Proatherogenic Role for NK Cells Revealed

MacRae F. Linton, Amy S. Major, Sergio Fazio

Atherosclerosis is now widely accepted as an inflammatory disease that involves both the innate and acquired immune systems. Several types of immune cells have been identified in the atherosclerotic lesions of humans and animal models. Numerous studies have shown a role for T and B lymphocytes in atherogenesis. Most studies have shown that T lymphocytes are proatherogenic, whereas B lymphocytes are associated with protection from atherosclerosis. The mechanisms for T and B lymphocyte-mediated modulation of atherosclerosis remain undefined, but may involve the production of proatherogenic cytokines, such as interferon (IFN)-γ, and the secretion of atheroprotective autoantibodies, respectively.

Macrophages are particularly important to the atherosclerotic disease process. They are among the first cells to infiltrate the artery wall and regulate lesion growth from its inception through uptake of modified lipoproteins, production of apolipoprotein (apo)-E and regulation of cholesterol efflux, secretion of matrix metalloproteinases, and contribution to the inflammatory process. The importance of macrophages to atherosclerosis has been emphasized in animal models with defective macrophage biology such as the op/op and monocyte–macrophage–deficient beige mice. Of specific relevance to atherosclerosis was probably not caused by either the perforin-mediated cytolytic function of NK cells or by NK cell interaction with the adaptive immune response, thus leaving cytokine production as the most likely mechanism for NK cell–mediated protection.

Directly studying the role of NK cells in immunity and atherosclerosis has been complicated by the lack of a specific cell marker for in vivo antibody-mediated depletions or animal models of NK cell deficiency. One accepted animal model of NK cell functional deficiency is the beige mutation in mice. Two studies have used the beige mutation in mice to examine the role of NK cells in atherosclerosis with opposing results. The first study, conducted more than a decade ago by Paigen et al, focused on these mice as a model for defective platelet function. The results demonstrated that beige mice fed a high fat diet containing cholate had no differences in atherosclerosis severity compared with controls. The second study conducted more recently by Schiller et al looked at the role of NK cells using mice harboring the beige mutation on the LDLR background. This study showed an increase in atherosclerosis in “beige” LDLR-/ mice compared with “wild-type” LDLR+/ animals. Therefore, NK cells were concluded to be antiatherogenic. Further analyses by this group showed that the increase in atherosclerosis was probably not caused by either the perforin-mediated cytolytic function of NK cells or by NK cell interaction with the adaptive immune response, thereby leaving cytokine production as the most likely mechanism for NK cell–mediated protection.

The study by Schiller et al was an initial step toward understanding the role of NK cells in atherosclerosis. Concerns regarding the use of the beige mouse model were detailed in an editorial by Getz, and will not be repeated again in this discussion. It is undisputed that the mutation in beige mice involves the Lyst gene, results in a complicated phenotype that goes beyond decreased NK cell activity. The Lyst gene encodes a protein involved in lysosomal trafficking, but the beige defect does not result in a total “lysosomal traffic jam.” Because of this, one could propose alternative NK cell–independent hypotheses for the increased atherosclerosis in beige mice. Of specific relevance may be functional changes in lysosomal trafficking in other cells, such as lesion macrophages or smooth muscle cells, which could lead to increased foam cell formation or apoptosis.

In this issue, Whitman et al have taken advantage of a new mouse model, the Ly49A transgenic mouse, to examine the role of NK cells in atherosclerosis. Ly49A, a cell surface receptor expressed by NK cells and some T cells, recognizes the MHC class-I molecule on antigen-presenting cells. Interaction of Ly49A with its ligand results in inhibition of NK cell–mediated cell lysis and cytokine secretion. The Ly49A transgenic mice used in this study were generated by expressing Ly49A under the control of the granzyme A promoter. Granzyme A is a major component of cytolytic granules abundantly expressed by NK cells and cytotoxic T lymphocytes. The result of this genetic manipulation was a
founder line that expressed Ly49A on all NK cells and half of all T cells. Expression of the Ly49A transgene led to a somewhat unexplained, but selective, deficiency in NK cell numbers, which correlated to decreased in vitro and in vivo cytolytic activity.\(^{31}\) Although the data were not provided in the original description of the Ly49A transgenic mice, the authors state that concentrations of T and B lymphocytes, as well as monocyte functions and numbers, were intact. These mice were dramatically different from previously described Ly49A transgenic mice, which showed inhibition of both NK and cytotoxic T cell function without a reduction in numbers of these cells.\(^{31,32}\) Therefore, the Ly49A mice represent the first opportunity to study atherosclerosis in an NK cell–deficient environment.

Using the now classical approach of bone marrow transplantation, Whitman et al transferred the NK cell deficient phenotype to the LDLR\(^{-/-}\) mouse model of atherosclerosis. Reconstituting LDLR\(^{-/-}\) mice with Ly49A transgenic bone marrow had no obvious effect on mouse body weight or serum lipoproteins.\(^{27}\) What resulted was an impressive 70% and 38% reduction in lesion size in the root and arch of the aorta, respectively, in Ly49A transgenic recipients compared with control LDLR\(^{-/-}\) mice, which were given nontransgenic marrow. The authors concluded that NK cells are proatherogenic; a diametrically opposite result of that in the beige mouse studies.\(^{24,34}\) Although the results of this study are different than those reported by Schiller et al.,\(^{24}\) one may argue that the Ly49A transgenic mouse is the more appropriate model in which to study NK cell function. Therefore, the report by Whitman et al.\(^{27}\) may be more applicable to the role of NK cells in atherosclerosis than previous work with the natural beige mutation. However, the Whitman study leaves some gaps in our understanding the mechanisms for NK cell–modulation of lesion progression.

