Chemokine CXCL10 Promotes Atherogenesis by Modulating the Local Balance of Effector and Regulatory T Cells

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Background—Studies to define the overall contribution of lymphocytes to lesion formation in atherosclerosis-susceptible mice have demonstrated relatively subtle effects; the use of lymphocyte-deficient mice, however, compromises both the effector and regulatory arms of the immune system. Here, we tested the hypothesis that deletion of CXCL10 (IP-10), a chemokine specific for effector T cells that has been localized within atherosclerotic lesions, would significantly inhibit atherogenesis.

Methods and Results—Compound deficient Apoe<sup>−/−</sup>/Cxcl10<sup>−/−</sup> mice fed a Western-style diet for either 6 or 12 weeks demonstrated significant reductions in atherogenesis as compared with Apoe<sup>−/−</sup> controls, as assessed by both aortic en face and cross-sectional analyses. Immunohistochemical studies revealed a decrease in the accumulation of CD4<sup>+</sup> T cells, whereas quantitative polymerase chain reaction analysis of lesion-rich aortic arches demonstrated a marked reduction in mRNA for CXCR3, the CXCL10 chemokine receptor. Although overall T-cell accumulation was diminished significantly, we found evidence to suggest that regulatory T-cell (T<sub>reg</sub>) numbers and activity were enhanced, as assessed by increased message for the T<sub>reg</sub>-specific marker Foxp3, as well as increases in immunostaining for the T<sub>reg</sub>-associated cytokines interleukin-10 and transforming growth factor-β1. We also documented naturally occurring T<sub>reg</sub> cells in human atherosclerotic lesions.

Conclusions—We provide novel evidence for a functional role for the effector T-cell chemoattractant CXCL10 in atherosclerotic lesion formation by modulating the local balance of the effector and regulatory arms of the immune system. (Circulation. 2006;113:2301-2312.)

Key Words: atherosclerosis ■ immunology ■ inflammation ■ leukocytes ■ chemokines

Studies to define the overall contribution of lymphocytes to lesion formation in atherosclerosis-susceptible mice have demonstrated relatively subtle effects. Mice with global lymphocyte ablation, achieved by crossing recombinase-activating gene–deficient mice into atherosclerosis-susceptible murine strains, have less pronounced plaque diminution than is seen with global monocyte deletion, particularly in the setting of a Western-style diet. However, complete ablation of T lymphocytes disturbs both effector and regulatory arms of the immune system, and the absence of B lymphocytes might also modify potentially protective antibody responses. Thus, a disruption that pinpoints effector T-cell–specific function might have more pronounced effects on atherogenesis.

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IP-10 (initially named as Interferon γ-induced Protein of 10 kDa and now also denoted as CXCL10), is a T-cell chemokine that was identified as an abundantly induced mRNA after interferon (IFN)-γ activation of monocytic U937 cells. CXCL10 is constitutively expressed at low levels in thymic, splenic, and lymph node stroma; however, expression can be highly induced by interferons in leukocytes, such as monocytes and macrophages, as well as in nonleukocytes, including endothelial and smooth muscle cells. A growing body of evidence suggests that CXCL10 may play a role in chronic inflammatory diseases, including coronary artery disease and related manifestations of atherosclerosis. Human atheromas express high levels of CXCL10 and 2 functionally related IFN-γ-inducible CXC chemokines, monokine induced by IFN-γ (MIG, CXCL9) and IFN-inducible T cell α-chemotactrant (ITAC, CXCL11), throughout all stages of plaque development. One observational study in humans found that higher levels of plasma CXCL10 correlate directly with restenosis after percutaneous coronary interventions.
Another study demonstrated increased expression of IFN-γ and CXCL10 in patients with coronary artery disease.13 CXCL10 exerts its functional effects through interactions with a specific high-affinity, G-protein–coupled receptor, CXCR3, which is abundant on activated effector T lymphocytes of the Th1 phenotype.14–16 Very recently, Veillard and colleagues demonstrated that CXCR3 deletion modulates early lesion formation in an atherosclerosis-prone background.17 However, the related chemokines CXCL9, CXCL10, and CXCL11 all activate the CXCR3 receptor.18 Furthermore, emerging data suggest that they confer differential signaling via CXCR3 in vitro and have unique patterns of expression, kinetics, and function in vivo. For example, anti-CXCL10 treatment inhibited demyelination and improved cognitive function in a murine model of multiple sclerosis, whereas anti-CXCL9 treatment had no effect.19 Monoclonal antibody (mAb) neutralization experiments have demonstrated that inhibition of CXCL10 alone results in increased mortality after infection of mice with Toxoplasma gondii.20 Of the 3 CXCR3 ligands, CXCL10 is unique in that its promoter has 2 functional nuclear factor-κB binding sites, whereas the CXCL9 and CXCL11 promoters have none.21 Here, we bred CXCL10-deficient mice into the hypercholesterolemic Apo−/− background to test the functional consequences of deletion of a specific effector T-cell chemokine, as opposed to more generalized abrogation of lymphocyte function, on atherosclerotic lesion development. Moreover, we define the dominant CXCR3-activating signal modulating T-cell trafficking and lesion formation.

