciences). For immunoblotting analysis, protein lysates from VSMCs were blotted with rabbit antibodies to IκBα, phospho-p38 mitogen-activated protein kinase (MAPK), and phospho-STAT1 (Cell Signaling Technology, Beverly, Mass) or with mouse monoclonal antibodies to β-actin (Sigma-Aldrich), followed by horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, Pa).

Statistical Analysis

Results were analyzed from 3 different projects: a patient-related serological study, an organ culture model, and a cell culture model. In the serological study, plasma cytokine levels were undetectable in a substantial proportion of subjects, and the distribution was skewed, even after log transformation. The 2 groups of patients with and without coronary atherosclerosis and a third group of healthy individuals were compared for a binary outcome of detectable versus undetectable cytokine plasma levels with Fisher’s exact test. Comparisons between individual groups were performed after establishing significance with a global test, ie, with 2 df for 3 groups using the χ² test. Correlations between cytokine plasma levels were examined by the nonparametric Spearman test. Continuous clinical variables were compared between groups with the 2-sample t test, and categorical clinical variables were analyzed by Fisher’s exact test. In the organ culture and cell culture models, the distribution of supernatant cytokine levels was gaussian, and parametric tests were performed. For in vitro assays with 2 experimental arms, comparisons were by the t test; for those with >2 groups, by 1-way ANOVA; and for assays over time, by repeated-measures ANOVA. All probability values were 2 tailed, and values of P<0.05 were considered to indicate statistical significance. Statistical analyses...
were performed with Prism 4 (GraphPad Software, San Diego, Calif).

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**IL-17 Is Detected Concomitantly With IFN-γ in the Plasma of Patients With Coronary Atherosclerosis**

Plasma cytokine levels were measured in patients presenting with symptomatic coronary atherosclerosis (n = 108), in referent outpatients without a diagnosis of coronary atherosclerosis (n = 59), and in healthy control subjects (n = 18). Circulating IL-17 levels were further compared in coronary atherosclerosis patients with lower disease extent scores between 1 and 22 (C; n = 60) and those with higher disease extent scores between 23 and 45 (n = 48) and between those with presenting symptoms of chronic stable angina (D; n = 87) and those with acute coronary syndrome (n = 21). E, Circulating IL-17 levels also were compared in referent outpatients with no current medical illnesses (n = 12) and patients with current noncardiac medical illnesses (n = 48). Individual data are shown. The proportion of patients with detectable levels of IL-17 and IFN-γ (>7.8 pg/mL) was compared between groups with Fisher’s exact test. CAD indicates coronary artery disease.

We examined for an association between circulating cytokines. Surprisingly, there was a strong correlation between IL-17 and IFN-γ plasma levels (particularly at higher concentrations) in patients with coronary atherosclerosis (Figure 2A) and somewhat less so in referent patients (r = 0.76, P < 0.0001). IL-17 also correlated with other markers of the IFN-γ cytokine axis in patients with coronary atherosclerosis such as the IFN-γ inducer IL-12 and the IFN-γ—inducible chemokine CXCL10 (Figure 2B and 2C). In contrast, IL-17 concentrations did not correlate with the inflammatory marker most frequently described in patients with coronary atherosclerosis, C-reactive protein (r = 0.13, P = 0.19), or with levels of cytokines known to promote human Th17 immune responses such as IL-1β, IL-6, and IL-23 (Figure 2D through 2F). Independently, measurements of the common p40 subunit for IL-12 and IL-23 revealed an intermediate association with IL-17 (r = 0.41, P < 0.0001). The close correlation between circulating IL-17 and IFN-γ suggested at least a partially shared, common trigger for production of these cytokines in coronary atherosclerosis.