In the original description of the Ly49A transgenic mice, Kim et al demonstrated a significant decrease in the NK cells, as represented by the NK1.1\(^{+}\)CD3\(^{+}\) population, in all tissues studied.\(^{29}\) Transfer of the Ly49A transgene to LDLR\(^{-/-}\) did result in a 50% decrease in NK1.1\(^{+}\)CD3\(^{+}\) cells in the spleen. However, the authors state that this was not a significant difference. No NK cell functional assays were conducted to confirm that the “nonsignificant” decrease in NK1.1\(^{+}\)CD3\(^{+}\) splenocytes resulted in decreased NK cell activity. Given the negative results in the perforin\(^{-/-}\)LDLR\(^{-/-}\) mice presented by Schiller et al.,\(^{24}\) the most likely role for NK cells in atherosclerosis may be via cytokine production (Figure). However, there were no obvious differences in NK cell numbers or IFN-γ production, as indirectly measured by I-Ab\(^{+}\) cells, in the atherosclerotic lesions of Ly49A or wild-type recipient mice. Interestingly, Kim et al reported that the Ly49A transgenic mice had decreased IFN-γ production in response to lipopolysaccharide (LPS) but not to IL-12 stimulation in vivo.\(^{29}\) Therefore, with no reported significant changes in NK cell numbers or functions, it is difficult to understand the mechanism(s) for reduced atherosclerosis in the Ly49A transgenic recipients. To understand the NK cell–mediated effects on atherosclerosis, it may be necessary to more directly address differences in the plaque cytokine environment. However, these functional examinations could prove frustrating because the cellular heterogeneity and the small NK cell number in atherosclerotic lesions would make it difficult to conduct in situ analyses, and in vitro experiments are notoriously hard to correlate to the in vivo situation.

Another question that needs to be addressed is what effect inhibition of other cell populations is having in the current study. In addition to the NK cell expression of the Ly49A transgene, Kim et al state that \(\approx 50\%\) of T cells express the Ly49A transgene.\(^{29}\) Whether these are CD8\(^{+}\) or CD4\(^{+}\) T cells was not discussed in either the original description of the transgenic animals or in the current report.\(^{27}\) Although little is known regarding the role of CD8\(^{+}\) T lymphocytes in atherosclerosis, their presence has been demonstrated in human lesions,\(^{33}\) and one study has shown that local, antigen-specific activation of CD8\(^{+}\) T lymphocytes can exacerbate atherosclerosis in apoE\(^{-/-}\) mice.\(^{34}\) Several studies in RAG\(^{/-/-}\) LDLR\(^{-/-}\) and apoE\(^{-/-}\) mice, which lack T and B lymphocytes but have enhanced NK cell activity, have demonstrated reductions in atherosclerosis.\(^{4,6}\) Data in the RAG\(^{-/-}\) mice suggest that NK cells, in the absence of adaptive immunity, are not sufficient to enhance atherosclerosis alone.

Of final note, in addition to NK cell deficiencies, the Ly49A mice have a slight (23%) decrease in NKT cell numbers and function (30%) in their spleens.\(^{29}\) These cells are potentially significant in atherosclerosis as they respond to glycolipid antigens by producing large amounts of cytokines that have been shown to regulate both innate and adaptive immunity, including NK cells.\(^{35,36}\) Recently, Tupin et al.\(^{37}\) and our laboratory (manuscript in review) have observed a
proatherogenic role for NKT cells in apoE \(^{-/-}\) mice. This observation would be consistent with the decrease in lesion size in Ly49A transgenic bone marrow recipients.\(^{22}\) Therefore, one may consider the possibility that NKT cells, acting upstream of NK cells, regulate their atherogenic potential and may also be implicated in the decreased atherosclerosis observed in Ly49A transgenic recipients.

Although these questions remain to be answered, the deficiencies of NKT and T lymphocytes are most likely secondary to the severe NK cell defect observed in the Ly49A mouse. The Whitman article provides a significant observation in the field of immunity and vascular disease, definitively placing the NK cell in the immunity–atherosclerosis axis. Although not perfect, the Ly49A mouse model is much more applicable than the beige mouse for studying NK cell function. Overall studies in this direction are extremely important to the understanding of the role of innate immunity in atherogenesis systems and to the development of novel therapeutics to treat vascular disease.

Acknowledgments

The authors thank Dr. Luc Van Kaer for helpful discussion. A.S. Major is supported by the American Heart Association Scientist Development Grant (0330412N) and the Vanderbilt Discovery Grant program. S. Fazio and M.F. Linton are supported by grants from the National Heart, Lung, and Blood Institute (HL57986, HL65709, HL65405, and HL53989).

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Arterioscler Thromb Vasc Biol. 2004;24:992-994
doi: 10.1161/01.ATV.0000128896.45976.f0

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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