Methods

Mice
Cxcl10−/− mice22 were backcrossed 10 times into a C57/BL6J background and subsequently bred with Apo−/− mice23 (Jackson Laboratory, Bar Harbor, Me). Intercrosses of Apo−/−/Cxcl10−/− mice yielded offspring that entered the study. Comparisons were done as part of an analysis of several genetic alterations on an Apo−/− background versus apolipoprotein E deficiency alone.24 Genotyping for CXCL1022 and apolipoprotein E25 was performed by polymerase chain reaction (PCR). The mice were fed a high-fat, triglyceride levels (Roche Diagnostics, Indianapolis, Ind).

Blood and Plasma Analysis
A total of 0.5 to 1.0 mL of blood was obtained from experimental mice by right ventricular puncture when the animals were euthanized. A small aliquot of blood was analyzed for a complete blood count (Drew Scientific Group, Oxford, Conn), and the remainder was used for enzymatic colorimetric analysis of total cholesterol and triglyceride levels (Roche Diagnostics, Indianapolis, Ind).

En Face Lesion Analysis
Aortas were dissected from mice of the indicated age, stained with oil red O (ORO), and analyzed as described previously.24 Images were obtained with a Nikon Coolpix camera (Nikon Inc, Melville, NY) attached to an inverted microscope. The percentage of ORO-stained lesion area was determined by Image Pro Plus image analysis software (Silver Spring, Md). Lesion analysis was conducted by a single observer blinded to the genotype of the mice.

Murine Aortic Root Lesion Analysis and Immunohistochemistry
The heart and aortic root were dissected and removed as described above and embedded in OCT. Serial 5-μm sections were cut through the aorta at the level of the aortic valve leaflets, and every fifth section through the aortic sinus (400 μm total length) was stained with ORO and hematoxylin. Immunohistochemistry was performed with antibodies to identify smooth muscle cells (α-actin, 1:50, DAKO, Glostrup, Denmark), macrophages (Mφ; F4/80, 1:25, Serotec, Oxford, United Kingdom), and T cells (CD4, 1:50, BD Biosciences, Franklin Lakes, NJ), detected with either DAKO ARK peroxidase (DakoCytomation, Carpinteria, Calif) or goat anti-rat HRP-conjugated IgG with DAB substrate (BD Biosciences, San Diego, Calif) as indicated. Negative controls were prepared with substitution with an isotype control antibody. Stained sections were digitally captured, and immunohistochemistry was analyzed with IP Laboratory image-analysis software (Scanalytics, Fairfax, Va). Threshold color analysis by a single observer quantified areas stained for a particular antigen and total plaque area. We divided areas stained for a particular antigen by total plaque area to account for variability in plaque size.

Immunohistochemistry of Human Atherosclerotic Lesions
Surgical specimens of human carotid atheroma were obtained by approved protocols. Serial cryostat sections (5 μm) were cut, air-dried onto microscope slides (Fisher Scientific Co, Pittsburgh, Pa), and fixed in acetone at −20°C for 5 minutes. Sections were preincubated with phosphate-buffering saline (PBS) containing 0.3% hydrogen peroxide were then incubated for 90 minutes with primary or isotype-matched control antibody, diluted in PBS supplemented with 5% appropriate serum. After being washed 3 times in PBS, sections were incubated with the respective biotinylated secondary antibody (for 45 minutes; Vector Laboratories, Burlingame, Calif) followed by avidin-biotin-peroxidase complex (VECTASTAIN ABC kit; Vector Laboratories). Immunostaining was viewed with 3-aminio-9-ethyl carbazole (Vector Laboratories) according to the recommendations provided by the supplier. Human plaques were characterized with the following antibodies: anti-smooth muscle actin mAb for SMC (Enzo Diagnostics, New York, NY), anti-CD31 mAb for endothelial cells (DAKO), anti-CD68 mAb for Mφ (DAKO), anti-CD3 antibody for T cells (BD Pharmingen, San Diego, Calif), anti-Foxp3 antibody for regulatory T cells (Abcam, Cambridge, United Kingdom), and anti-human interleukin (IL)-10 (R&D Systems, Minneapolis, Minn), anti-human transforming growth factor (TGF)-β1 (R&D Systems), anti-human CCR4 (Abcam), and anti-human CCL17 (R&D Systems).