**Both IL-17 and IFN-γ Are Produced by T Cells Within Atherosclerotic Coronary Arteries**

We used an organ culture system to determine stimuli that can increase IL-17 production within human coronary arteries. We first characterized the cytokine polarization of artery-infiltrating T cells in response to polyclonal T-cell receptor activation with agonistic antibodies to CD3 and CD28. Optimal concentrations of the T-cell—stimulating antibodies elicited IL-17 and even greater IFN-γ secretion from artho-
sclerotic coronary artery rings in a time-dependent fashion (Figure 3A and 3B). A lesser secretion of cytokines in response to anti-CD3/CD28 was detected from coronary arteries of donors without gross signs of atherosclerosis. In the absence of cognate stimuli, inflammatory factors known to promote the differentiation of Th17 cells or to maintain Th17 immune responses such as IL-1, IL-6, IL-15, IL-21, IL-23, and TGF-β1 did not result in detectable IL-17 production either alone or in various combinations (Figure 3C). These data demonstrate that antigen but not inflammatory pathways effectively activated Th17 immune responses in human coronary arteries.

We further investigated cytokine cross-regulation of receptor-activated T cells present within atherosclerotic coronary arteries. T cells resident in plaques were polyclonally stimulated in situ under pathologically relevant conditions in the presence of various treatment conditions. Inhibiting IL-17 responses with neutralizing antibody to IL-17RA decreased IFN-γ secretion without modulating IL-17 secretion. In contrast, inhibiting IFN-γ responses with neutralizing antibody to IFN-γR1 increased IL-17 secretion but decreased IFN-γ production (Figure 4A). TGF-β1 inhibited the production of both IL-17 and IFN-γ after T-cell receptor engagement (Figure 4B). Finally, the combination of IL-12 and IL-18, known to induce IFN-γ production, did not stimulate IL-17 secretion either with or without CD3/CD28 antibody treatment (Figure 4C). The results indicated distinct regulation of Th17 and Th1 cytokine production in atherosclerotic coronary arteries.

A Subset of Coronary Artery–Infiltrating CD4+ T Cells Are IL-17/IFN-γ Double Producers
To extend the characterization of artery-infiltrating IL-17–producing T cells, we isolated CD4+ T cells from enzymedigested atherosclerotic coronary vessels using antibody-coated magnetic beads. An analysis of one of the purified preparations is presented in Figure 5A; the resultant population appears uniformly positive for CD4 expression. We analyzed these artery-infiltrating T helper cells by flow cytometry to assess cytokine production after polyclonal stimulation. Analysis at the single-cell level demonstrated that a major population of T helper cells produced only IFN-γ, whereas minor populations produced only IL-17 or both IFN-γ and IL-17 (Figure 5B and 5C). The corresponding fractions of cytokine-producing CD4+ T cells were not significantly different in the peripheral blood of healthy donors except for fewer IL-17/IFN-γ dual producers (Figure 5B and 5D). These findings confirmed that artery-infiltrating T helper cells were a source of IL-17, and the readily detectable double producers of IL-17 and IFN-γ may explain, at least in part, the parallel elevations in Th1 and Th17 cytokines in patients with coronary atherosclerosis.

IL-17 Does Not Modulate VSMC Growth or Survival
To determine whether IL-17 produced by artery-infiltrating T cells may have direct effects on VSMCs, we first investigated whether it modulated cell growth and survival because VSMC proliferation and apoptosis are known to cause disease progression or complications of atherosclerosis. IL-17
had no effect on the number of VSMCs cultured under optimal (20%) or low (0.5%) serum conditions over a period of 8 days (supplemental Figure IA). Similarly, IL-17 treatment did not alter the frequency of dying (subdiploid population), nondividing (G0/G1 phases), or dividing (S/G2/M phases) VSMCs as determined by flow cytometric analysis of DNA content with propidium iodide staining (supplemental Figure IB). IL-17 also had no effect on VSMC death as assessed by flow cytometric analysis of phosphatidylserine membrane translocation using annexin-V labeling and loss of membrane integrity using 7-amino-actinomycin D uptake (supplemental Figure IC). Furthermore, IL-17 did not interact with IFN-γ/H9253 in modulating VSMC growth or survival (supplemental Figure ID and IE). Collectively, these data indicated that IL-17 did not have significant mitogenic or proapoptotic effects on cultured VSMCs.

