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Natural Killer cells at ease-Atherosclerosis is not affected by genetic depletion or hyperactivation of Natural Killer cells

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Atherosclerosis is a lipid-driven, chronic inflammatory disorder that is characterized by the formation of leukocyte-rich plaques in large and medium sized arteries. Plaque macrophages form lipid-laden foam cells and eventually fail to clear the overwhelming number of apoptotic cells (failure of efferocytosis), forming a necrotic core. Other immune cells like T and B cell subsets contribute to atheroprogression by controlling the inflammatory milieu. The subject of the present work[1] concerns the role of NK cells in atherosclerosis progression.

Natural killer (NK) cells are found at an average of 1–2 cells per lesion section.[1] NK cells are potent immune cells protecting the host from viral infections and tumor formation. If a host cell lacks surface major histocompatibility complex I (MHC-I), being in a state of "missing self", NK cells will kill this cell. NK cells also display immune-regulatory features and are capable of influencing antigen-specific T cell responses. In the course of cardiovascular disease, the number of NK cells decreases in patients with stable angina or non-ST elevation myocardial infarction (non-STEMI) without affecting their cytokine expression profile.[2] Until now[1], the role of NK cells in atherosclerosis had remained unclear and controversial.

In this issue of *Circulation Research*, Nour-Eldine et al[1]. show that NK cells are not involved in atherosclerosis.

A first study investigating NK cell activity in atherosclerosis assessed *beige* mice. *Beige* mice carry a mutation of the *Lyst* gene impairing NK cell activity. *Beige* mice fed a high fat diet with cholate did not display altered atherosclerotic lesion formation whereas low-density lipoprotein receptor deficient ($Ldlr^{-/-}$) mice crossbred to beige mice harbored smaller lesions. This was interpreted to mean that NK cells may be pro-atherogenic.[3]

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Disclosures

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However, *beige* mice have additional defects (table 1) such as lysosomal storage impairments which might affect other immune cells and thus atherosclerosis.[4]

Further studies delineating NK cell activity involved transgenic mice expressing Ly49A under control of the granzyme A promotor. Ly49A is an MHC-I binding receptor that inhibits NK cell function and survival. These mice have fewer NK cells.[5] The transplantation of bone marrow from these transgenic mice into $Ldlr^{-/-}$ mice reduced atherosclerosis, suggesting that NK cells may be pro-atherogenic.[6] However, granzyme A is also expressed by NKT cells and CD8 T cells, which have been both identified as pro-atherogenic.[7–9] Thus, this model is not suitable to isolate the role of NK cell function.

A third set of studies applied rabbit anti-asalio GM serum. Injection of this serum into apolipoprotein deficient ($Apoe^{-/-}$) mice depleted NK cells and significantly reduced atherosclerosis formation, again ascribing NK cells a pro-atherogenic role.[10] However, the glycolipid asialo GM-1 is not exclusively expressed by NK cells, but also by myeloid cells, T cells, and even epithelial cells. This suggests that anti-asialo-GM1 may have extraneous effects that confound the interpretation. Adoptive transfer of NK cells into lymphopenic and NK cells deficient $Apoe^{-/-}$ $Rag2^{-/-}$ $Il2rg^{-/-}$ mice suggested that NK cells contribute to necrotic core formation and atheroprogression.[10]

Nour-Eldine et al[1] looked at NK cell functionality in atherosclerosis using precise and specific genetic approaches. In the first model, Cre recombinase was controlled by the internal Ncr1 promotor (Ncr^{iCre}). The *Ncr1* gene encodes the NK cell-specific inhibitory receptor Nkp46. These mice were crossed with transgenic mice expressing a flox-STOP-flox controlled diphtheria toxin a (DTA) fragment in the Rosa26 locus ($R26^{lsl\text{-}DTA}$). Cre-driven excision of the stop codon induced NK cell death by DTA expression. It should be noted though that a minor fraction of innate lymphoid cells in the liver and in the small intestine also express this marker and will be affected by the described deletion strategy. Nour-Eldine et al[1] transplanted bone marrow from Ncr^{iCre} $R26^{lsl\text{-}DTA}$ mice into $Ldlr^{-/-}$ mice. They found that atherosclerotic burden did not differ compared to control mice after 8, 12, or 15 weeks of high fat diet (HFD).