RNA Isolation and Quantitative PCR
Total RNA was isolated from aortic arches from mice perfused with RNA Later (Ambion, Austin, Tex) with Trizol reagent (Invitrogen, Carlsbad, Calif) and RNeasy columns (Qiagen, Valencia, Calif).24 Briefly, after DNase I digestion, 1 μg of RNA from each sample was reverse-transcribed with Taqman reverse-transcription reagents, including oligo (dT)12, random hexamers, and Multiscribe reverse transcriptase (Applied Biosystems, Foster City, Calif). Quantitative reverse-transcription PCR (QPCR) reactions were conducted with the Multiplex QPCR system (Stratagene, La Jolla, Calif) as described previously.26 Amplification plots were analyzed with MX4000 software version 3.0. Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase as an internal control.

Flow Cytometry Studies of CD4+ CD25+ Regulatory T Cells
Lymphocytes were isolated from the spleen, lymph nodes, and peripheral blood of 8- to 10-week-old and 6-month-old Apo−/− and Apo−/−/Cxcl10−/− mice with Lympholyte (Cedarlane Laboratories, Ltd, Hornby, Canada), and were resuspended in RPMI-1640 (Cellgro, Herndon, Va) containing 1% FCS (Sigma, St. Louis, Mo). Cells...
were then stained with a cocktail of directly conjugated monoclonal antibodies including CD45RB-FITC (clone 16A; BD Pharmingen), CD25-PE (clone PC61; BD Pharmingen), CD3-APC (clone 145-2C11; BD Pharmingen), and CD4-PE-Cy5.5 (clone RM4–5; Caltag Laboratories, Burlingame, Calif), and isotype matched control monoclonal antibodies, respectively. The CD4+CD25+RB56+ cells were isolated with a MoFlo high-performance cell sorter (DakoCytomation).

Statistical Analysis
Group means were compared with Student t tests. A probability value of <0.05 was considered statistically significant. All data are reported as mean±SEM or SD as indicated.

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

Results
Atherosclerotic Lesion Formation in CXCL10-Deficient Mice
To directly assess the role of CXCL10 in atherogenesis, we studied Cxcl10−/− mice22 that were backcrossed 10 times into a C57BL/6J background and subsequently bred with ApoE−/− mice. Intercrosses of ApoE−/−/Cxcl10−/− mice yielded offspring that entered the study. Mice were placed on a high-fat, high-cholesterol Western diet for 6 or 12 weeks. ApoE−/− and ApoE−/−/Cxcl10−/− mice had comparable cholesterol levels and circulating white blood cell counts (Table 1), which thus excluded several obvious confounding variables.

Lesion area was first quantified in aortas pinned out en face and stained with ORO. This analysis revealed a significant reduction in aortic plaque burden at 6 weeks in the compound deficient ApoE−/−/Cxcl10−/− mice as compared with the ApoE−/−/Cxcl10+/+ animals (49% reduction, P<0.001; Figure 1, A and B). Plaque reductions were concordant across the aortic arch (55.6% reduction, P<0.001), thoracic aorta (43.1% reduction, P=0.006), and abdominal aorta (41.4% reduction, P=0.04; Figure 1C). We observed decreased lesion formation in both male (50.7% reduction, P<0.001) and female (43.5% reduction, P=0.02) mice (Figure 1D) without statistically significant intergender differences (P=NS). A consistent decrease in lesion area was observed in the ApoE−/−/Cxcl10−/− mice after 12 weeks on the Western-style diet, which indicates that loss of CXCL10 continues to have an impact on more mature lesions in older mice (25.3% reduction, P=0.02; Figure 1E).

