Do Natural Killer Cells Participate in a Killer Vascular Disease?

Godfrey S. Getz

The article reporting on the exacerbated atherosclerosis in the double mutant, Lyst<sup>beige</sup>, LDL receptor (LDLR) knockout is important in drawing attention to the possible involvement of natural killer (NK) cells in atherogenesis. It is appropriate that NK cells come into focus: a recent review on immune mechanisms in atherosclerosis made scant mention of NK cells, and they are important cytolytic cells of the innate immune system. The Lyst beige mutation results in a defect in the cytolytic function of NK cells, and this was demonstrated in the double mutant after consumption of a high-fat diet for 16 weeks.

So the authors asked whether the impaired cytolytic activity of NK cells in beige mice may account for the increased atherosclerosis they observed in these mice. To test this, they generated a second complex knockout mouse, one lacking both the LDLR and the perforin gene involved in the cytolytic activity of both cytotoxic T cells, NK T cells, and NK cells. The absence of the perforin gene had no effect on atherosclerosis, even though the total serum cholesterol was increased in these double knockout mice at 16 weeks of diet treatment. Thus the cytolytic defect of NK cells in the Lyst beige LDLR/- strain does not by itself seem to account for the increased atherosclerosis in this strain. NK cells are important components of the innate immune system. To ascertain whether the adaptive immune system might be implicated in the pro-atherogenic influence of the beige mutation, these experiments were repeated in the absence of an adaptive immune system, ie, with a defective Rag (recombinase activating) gene. A comparison was made of the LDLR/-RAG1-/- strain with the triple knockout including the Lyst beige mutation. In this context too, the addition of the beige mutation resulted in an increase in atherosclerosis, so the adaptive immune system is not required for the manifestation of the pro-atherogenic influence of the beige mutation.

The conclusion of these studies is that the beige mutation results in an increase in atherosclerosis, but this appears to involve neither the cytolytic function of NK cells nor an interaction with the system of adaptive immunity. We are thus left with two questions. Is some function of NK cells other than their cytolytic function operative in atherogenesis and is this function disturbed in the cells lacking the Lyst protein? Alternatively, is this mutant protein operating in another cell involved in atherosclerosis such that its defect could increase the vascular disease? A major candidate for this latter cell is the monocyte/macrophage.

Before discussing these questions, there are a number of aspects of these studies that deserve comment. First, the effects on increased atherosclerosis are modest, albeit statistically significant. A similar increase in lesions was seen both in the aortic valve lesion and throughout the aorta. The diet used for these studies contained 1.25% cholesterol, which is quite high for atherosclerosis studies and raises the question of whether the same observations would be made in animals fed the frequently used Western diet, containing 0.15% cholesterol.

Though NK cells are found in the lesions, their presence alone gives no clear indication of the extent of their involvement nor how they may be involved in atherogenesis. Their absence from the more mature lesions of the LDLR-deficient mice is unexplained. If one infers that proximity suggests pathogenetic involvement, one might conclude that these cells play no role in the later features of lesion evolution. Because many viral sequences have been detected in atherosclerotic plaques and because NK cells represent important components of the innate response to viral infections, it might be of interest to ascertain whether those plaques containing evidence of cytomegalovirus or herpes viruses also contain increased numbers of NK cells.

The ontogeny of mature NK cells, unlike B and T cells, does not involve the rearrangement of germ line genes, so these are the only functional lymphocytes in RAG-deficient animals. It is possible that there is enhanced activity of the NK cells in this mouse model representative of adaptive changes in response to the global immune deficiency. It is very likely that this is mediated by changes in the cytokine spectrum of immune-deficient mice. Some such changes may even operate in the presence of the adaptive immune system, albeit one altered by the beige mutation. Two of the cytokines known to stimulate NK cell function are interleukin (IL)-12 and IL-18, both of which have been implicated in atherosclerosis. Thus, whether the NK cells are involved in atherogenesis or are mainly markers of an altered cytokine spectrum remains to be established.