**IL-17 Acts Synergistically With IFN-γ to Induce Inflammatory Responses in VSMCs**

We then investigated whether IL-17 may interact with IFN-γ in the induction of proinflammatory responses in VSMCs using a cytokine array. The production of several cytokines and chemokines was elicited by IL-17 or IFN-γ, and the detection of IL-1 receptor antagonist, IL-6, IL-8, CCL5, CXCL1, CXCL10, C5a, and soluble ICAM-1 was enhanced in the presence of both factors compared with twice-higher concentrations of either IL-17 or IFN-γ alone (supplemental Table II and Figure II). The induction of other cytokines and chemokines such as IL-13 and CCL2 was not augmented by the combination treatment, and the variable detection of IL-17 and IFN-γ in the supernatant reflected the different concentrations of exogenous cytokines added to the cell cultures. More detailed dose-response experiments with ELISA confirmed synergistic interactions of IL-17 with IFN-γ in the production of IL-6, CXCL8, and CXCL10 (Figure 6). These results suggest that IL-17 production within the vessel wall may contribute to the proinflammatory milieu of atherosclerosis even in the presence of dominant Th1 immune responses.

To gain insight into the mechanisms for synergy of IL-17 and IFN-γ effects in VSMCs, we assessed their signaling responses (Figure 7). IL-17 activated nuclear factor-κB and p38 MAPK signaling pathways as evidenced by rapid degradation (followed by resynthesis) of IkBα and phosphorylation of p38 MAPK, respectively. However, IL-17 did not induce phosphorylation of STAT1. In contrast, IFN-γ activated STAT1 but not nuclear factor-κB or p38 MAPK. Combined cytokine treatment demonstrated that IFN-γ only modestly enhanced nuclear factor-κB and p38 MAPK activation by IL-17 at early time points and at high doses, whereas IL-17 had minimal, if any, effect on phosphorylation of STAT1 by IFN-γ. Thus, the interactions between the proinflammatory effects of IL-17 and IFN-γ likely occur at transcriptional and/or translational responses by VSMCs, a subject of future studies, rather than at the level of signaling pathways.

**Discussion**

There currently is a great deal of interest in defining the pathogenetic consequences of aberrant Th17 immune re-

---

**Figure 3.** Coronary artery–infiltrating T cells produced both IL-17 and IFN-γ. Equal-length segments of atherosclerotic or macroscopically normal coronary arteries in organ culture were treated with agonistic antibodies (a) to CD3 (0.1 μg/mL) and CD28 (1 μg/mL) for 24 to 72 hours or no antibodies for 72 hours (control), and supernatant levels of IL-17 (A) and IFN-γ (B) were measured by ELISA. Alternatively, atherosclerotic coronary artery rings were treated with cytokines either alone or in various combinations, including IL-1α (1 ng/mL), IL-6 (30 ng/mL), IL-15 (30 ng/mL), IL-21 (30 ng/mL), IL-23 (30 ng/mL), and TGF-β1 (10 ng/mL) or with the well-characterized T-cell activators PMA (1 μg/mL) and ionomycin (1 μmol/L), for 48 hours, and IL-17 supernatant levels were measured by ELISA (C). The data are mean±SEM (n=6 to 12) and are pooled from independent experiments using arteries from 6 different donors. The dotted line represents the lower limit of detection of the assay, and statistical analyses were not performed for responses to inflammatory cytokines resulting in IL-17 concentrations below this level. *P<0.05 and **P<0.001, treated vs control; #P<0.05, atherosclerotic vs nondiseased (repeated-measures ANOVA).
IL-17 has been hypothesized to potentially play a role in the development and complications of atherosclerosis, although there is little direct evidence to date to support this position. We investigated the production and effects of IL-17 in patient-related studies and experimental systems of human coronary atherosclerosis. We anticipated that IL-17 production would be minimal because of the described antagonism between the Th1 and Th17 subsets of T helper cells, but we unexpectedly found a positive correlation between IL-17 and IFN-γ plasma levels. Furthermore, a significant population of artery-infiltrating T helper cells produced both IL-17 and IFN-γ, and we observed a synergy between IL-17 and IFN-γ proinflammatory effects on VSMCs.