We performed cross-sectional analysis of aortic roots in parallel with the en face studies. At the 6-week time point, the average cross-sectional lesion area for ApoE−/−/Cxcl10−/− mice was 56% less than the cross-sectional lesion area for ApoE−/− controls (P=0.005; Figure 2, A and B), which was an even greater reduction than that observed by surface lipid staining. As in the en face analysis, a persistent decrease in aortic sinus lesion area was observed in the ApoE−/−/Cxcl10−/− mice after 12 weeks on the Western-style diet (38.6% reduction, P=0.009; Figure 2C).

Decreased T-Cell Accumulation in ApoE−/−/Cxcl10−/− Mice
We next performed immunohistochemical studies of atherosclerotic vessel-wall constituents to further delineate the effects of CXCL10 on lesion formation. Analyses were performed on the mice fed a Western-style diet for 6 weeks and were normalized to lesion area in light of the significant differences in plaque involvement between the 2 groups as noted above. Intimal area was significantly decreased in cross sections of aortic root lesions from ApoE−/−/Cxcl10−/− mice. There was a marked decrease in CD4+ T-cell accumulation in the plaques of the double-knockout mice compared with ApoE−/− controls (44% reduction, P=0.001; Table 2; Figure 3A). In addition, there was also a 3-fold reduction in mRNA for the CXCL10 receptor, CXCR3, consistent with decreased infiltration of effector T cells (66% reduction, P=0.004; Figure 3D). In addition to the observed effects on T-cell accumulation, there were also more modest reductions in lesional macrophage and smooth muscle cell accumulation (Table 2; Figure 3, B and C).

Enhanced Regulatory T-Cell Activity in ApoE−/−/Cxcl10−/− Mice
We hypothesized that in the setting of diminished CXCL10-dependent effector T-cell accumulation, there would be a concomitant enrichment for Treg activity in the lesions. The forkhead/winged transcription factor Foxp3 is specifically expressed in CD4+CD25+ naturally occurring Tregs27–30. Tregs are believed to exert their antiinflammatory and immunosuppressive effects by secretion of soluble IL-10 and TGF-β1, as well as by cell-cell contact via membrane-bound TGF-β1 and cytotoxic T-lymphocyte–associated protein 4 (CTLA4).31,32 We thus evaluated aortas from ApoE−/−/Cxcl10−/− mice and ApoE−/− controls for a naturally occurring Treg expression profile by QPCR and immunohistochemistry. We observed a 2-fold increase in Foxp3 mRNA (P=0.005; Figure 4A) in the proximal aortas of ApoE−/−/Cxcl10−/− compound deficient mice compared with ApoE−/− controls, despite the signifi-

### Table 1. Analysis of Baseline Characteristics Between ApoE−/− and ApoE−/−/Cxcl10−/− Mice

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cantly decreased number of lesional T cells in the \textit{Apoe}^{-/-} \textit{Cxcl10}^{-/-} mice. Concordant with the findings of augmented Foxp3 mRNA, there was also a 2.3-fold increase in IL-10 message (\(P < 0.027\); Figure 4B), a 2.8-fold increase in TGF-\(\beta\)1 message (\(P < 0.008\); Figure 4C), and a trend toward increased CTLA4 message (1.8-fold increase, \(P = 0.14\); Figure 4D) in the \textit{Apoe}^{-/-} \textit{Cxcl10}^{-/-} aortas compared with \textit{Apoe}^{-/-} controls. Importantly, the QPCR findings were corroborated by quantitative immunostaining that demonstrated significant increases in both IL-10 (2.1-fold increase, \(P = 0.0001\)) and TGF-\(\beta\)1 (1.9-fold increase, \(P = 0.02\)) protein levels in the lesions of the double knockouts, even in plaques of comparable size (Figure 4, E and F). Taken together, the present studies revealed a relative increase in regulatory
T-cell accumulation and activity in the lesions of the $\text{Apoe}^{-/-}/\text{Cxcl10}^{-/-}$ mice.