Although a consideration of the role of NK cells in atherogenesis is certainly appropriate, other possible interpretations must be examined. The beige mouse has a complex phenotype, involving partial albinism, an immunodeficiency resulting in susceptibility to pyogenic infections, a bleeding diathesis related to platelet function, and neuropathy. The mutation in the beige mouse involves the Lyst gene, which is a homologue of the protein implicated in the autosomal recessive human Chediak Higashi syndrome. The function of
this protein is incompletely understood, but there is much evidence indicating its role in lysosomal fusion/fission and trafficking, hence the designation Lyst (lysosomal trafficking regulator). A feature of both the beige mouse and the Chediak Higashi syndrome is the presence within NK cells, neutrophils, and melanocytes of giant organelles that congregate around the nucleus. These organelles are derived from late endosomes and lysosomes. The beige/Chediak Higashi gene encodes a widely expressed cytosolic protein. The overexpression in mutant cells of the wild-type protein leads to small lysosomes distributed at the periphery of cells. Lysosomes, melanosomes, lytic granules, the MHC class II compartment, platelet-dense granules, and neutrophilic azurophil granules are all lysosome-related organelles that are affected in the Chediak Higashi syndrome, accounting for the protean phenotype of the syndrome and of the beige mouse. The cytolytic function of NK cells is defective in the beige mouse and in Chediak Higashi syndrome. Peptide loading and MHC class II endosomal sorting and therefore antigen presentation to T cells are also deficient. However, endocytosis of α2 macroglobulin is normal in mutant cells. Thus the defective lysosomal or related organelle trafficking does not disrupt all functions involving endosomes and lysosomes. It is clear that in the beige mouse, there may be disturbances in cellular functions other than in NK-mediated cytolysis or even other potential functions of NK cells. Schiller and colleagues recognize that the effects of the beige mutation may affect cells other than NK cells, so the plausibility of their observations on NK cells notwithstanding, much more work is required before we can understand the role of the Lyst protein on atherosclerosis whether direct or indirect. It is notable that whatever effects the beige mutation has in accentuating atherosclerosis, this occurs in the face of a reduction in the hyperlipidemia of the LDLR-deficient mouse.

If the enhanced physiological function of the NK cells contributes to the reduction of atherosclerosis in the aorta of the LDLR, RAG1 doubly deficient mouse, this influence is either not effective or is counterbalanced in the innominate artery, where atherosclerosis is unchanged in comparison to the immune-competent LDLR-deficient animal (Reardon and Getz in preparation). A similar finding has been reported for the immunodeficient apolipoprotein E–deficient mouse.

NK cells are most often studied for their effect on cytolyis or cytotoxicity. Although perforin-dependent cytolyis does not appear to influence atherosclerosis, a perforin-independent pathway such as Fas/FasL cannot be excluded in this model. FasL is constitutively expressed in NK cells. NK cells when appropriately activated are capable of cytokine production, particularly interferon (IFN)γ, IL-1, IL-10, and tumor necrosis factor-α, all of which may have an impact on atherogenesis. They also produce granulocyte macrophage colony stimulating factor (GM-CSF) and the beta chemokines macrophage inflammatory proteins 1α and 1β. NK cell proliferation and activity may be regulated by a variety of cytokines, including IFNα/β IL-12, IL-15, IL-18, and TGFβ. IFNα/β inhibits IL-12–induced IFNγ production by NK cells. IFNγ production is probably central to NK cell function, at least as innate defender against infections. If NK cells do in fact result in the attenuation of atherosclerosis, this is unlikely to be mediated by IFNγ as it is pro-atherogenic. Thus NK cells not only have multiple possible functions but are regulated in complex ways. It is not clear what is the state of the cytokine environment of NK cells in the beige model coupled with LDLR deficiency, and this uncertainty should be resolved. With the widespread functions of the Lyst protein, it might be most appropriate to attempt to understand the contribution of NK cells to atherogenesis in the LDLR, RAG1 double knockout. As stated, in this model, the NK cells are the only active lymphocyte population. One needs to use reagents that will target NK cell function in these animals. It might be interesting initially to focus on the cytokine context in which the NK cells of the lesion are functioning, including what they are producing. Do beige NK cells in atherosclerotic lesions produce more or less IFNγ? It would also of interest to know more about macrophage function in the beige mouse, particularly how this cell handles lipids and lipoproteins. Some of these questions could be readily answered in the available models.

In summary, the interesting article published in this issue, authored by Schiller and colleagues, draws attention to the potential interaction of NK cells with the atherogenic process. It has not yet been proven that these cells play a role in this process, though their presence and the fact that they are, under some circumstances, major cytokine producers, at least on a per cell basis, would argue strongly for their likely involvement. But the data does not yet allow for clear conclusions on this theme. The work reported here should stimulate further studies on this issue, and many of the models and techniques are at hand.

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References


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