Circulating IL-17 and IFN-γ was not detected in the healthy subjects who were also much younger, and the differences in inflammatory markers may be due to either age or state of health. Our data from 185 subjects show no difference between elevated plasma levels of IL-17 in patients with coronary atherosclerosis and referent patients without coronary atherosclerosis, perhaps because of Th17 immune responses in other chronic disease that may obscure biomarkers of cardiovascular disease. In contrast, subgroup analyses in a previously published study of 78 individuals found that circulating IL-17 was modestly higher in patients with acute coronary syndrome compared with referent patients but not between patients with stable angina and referent patients. We did not find a correlation between plasma levels of IL-17 and those of cytokines known to be inducers (eg, IL-1β, IL-6, and IL-23) or products (IL-1β, IL-6, and C-reactive protein) of Th17 immune responses. However, there is a strong correlation between circulating IL-17 and the IFN-γ cytokine axis. Our results do not provide evidence for a serological IL-17 cytokine axis in patients with coronary atherosclerosis. Instead, our findings are consistent with broad activation of T cells with systemically detectable lymphokines in a subgroup of patients that is unrelated to the onset of acute coronary syndrome. The putative antigen(s) for such immune responses are unknown but may also reflect antigen-independent activation of bystander T cells within the artery wall by circulating or local proinflammatory factors.

Caveats to our conclusions include the relatively small number of patients studied, requiring verification in large epidemiological clinical trials, and the possibility that serological measurements may not reflect local vascular events.

Our in vitro studies on the production of IL-17 are in keeping with our observations in patients. IL-17 was secreted synchronously with IFN-γ in response to polyclonal T-cell activators, suggesting that either Th1 and Th17 cells or dual IL-17/IFN-γ producers infiltrated atherosclerotic coronary arteries. This response was also seen in arteries without macroscopic disease, confirming our previous observations of at least minimal T-cell accumulation in human arteries regardless of disease. We did not find that cytokines such as IL-23 induced detectable IL-17 production by effector T cells resident in atherosclerotic coronary arteries as previously reported for human peripheral blood memory T helper cells. This finding may reflect differences in T-cell phenotype, cell numbers, or donor variation. Our inability to determine antigen-independent inflammatory stimuli for IL-17 production in human T cells was not an exhaustive investigation and does not exclude other cytokines that may fulfill these criteria. Our results also do not contradict critical
roles for these cytokines in the differentiation or maintenance of Th17 cells. The combination of IL-12 and IL-18 robustly and specifically induced IFN-γ, not IL-17, secretion, which may reflect activation of distinct signaling pathways or the selective upregulation of IL-12Rβ2 in naïve T cells under Th1- but not Th17-polarizing conditions.10,12 This suggests that the expression of IL-12Rβ2 may differentiate human Th1 from IL-17/IFN-γ double producers, similar to the reported distinctions in CCR4, CCR6, and CXCR3 expression between these T-cell subtypes.24

We have adapted a method of extracting leukocytes from mouse aorta25 and applied it to atherosclerotic human coronary arteries with sufficient yield of CD4+ T cells to enable flow cytometric intracellular cytokine staining analysis. Th1 cells predominated and were ∼10- to 20-fold more frequent than IL-17– and IL-17/IFN-γ–producing T helper cells. The latter double producers have been described for human effector T cells, although their lineage identity is unclear. Peripheral blood CCR6/CXCR3+ memory T helper cells producing both IL-17 and IFN-γ express more abundant TBX21, the human ortholog of the murine Th1 transcription factor T-bet, than RORC, the human ortholog of the murine Th17 transcription factor ROR-γt.8 On the other hand, IL-23 stimulates both IL-17 and IFN-γ secretion from peripheral blood CD4+/CD45RO+ T cells.9 The production of IL-17 by Th1 cells or IFN-γ by Th17 cells may represent plasticity in cytokine production by T-cell lineages that are not always distinct, particularly in humans. The increased frequency of IL-17/IFN-γ double producers to true Th17 cells in atherosclerotic coronary arteries may also reflect the predominance of Th1 immune responses in this disease process.