**Enhanced Expression of T\textsubscript{reg} Receptors and Their Cognate Ligands in $\text{Apoe}^{-/-}/\text{Cxcl10}^{-/-}$ Aortas**

Recent studies have demonstrated the expression of the chemokine receptors CCR4 and CCR8 on the surface of T\textsubscript{reg}\textsuperscript{33-35}. The CCR4 ligands CCL17\textsuperscript{36} and CCL22 (macrophage-derived chemokine [MDC]), in turn, have been identified as critical chemokines directing the trafficking of regulatory T cells\textsuperscript{33,34}. We therefore performed QPCR of RNA isolated from aortas of both genotypes to examine expression of chemokine receptor ligands that might be responsible for recruiting T\textsubscript{reg} to the lesions. QPCR analysis demonstrated a 4.6-fold increase in CCR4 mRNA levels ($P=0.007$; Figure 5A) and a 6.3-fold increase in CCR8 mRNA levels ($P=0.003$; Figure 5B) in $\text{Apoe}^{-/-}/\text{Cxcl10}^{-/-}$ aortas compared with $\text{Apoe}^{-/-}$ controls. Furthermore, we observed a 4.8-fold increase in CCL17 mRNA levels ($P=0.003$; Figure 5C) and a 3-fold increase in CCL22 mRNA levels ($P=0.008$; Figure 5D) within aortas of the double-knockout mice. Thus, in the absence of CXCL10, there is upregulation of 2 chemokines and 2 receptors consistent with enhanced accumulation of T\textsubscript{reg} within lesions. In contrast to the upregulation of these specific chemokines and receptors, there were no differences between $\text{Apoe}^{-/-}/\text{Cxcl10}^{-/-}$ and $\text{Apoe}^{-/-}$ aortas in the expression of other relevant chemokines, such as CCL2, CX3CL1, CXCL9, or CXCL11, or the receptors CCR2, CX3CR1, or CXCR2 ($P=\text{NS}$ for all, data not shown).

**T\textsubscript{reg} Levels and Activity in Lymphoid Tissue and Circulating Blood Do Not Differ Between $\text{Apoe}^{-/-}$ and $\text{Apoe}^{-/-}/\text{Cxcl10}^{-/-}$ Mice**

We next investigated whether CXCL10 deletion had global effects on T\textsubscript{reg} in addition to the local enhancement of the T\textsubscript{reg} expression profile we observed within the atherosclerotic lesions of $\text{Apoe}^{-/-}/\text{Cxcl10}^{-/-}$ mice. We performed flow cy-
tometry studies of splenic/lymph node and peripheral blood T cells but observed no differences in the percentages of CD4+ CD25+ RBlow regulatory T cells between genotypes (Figure 6, A and B). Furthermore, we observed no significant differences in Foxp3 levels in flow-sorted CD4+ CD25+ RBlow cells from peripheral blood or lymphoid tissue (Figure 6, C and D). Finally, we saw no differences in IL-10 and TGF-β1 expression in these cells, nor did we observe modulation of Foxp3 levels or CD4+ CD25+ RBlow cell survival after CXCL10 treatment (100 nmol/L, 0 to 72 hours; data not shown). These studies therefore suggested that the effect of CXCL10 deletion of the balance of regulatory and effector T cells is not global but rather is restricted to the vascular lesions.

Expression of Foxp3 in Human Atherosclerotic Lesions
We performed immunohistochemistry on human atherosclerotic plaques using a recently developed anti-human Foxp3 antibody37 (Figure 7). Our analysis revealed the expression of Foxp3-positive cells on a subset of the CD3+ T cells within atherosclerotic lesions, which in turn colocalized with both IL-10 and TGF-β1 expression. Furthermore, we demonstrate colocalization of the Foxp3-expressing Treg with the Treg-associated chemokine receptor CCR4 and its ligand, CCL17. Importantly, there was no staining for Foxp3 in fibromuscular plaques that were devoid of T cells.

Discussion
Emerging evidence has suggested a functional role for the T-cell chemoattractant CXCL10 in chronic inflammatory diseases, including atherosclerosis.11 Here, we have demonstrated that the absence of CXCL10 confers a 2-fold reduction in early lesion formation in Apoe+/−/Cxcl10−/− mice compared with Apoe+/−/− controls, as assessed by both aortic en face and cross-sectional analysis of plaque burden. Reduction in lesion formation was evident in all regions of the aorta, was similar in both male and female mice, and persisted, although it was attenuated, in mature inflammatory plaques. Immunohistochemistry and QPCR studies revealed a marked reduction in CD4+ and CXCR3-expressing T cells in CXCL10-deficient mice. Although overall T-cell accumulation was diminished significantly, we found evidence to suggest that markers associated with regulatory T-cell function were enhanced, including increased message for Foxp3 and robust increases in Treg-associated cytokines, including IL-10 and TGF-β1. Furthermore, in the aortas of the compound deficient animals, we observed increases in CCL17, CCL22, CCR4, and CCR8, important chemokines and receptors involved in regulatory T-cell trafficking. Finally, immunohistochemistry studies performed with a recently developed anti-human Foxp3 mAb confirmed the presence of this protein in a subset of T cells in human atherosclerotic lesions, where it colocalized with IL-10, TGF-β1, CCL17, and CCR4. We thus provide novel evidence for a functional role of the T-cell active chemokine CXCL10 in lesion formation by modulating the local balance of the effector and regulatory arms of the immune system.

