Targeting Interferon-γ to Treat Atherosclerosis

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The chronic inflammatory nature of atherosclerotic disease is now widely appreciated. Abnormal deposition and oxidative modification of serum lipoproteins in arterial walls stimulates the recruitment of monocytes and their conversion into foam cells. Concurrently, both innate and adaptive immune responses are induced. Cytokines and growth factors produced as part of these responses lead to the remodeling of the arterial wall that typifies atherosclerotic lesions, including SMC accumulation and collagenous matrix deposition in the intima. The predominant inflammatory remodeling of the arterial wall that typifies atherosclerotic growth factor production as part of these responses leads to the conversion into foam cells. Concurrently, both innate and walls stimulates the recruitment of monocytes and their oxidative modification of serum lipoproteins in arterial processes that enhance lesion development. The majority of these T cells have a phenotype characteristic of the proinflammatory T-helper 1 (Th1) subset, and Th1-mediated immune responses have been shown to correlate with the development of atherosclerosis in mouse models.

The signature cytokine produced by Th1 cells is interferon (IFN)γ. Immunohistochemical and in situ hybridization studies indicate that IFNγ is present in human atherosclerotic plaques. Numerous studies in mice have demonstrated a central role of IFNγ in atherosclerosis. Genetic ablation of IFNγ or IFNγ receptor (IFNγR) expression in mice significantly reduces atherosclerosis, whereas administration of exogenous IFNγ potentiates atherosclerosis. IFNγ has many different biologic effects that contribute to lesion development, including enhancement of antigen presenting capabilities of endothelium and macrophages, enhancement of inflammatory cell recruitment via upregulation of endothelial adhesion molecules, stimulation of proinflammatory cytokine and chemokine secretion by macrophages, production of reactive oxygen species by macrophages, and the inhibition of cholesterol efflux from foam cells. IFNγ also contributes to destabilization of mature lesions by blocking smooth muscle cell (SMC) proliferation and collagen synthesis and by stimulating matrix metalloproteinase synthesis by macrophages.

Given the central role of IFNγ in atherosclerotic-plaque pathology, therapeutically targeting this cytokine could reduce the atherosclerotic burden and/or enhance the stability of plaques. The study by Koga et al in the current issue of Circulation Research addresses this option in a mouse model. The study elegantly demonstrates that IFNγ neutralization, by repeated injections of a plasmid encoding a soluble INF-γR construct (sIFNγR), reduces lesion progression. In 1 experiment, apolipoprotein E (apoE)-deficient mice were fed a high-fat diet for 8 weeks and received 2 intramuscular injections of control or sIFNγR-encoding plasmid DNA after 4 and 6 weeks of the diet regimen before euthanasia. The efficacy of the sIFNγR treatment in neutralizing IFNγ was confirmed in a biological assay, and the effects of the treatment on aortic lesion size and phenotypes were assessed. The treatment was found to significantly reduce lesion size. Furthermore, the plaque burden reduction was accompanied by a change to a more stable phenotype with reduced lipid and macrophages and increased SMCs and collagen deposition. In support of a direct effect of IFNγ blocking, reduced phosphorylation of Stat1, a downstream signaling molecule activated through the IFNγR, was seen in the lesions of the treated mice. Koga et al also provide some mechanistic explanation for these findings. They show that sIFNγR treatment downregulates lesional gene expression of the proinflammatory molecules interleukin-1β, interleukin-6, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1α. All of these molecules have been shown to be important in the development of the atherosclerotic lesion. In addition, the treatment reduced the gene expression of matrix metalloproteinase-9 and -13, enzymes important in extracellular matrix degradation, while upregulating procollagen type I. These findings provide a plausible explanation for the stabilization of the plaque. They also show that CD40 and CD40L, which exert proatherogenic effects in mice, are downregulated in the lesions of the treated mice.

Although it is now well established that IFNγ plays a central role in the propagation of atherosclerosis and genetic ablation significantly reduces atherosclerosis, this is the first published study that targets IFNγ in a pharmacological manner (using a gene transfer technology) to treat atherosclerosis. The use of sIFNγR gene transfer and the effects of this approach on atherosclerotic lesion development and phenotype are of significant interest. This novel approach has several advantages. In experimental animals it avoids developmental compensations for mutant genes that may arise in gene knock out mice. The use of plasmid injection allows some control over the time course of IFNγ neutralization. Importantly, gene transfer is a potential treatment strategy in humans, and therefore this study helps in defines the feasibility of targeting a proatherogenic cytokine.

Although the prospect of targeting INF-γ as a potential therapeutic avenue for atherosclerosis, as suggested by this
article, is very appealing, several issues must be addressed before such an approach can be considered in the clinical arena. In a second experiment focusing on what happens after treatment cessation, Koga et al fed the apoE−/− mice a high-fat diet for 16 weeks, and sIFNγR treatment was administered to 1 group at 4, 6, 8, and 12 weeks and administered to another group only at 4 and 6 weeks. In this experiment, lesion progression resumed, possibly even at a faster rate than in mock-treated animals, after sIFNγR treatment was stopped. This raises concerns about the potential limitations of cytokine blockade in general for the treatment of atherosclerosis, because temporary blockade of plaque-based inflammation apparently does not effectively remove the stimuli driving the inflammation or the cells that respond to these stimuli. Of course, IFNγ has a central role in the function of the adaptive immune response, and long-term systemic inhibition of IFNγ may result in increased susceptibility to intracellular infections and possibly neoplasia. As suggested by the authors, it is very appealing to consider local therapeutic avenues such as local delivery of recombinant sIFNγR protein using nanoparticles, thus avoiding the possible systemic side effects.

Another important finding of this study is the potential stabilizing affect of sIFNγR treatment on the atherosclerotic plaque. As the majority of acute coronary events are postulated to occur when unstable plaques rupture, stabilization of the lesion is of significant clinical importance. Koga et al demonstrated that sIFNγR-treated animals had a more stable phenotype, with reduced inflammatory cells and increased collagen and SMC content. Indeed, IFNγ has been shown to reduce collagen type I deposition by SMCs affecting the stabilizing affect of sIFNγR. 

IFNγ may have the reverse effect on vascular remodeling. IFNγ may directly cause SMC proliferation and activation, inhibit SMC apoptosis, and increase formation of neointimal proliferation after vascular injury. Indeed, in a recent publication by the same group, IFNγ blockade using the same sIFNγR agent used in the present study, caused reduced proliferation of intimal SMC, attenuating neointima formation in a rat model of vascular injury. Further work will be required to understand these conflicting effects of IFNγ blockade on neointimal SMC accumulation in different vascular pathologies.

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None.

**References**


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