**TGF-β in Atherosclerosis**

*To the Editor:*

In his excellent review on the role of transforming growth factor (TGF)-β in atherosclerosis in the March 2004 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology,* David J. Grainger discusses the multiple roles of this growth factor and suggests that direct effects on vascular cells as well as effects exerted primarily on immune cells may account for its antiatherogenic properties. He points out the discrepancy between the experiments applying generalized TGF-β suppression and those using dominant-negative (dn) TGF-β receptors in T cells. Using neutralizing anti–TGF-β antibodies in apoE−/− mice, Mallat et al observed an increased lesion size, whereas transplantation of bone marrow containing T cells with dn–TGF-β receptors led to reduced lesions. Therefore, Dr. Grainger concludes that TGF-β signaling in the inflammatory cell population cannot be the whole story, and he suggests that the effects of this cytokine on smooth muscle cells (SMCs) determine the formation of matrix-rich, stable plaques. However, new data published after the acceptance of his review article suggests that immunosuppressive effects of TGF-β modulate the growth and stability of the atherosclerotic lesion as well as its inflammatory properties.

In an article published in the November 2003 issue of the *Journal of Clinical Investigation,* we show that apoE−/− mice with abrogated TGF-β signaling in T cells develop dramatically accelerated atherosclerosis with a several-fold increase in lesion size as well as a more vulnerable lesion phenotype with reduced collagen and increased inflammation. These proatherogenic effects were entirely caused by the lack of functional TGF-β receptors on T cells, because all other aspects of TGF-β signaling remained intact, including circulating cytokine levels and receptor expression by other cells. Some of our results are in agreement with the findings by Gojova et al., who studied the effects on atherosclerosis in low-density lipoprotein receptor (LDLR)−/− mice after transplantation of bone marrow from mice lacking functional TGF-β receptors in T cells. Thus, both we and Gojova et al found that abrogation of TGF-β signaling in T cells caused a more vulnerable lesion structure with reduced collagen and increased signs of inflammation. However, we registered a dramatic increase in lesion size in apoE−/− mice carrying dn–TGF-β receptors in T cells, whereas Gojova et al found a modest decrease in lesion size after bone marrow transplantation (BMT) of such T cells into LDLR−/− mice. The reason for this discrepancy may be caused by the different experimental designs. Although the two models of atherosclerosis may contribute to the discrepancy, we believe that the different strategies used to create a selective defect in TGF-β signaling is a more plausible explanation.

By mating mice carrying dn–TGF-β receptors under a T-cell specific promoter with atherosclerosis-prone animals, a situation of deficient TGF-β signaling is created from early life and onwards. Analogous experiments using other strains show that this setting leads to autoimmune, inflammatory disease by creating a situation of uncontrolled T-cell activity. A potential drawback of the cross-breeding design is the risk that a congenital defect may not faithfully reproduce regulation in adult life. A major advantage is that no manipulation of the offspring is required before lesions are analyzed.

BMT, on the other hand, requires substantial manipulation of the recipient mouse. Firstly, it is lethally irradiated before cell transfer; this is likely to interfere with cell proliferation throughout the organism, reduce endothelial viability, and impair vascular repair processes. Secondly, irradiation causes a situation of general cytopenia; the transferred cells including T cells will therefore divide vividly in order to expand into the empty blood cell compartment, which can have substantial effects on immune regulation. It is therefore likely that the BMT strategy affects the behavior of the transferred cell population as well as the vessel wall of the recipient. In order to interpret the experiments, it is important to consider the possible effects of pretransplant irradiation on the vessel wall. Could it be that this treatment inhibits SMC (and perhaps endothelial cell) proliferation in such a way that lesion expansion is inhibited? At any rate, our recent findings in the CD4− dn–TGFβRII × apoE−/− mice show that the T-cell inhibitory effect of TGF-β can explain the capacity of this cytokine to promote the formation of stable lesions in murine atherosclerosis.

We suggest that TGF-β–mediated inhibition of T-cell activity increases lesion stability by reducing the production of interferon (IFN)-γ in the atherosclerotic artery. In support of this suggestion, we observed a 100-fold increase in IFN-γ mRNA in the aortas of dn–TGFβRII × apoE−/− mice. Because IFN-γ powerfully inhibits collagen synthesis by SMCs and promotes macrophage activation, the loss of TGF-β inhibition of IFN-γ production by activated Th1 cells may explain all the features of the large, inflamed, and vulnerable lesions in the CD4− dn–TGFβRII × apoE−/− mice. Although these experiments focus selectively on TGF-β effects on T cells, others using global TGF-β inhibition have generated diverging results concerning plaque size but similar findings regarding plaque inflammation. Variable effects on SMCs, depending on experimental design, might account for these differences.

In summary, our recent findings are in line with the hypothesis that major antiatherogenic effects of TGF-β are caused by its inhibitory action on T cells. Whether direct effects on SMC also impact on the development of experimental atherosclerotic lesions remains to be determined, and as Dr. Grainger pointed out, it will be important to deduce whether TGF-β plays an important role in human disease.

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In Response:

The detailed studies of the impact of T-cell specific abrogation of TGF-β signaling through introduction of a dominant-negative TGF-β receptor which Drs Hansson and Robertson describe emphasizes the importance of the T cell as a target for the antiatherogenic effects of TGF-β. Their claim that the proatherogenic effects that they observed in their well-designed study were “entirely caused by the lack of functional TGF-β receptors on T cells”, however, is...
too definitive. Circulating levels of TGF-β were not different between the two groups, but this is a notoriously difficult measure to interpret.² The levels of at least some TGF-β receptors were also apparently unaffected, but it would be difficult to exclude an impact on local tissue levels of TGF-β ligand capable of binding to receptors on other cell types, such as smooth muscle cells or endothelial cells. There is some evidence that TGF-β can positively auto-regulate its own production,³ and if T cells were a significant local source of TGF-β, then some of the proatherogenic changes they describe could plausibly depend on a reduced level of TGF-β acting on cells other than T-lymphocytes.

Along similar lines, deletion of apolipoprotein-E (apoE) may have direct immunological consequences,⁴ as well as effects on lipoprotein trafficking. If that is the case, it is possible that the central role of the T cell is emphasized in this particular model, and that under other conditions (in the human population, for example, where apoE deficiency is not the dominant cause of atherosclerosis), other pathways such as the ones we have described⁵ are at least as important in the T-cell mediated pathways revealed by their experiments.

Although it is essential to keep these caveats in mind, they do not diminish the central conclusions of this work: the inhibitory effects of TGF-β on T cells is an important antiatherogenic pathway, one which may ultimately be susceptible to therapeutic intervention.

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