Prior studies have demonstrated the differential expression of the 3 T-lymphocyte active CXC chemokines (CXCL9,
Figure 4. Cytokine expression in aortas. QPCR was performed on mRNA from the aortic arch of ≥4 mice of each genotype, and data are presented as mean±SEM. A, Foxp3 (*P=0.0005). B, IL-10 (*P=0.027). C, TGF-β1 (*P=0.008). D, CTLA4 (*P=0.14). Quantitative immunostaining of plaques from mice after 6 weeks on the Western diet was performed to confirm changes observed at the mRNA level. Probability values refer to cumulative data from ≥6 mice of each genotype, with representative images shown. E, IL-10 (*P=0.0001). F, TGF-β1 (*P=0.02).
CXCL10, and CXCL11) by human atheroma-associated cells. Moreover, Veillard and colleagues recently established a functional role for the CXCR3 receptor in atherogenesis, thus raising the critical question of which of the multiple potential ligands are contributing to lesion formation. We were interested to find that the degree of inhibition of atherosclerosis we observed with the Apoe/H/H/H/Cxcl10/H/H mice was comparable to results seen in the studies with Apoe/H/H/H/Cxcl10/H/H mice, which suggests that CXCL10 is the dominant CXCR3 agonist in this pathology. Our studies are consistent with the emerging notion that although CXCL10 and the related chemokines CXCL9 and CXCL11 all activate the same receptor, they exhibit unique patterns of expression, kinetics, and function in vivo. The present studies, of course, do not totally preclude functional roles for the other CXCR3 ligands in atherogenesis, because CXCL10 deletion did not entirely abrogate lesion formation. Future mouse atherosclerosis studies using targeted deletion of these ligands will thus be required to address the potential roles of these proteins in atherogenesis.

Initial studies examining the role of lymphocytes in atherosclerotic lesion development used recombinase-activating gene–knockout mice to confer global T- and B-cell deletion. Several of these studies revealed lymphocyte-dependent effects on lesion generation, although the studies suggested gender and regional effects of lymphocyte depletion, and the differences were less evident under conditions of severe hypercholesterolemia. More recent investigation by Lichtman et al has extended initial observations using recombinase-activating gene mice by specifically examining the role of T-cell differentiation and activation on atherosclerotic lesion development. In 1 study, B7-1/B7-2 costimulation was shown to regulate atherogenesis in LDL receptor–deficient mice, potentially by altering the priming of lesion antigen-specific T cells. A second line of investigation demonstrated that atherosclerosis-susceptible strains also deficient in T-bet, a member of the T-box family of transcription factors that induces Th1 differentiation and suppresses Th2 differentiation, have reduced lesions and altered plaque antigen-specific immune responses. The consistency of our findings in the CXCL10 knockout mice using multiple metrics of lesion formation provide further evidence that interference with T-cell signaling modifies the atherosclerotic process.
Recent studies have begun to identify immune pathways that prevent or minimize overexuberant responses to pathogens or other insults. In particular, studies have demonstrated that avoidance of host damage is achieved by active immune suppression mediated by regulatory T-cell populations.

Functionally, CD4+ T-cell subsets were identified that inhibited antigen-specific T-cell responses and prevented colitis in murine models. The forkhead/winged helix transcription factor Foxp3 is exclusively expressed by one regulatory T-cell subset, and this subset is further characterized by the surface markers CD4+CD25+RB1low. Recent studies by Lee et al have also suggested a functional role for CD25 Foxp3 T cells in mediating allograft tolerance in a cardiac transplantation model, because pretransplantation thymectomy or pretransplantation depletion of CD25+ cells prevented long-term survival, as did anti-CD25 mAb therapy in established grafts. With respect to atherosclerotic vascular disease, adoptive transfer of an exogenously generated clonal population of regulatory T cells (Tr1), along with their cognate presenting antigen (ovalbumin), limited lesion development in Apoe-/- mice. Of note, Tr1 cells appear to differ from endogenous regulatory CD4+ CD25+ RB1low T cells in that they are Foxp3 negative. Furthermore, it is difficult to discern whether the transfer of Tr1 cells diminished the inflammatory response to a specific lesional antigen or whether the effect on lesion development was simply due to overexpression of IL-10, which itself is antiatherogenic.