Similar to studies on endothelial cells,26 we did not find mitogenic or cytopathic effects of IL-17 on cultured VSMCs, arguing against direct pathogenetic roles in intimal expansion or plaque rupture. Rather, IL-17, alone and cooperatively with IFN-γ, induced the production of proinflammatory cytokines and chemokines in VSMCs, which may promote secondary proartherosclerotic vascular changes.1 The production of cytokines and chemokines in response to IL-17 has been reported in other cell types, including endothelial cells,6 and we have recently described proinflammatory effects of IL-17 on VSMCs that were considerably greater than in endothelial cells.27 The only other report of IL-17 effects on VSMCs described the induction of the acute-phase reactant C-reactive protein,28 a biomarker that may also contribute to the pathogenesis of atherosclerosis. Synergy between IL-17

Figure 5. A subset of coronary artery–infiltrating T helper cells were IL-17 and IFN-γ double producers. CD4+ T cells from atherosclerotic coronary arteries or from peripheral blood of healthy subjects were isolated with antibody-conjugated magnetic beads (A). The cells were stimulated with PMA (1 μg/mL) and ionomycin (1 μmol/L) for 6 hours in the presence of brefeldin A; fixed and permeabilized; labeled with anti-IL-17-PE, anti-IFN-γ-FITC, or fluorescein isothiocyanate, isotype-matched control antibodies; and analyzed by flow cytometry. Composite results (B) and representative histograms of intracellular cytokine staining, including that of isotype-matched antibody controls, are shown for artery-infiltrating (C) and circulating (D) T helper cells. The data are mean ± SEM (n = 3 to 4) from independent donors, with percent IL-17+ T cells representing those detected in the upper left quadrant, percent IFN-γ+ T cells representing those detected in the lower right quadrant, and percent IL-17+ IFN-γ+ T cells representing those detected in the upper right quadrant of the flow cytometry histograms. *P < 0.05, blood vs artery-infiltrating CD4+ T cells (t test).
and tumor necrosis factor-α or IL-1β but not IFN-γ has also been reported in cell types other than VSMCs.6 The basis for enhanced production of proinflammatory factors by 2 cytokines may reflect the activation of distinct signaling pathways and transcription factors with positive interactions on transcription rates as first described for IFN-γ and tumor necrosis factor-α.29 Our observation that blockade of IL-17 signaling reduces IFN-γ production in atherosclerotic arteries indicates that IL-17 may promote IFN-γ production in addition to IFN-γ responses, suggesting additional layers of interaction between these 2 cytokines.

Our findings may be of relevance regarding recommendations for anti–IFN-γ biological therapy in coronary atherosclerosis and graft arteriosclerosis.4,30 Neutralizing IFN-γ activity within the vessel wall may be expected to greatly increase IL-17 production as a result of a reversal of its anti-Th17 property, although this may be offset by loss of synergy between IFN-γ and IL-17 for proinflammatory effects on VSMCs. Antiinflammatory therapeutic strategies need to consider a network of interactive cytokines and chemokines rather than individual proinflammatory factors in isolation.

**Source of Funding**

This work was supported by the National Institutes of Health (grant PO1 HL70295).

**Disclosures**

None.

**References**


7. Mangan PR, Harrington LE, O’Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Walsh SM, Schoeb TR, Weaver CT. Transforming...

CLINICAL PERSPECTIVE
Coronary atherosclerosis is considered an inflammatory disease in which T cells play a key role. Different lineages of T cells are characterized by the production of specific cytokines. Interferon-γ, the signature cytokine of Th1 cells, is detected in clinical specimens of coronary atherosclerosis and has a proarteriosclerotic effect in experimental models. More recently, a new lineage of interleukin (IL)-17–producing Th17 cells has been described. There is currently a great deal of interest in defining the pathogenic consequences of IL-17 in diverse inflammatory disease processes, although the role of IL-17 in atherosclerosis remains unknown. We investigated the production and effects of IL-17 in patient studies and experimental models of human coronary atherosclerosis. Although we anticipated that IL-17 production would be minimal owing to the described antagonism between Th1 and Th17 cells, we unexpectedly found a positive correlation between IL-17 and interferon-γ plasma levels. A distinct population of coronary artery–infiltrating CD4+ T helper cells produced both IL-17 and interferon-γ that differed from classic Th1 and Th17 cells. Finally, we observed a synergy between IL-17 and interferon-γ proinflammatory effects on vascular smooth muscle cells. Our findings underscore the interactions between a network of cytokines present in coronary atherosclerosis and may be of relevance regarding proposals for anticytokine biological therapy.