

T Lymphocytes in Atherosclerosis The Yin-Yang of Th1 and Th2 Influence on Lesion Formation

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The presence of activated T lymphocytes in all stages of human atherosclerotic lesion development implies their involvement in this vascular disease process.¹ However, the specific role T lymphocytes play in atherogenesis remains unclear. It is not feasible to regulate the immune system in humans to determine its association with atherosclerotic-related diseases. Therefore, dissection of the role of T lymphocytes in lesion development will be dependent on animal models. Appropriate animal models need to mimic the cellular composition of human lesions, particularly in content of T lymphocytes. In this respect, the most commonly used mouse models of atherosclerosis, such as apolipoprotein E $-/-$ and low-density lipoprotein (LDL) receptor $-/-$ mice, contain T lymphocytes, although the number of cells is less than in human lesions.² T-lymphocyte presence has functional consequences, because their complete absence reduces lesion formation during moderate hypercholesterolemia.^{3,4}

The major class of T lymphocytes present in atherosclerotic lesions is CD4⁺. In response to the local milieu of cytokines, CD4⁺ cells differentiate into the Th1 or Th2 lineage. Among the principal inducers of the Th1 and Th2 cells are interleukin (IL)-12 and IL-10, respectively. Activated T lymphocytes are functionally defined by the cytokines produced with interferon (IFN)- γ secreted from the Th1 cells and IL-4 from the Th2 cells.

Much of the emphasis in atherosclerosis research in relation to T lymphocytes has focused on the role of Th1-type responses. The evidence for the role of Th1 cells includes the detection of IFN- γ mRNA and protein in lesions.^{5,6} A direct role in the disease process has been defined in atherosclerotic-susceptible mice that are deficient in either IFN- γ receptors⁷ or the cytokine itself.⁸ Conversely, injection of IFN- γ ⁹ or the IFN- γ -releasing factors IL-12¹⁰ and IL-18¹¹ enhances the extent of disease in apolipoprotein E $-/-$ mice.

Although Th1 cells may be the major regulators of the lymphocytic influence on the atherogenic process, the cytokine expression of human atherosclerotic lesions suggests there is local regulation of Th1 versus Th2 subtypes. In the human disease, the mRNA and protein and the Th1 inducer

IL-12 are abundantly expressed in the majority of lesions. The Th2 inducer IL-10 is also present, albeit in a more limited number of samples.¹² The source of this IL-10 within atherosclerotic lesions could potentially include Th2 cells, B cells, and macrophages. IL-10 exerts its effect on Th1:Th2 balance by downregulating IL-12 and IL-18 production and inhibiting any Th1-based immune responses.¹³

The role of IL-10 in the development of atherosclerosis was initially approached in mouse models by two independent groups.^{14,15} Both groups defined the effect of IL-10 deficiency in C57BL/6 mice fed a diet enriched in saturated fat, cholesterol, and cholate. In both studies, IL-10 deficiency led to marked reductions in plasma HDL-cholesterol concentrations and increased size of atherosclerotic lesions. The lesions developed in IL-10-deficient animals had equivalent macrophage content to those in wild-type animals but increased numbers of T lymphocytes and greatly decreased collagen content.¹⁴ Interestingly, Mallat et al¹⁵ observed that the effect of IL-10 deficiency on lesion formation was more pronounced in animals housed in a conventional facility compared with mice maintained in a specific pathogen-free environment. This infers that the effects of IL-10 on atherosclerosis may have greater consequences in a natural environment where individuals are routinely exposed to pathogens.

Both groups also defined the converse condition of increased IL-10 secretion on atherosclerosis development. The study of Mallat et al¹⁵ used a gene transfer procedure to introduce a plasmid into skeletal muscle and increased systemic IL-10 concentrations. Pinderski-Oslund et al¹⁴ used transgenic mice generated with the human genomic sequence of IL-10 under the control of the IL-2 promoter to augment local IL-10 secretion.¹⁶ This promoter will restrict expression of the transgene to activated lymphocytes. A recent publication from an additional group has increased plasma concentrations of IL-10 by adenoviral gene transfer in LDL receptor-deficient mice.¹⁷ All three strategies of increasing IL-10 secretion led to reductions in atherosclerotic lesion size.

The results of Pinderski et al¹⁸ in this issue of *Circulation Research* provide further evidence that overexpression of IL-10 attenuates lesion formation and associates this with alterations in lymphocyte and macrophage phenotypes. Transplantation of bone marrow cells was used to generate chimeric LDL receptor-deficient mice, a procedure that has become increasingly popular since its initial description in the field of atherosclerosis research.^{19,20} Donor bone marrow cells were obtained from either wild-type mice or IL-10 transgenic mice in which the cytokine DNA was under the control of the IL-2 promoter, as described in their earlier publication. The lethally irradiated LDL receptor-deficient

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recipient mice were repopulated with donor cells. After a recuperation period to enable engraftment of bone marrow cells, mice were fed a diet enriched in saturated fat and cholesterol for 20 weeks.

The combination of LDL receptor deficiency and fat-enriched diet led to a greater hypercholesterolemia than in the previous studies using C57BL/6 mice. Contrary to previous studies in C57BL/6 mice, overexpression of IL-10 in LDL receptor $-/-$ mice led to a reduction in plasma concentrations of cholesterol. Although plasma IL-10 concentrations were not increased in the transgenic mice, there were several measurements that indicated a functional influence. These include class switching of antibodies to malondialdehyde-modified forms of LDL from IgG₁ to IgG_{2a} and decreased expression of IFN- γ in peripheral blood lymphocytes and splenocytes. In addition, a novel finding was the reduction of IFN- γ expression in circulating monocytes from IL-10 transgenic mice.

Most importantly, overexpression of IL-10 in this restricted cell type led to a marked reduction in the size of aortic root lesions. In addition to size, changes were also noted in the characteristics of the atherosclerotic lesions of IL-10 transgenic mice. The differences included an increase in cell density and a decrease in extracellular matrix and necrotic cores. The decrease in necrotic cores in the IL-10 transgenic mice was consistent with the low expression of caspase-3, a regulator of apoptosis. Although it may be assumed that the effects of IL-10 occurred via local secretion within lesions, no T lymphocytes were detectable at the time of tissue acquisition. Therefore, the influence on lesion development may be attributable to early T-lymphocyte infiltration or peripheral effects.

These studies demonstrate that IL-10 has a profound effect on atherosclerosis that is associated with phenotypic modulation of selected leukocyte populations. Further experimentation is needed to determine the mechanism(s) by which IL-10 exerts this effect. In addition to regulating the balance of Th1 and Th2 cells, IL-10 also exerts direct effects on several pathways that could influence the atherogenic process. This includes effects on matrix metalloproteinases,²¹ inducible nitric oxide synthase,²² tissue factor,²³ and cyclooxygenase.²⁴ While it is desirable to define a specific pathway that leads to the development of atherosclerotic lesions, this represents a formidable challenge. The combination of the chronicity, variability, and complexity of lesions provides substantial difficulties in linking a selected cytokine to a specific atherogenic pathway. Irrespective of the unknown mechanistic basis underlying this highly consistent data of IL-10 on atherogenesis, there is potential therapeutic benefit in increasing its expression, either locally or systemically.

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