Very recently, Ait-Oufella and colleagues also demonstrated that mice reconstituted with bone marrow deficient in Cd80, Cd86, and Cd28, costimulatory molecules necessary for the generation and homeostasis of CD4+CD25+ Treg, had enhanced atherogenesis compared with controls. Our studies are also consistent with recently published work in CXCR3 knockout mice, which demonstrated enhanced expression of antiinflammatory molecules within the lesions of the double knockouts compared with apolipoprotein E deletion alone. Taken together, these studies have unmasked a role for the CXCR3-CXCL10 receptor-ligand pair in effector T-cell trafficking, which in turn modulates endogenous immunoregulatory cells within plaques. Importantly, the present studies further extend prior work by demonstrating the presence of FoxP3 protein in a subset of T cells in human atherosclerotic lesions as well.

Figure 6. Treg expression in lymphoid tissue and blood. Lymphocytes were isolated from the (A) spleen/lymph nodes or (B) circulating blood, and the percentage of CD4+CD25+RB1low cells was assessed by flow cytometry (n=3, P=NS for both). Next, QPCR for Foxp3 was performed on sorted CD4+CD25+RB1low cells from the (C) spleen/lymph nodes or (D) peripheral circulating blood (n=3, P=NS for both).
phenotype, as evidenced by enhanced expression of Foxp3 and Treg-associated cytokines such as IL-10 and TGF-β1, both of which inhibit atherogenesis. Increased Foxp3 and IL-10/TGF-β1 in the lesions of the Apoe−/−/Cxcl10−/− mice might be due to either enhanced activity or numbers of regulatory T cells, although Foxp3 levels appear to correlate with the number of CD4+CD25+RBlow cells.33 Our data also suggest that in the absence of CXCL10 and signals derived from effector T cells, there is enhanced CCL17 and CCL22 expression, likely from lesional macrophages, which may lead to trafficking of regulatory T cells. This appears to be a local lesional effect, because analysis of lymphocytes isolated from the spleen, lymph, nodes, and circulating blood revealed no differences in the percentages of CD4+CD25+RBlow cells or Foxp3 expression in Apoe−/−/Cxcl10−/− mice as opposed to Apoe−/− controls. Furthermore, we saw no direct effects of CXCL10 on Foxp3 expression or Treg survival ex vivo. The present studies do not preclude a role for CXCL10 in Foxp3 expression or in the survival of Treg, requiring additional signals that might be present in the vessel wall. Further investigation will be needed to clarify the mechanisms by which CXCL10 leads to increased accumulation of Treg.

The present study thus provides further evidence that lymphocytes indeed contribute to atherogenesis and has identified the T-cell chemokine CXCL10 as potentiating this process. Our findings underscore a novel pathway by which CXCL10 modulates both effector and regulatory T cells in atherosclerotic lesion formation. These studies suggest that interventions that differentially modulate discrete subsets of lymphocytes have potential therapeutic implications for coronary artery disease and related manifestations of atherosclerosis.

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Disclosures

None.

References


Recent studies have begun to identify immune pathways that prevent or minimize overexuberant responses to pathogens or other insults. In particular, studies have demonstrated that avoidance of host damage is achieved by active immune suppression mediated by regulatory T-cell populations; however, evidence for an imbalance of regulatory versus effector T cells in atherosclerosis has been lacking. In this study, we tested the hypothesis that deletion of CXCL10 (IP-10), an attractant specific for effector T cells, would significantly inhibit atherogenesis in a mouse model. Indeed, hypercholesterolemic mice that were deficient for this cytokine had a >2-fold reduction in atherosclerotic lesion formation. Furthermore, in the lesions in the CXCL10-deficient mice, there appeared to be a shift in the balance of T cells away from the proinflammatory effector T cells and toward the regulatory or protective T-cell population, which in turn appeared to have inhibited the atherosclerotic process. These studies suggest that interventions that differentially modulate discrete subsets of lymphocytes have potential therapeutic implications for coronary artery disease and related manifestations of atherosclerosis.
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