To test whether anti-asialo GM1 treatment is specific for NK cells[10], the authors performed bone marrow transplantations of wildtype or Ncr^{iCre} $R26^{lsl\text{-}DTA}$ bone marrow into $Ldlr^{-/-}$ mice. Again $Ldlr^{-/-}$ mice receiving either bone marrow and a control antibody displayed similar levels of atherosclerosis, but injection of anti-asialo GM1 serum into both types of mice significantly reduced atherosclerosis. Thus, anti-asialo GM1 had significant effects on cells other than NK cells.

A third model carried the *Noe* mutation.[11] This point mutation generated by random mutagenesis prohibits NKp46 expression on the cell surface, thus rendering NK cells hyperresponsive, which leads to elevated production of the pro-inflammatory cytokine IFNγ and a higher potential of degranulation. Nour-Eldine et al[1] transplanted bone marrow from *Noe* mice on a C57BL6/J background into $Ldlr^{-/-}$ mice. After 8 weeks of HFD, no difference in lesion formation was observed between mice harbouring hyperresponsive NK

cells and controls. As expected, the production of IFNγ by splenic NK cells derived from *Noe* transplanted *Ldlr*$^{-/-}$ mice was higher.

As a positive control, the authors studied to what extent poly(I:C) injections as a model of chronic viral infection would reveal a role of hyperresponsive NK cell function contributing to atherosclerosis. The TLR agonist poly-I:C enhances perforin, granzyme B, and IFNγ. Indeed, poly(I:C) treated mice lacking NK cells were protected from elevated atherosclerosis. Thus, NK cells are pro-atherogenic under conditions of chronic viral infections, which might have an implication on the cardiovascular health status of patients suffering from chronic viral infections such as HIV.

The study by Nour-Eldine et al[1] elegantly shows that NK cells are not involved in atherosclerosis in the *Ldlr*$^{-/-}$ mouse model, except under conditions of modelled chronic viral infection. Atherosclerotic burden neither in the aortic sinus nor in the descending aorta changed in mice lacking NK cells or having hyperresponsive NK cells.

A strength of the present study is that three time points were studied (feeding HFD for 8, 12, or 15 weeks). A limitation is that type 1 and type 3 ILC in the small intestine and in the liver also express NKp46 and thus were likely depleted or hyperactivated, respectively, in the mouse models used. Whereas CD25^{+} type 2 ILCs curb the development in atherosclerosis[12], the role of ILC1 and ILC3 is unknown. The authors of the present study confirm that anti-asialo GM1 treatment protects from atherosclerosis, but this is true even in mice lacking NK cells. Thus, previous results utilizing anti-asialo GM1 treatment must be reinterpreted. Another limitation is that bone marrow transplantations into *Ldlr*$^{-/-}$ mice can yield different results than mice in which the transgenes were crossed into the *Ldlr*$^{-/-}$ background.[13, 14]

In conclusion, Nour-Eldine et al[1] find no effect of NK cell depletion or hyperactivation on atherosclerosis in the *Ldlr*$^{-/-}$ mouse model under HFD conditions. This resolves a long-standing controversy in the field.

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Table 1

Overview of models used to assess NK cell function in atherosclerosis.

Model	NK cells	Atherosclerosis	Other cells targeted
Anti-asialo GM1 serum[10]	80% depleted	Reduced	Myeloid cells, epithelial cells, CD8 T cells 60% depleted, NKT cells 60% depleted
Granzyme A-Ly49A transgenic mice[6]	Depleted	Reduced	Some CD, CD8 T cells, NKT cells
Beige mice[3]	Function impaired	Reduced	Neutrophils, smooth muscle, macrophages
Ncr^{iCre} $R26^{lsl\text{-}DTA}$ mice[1]	>90% depleted	Unaffected	ILC1 cells in liver, ILC3 cells in small intestine
Noe mice[1] (gain-of-function point mutation in Ncr1)[1]	Hyperreactive (more IFNγ)	Unaffected	None known, maybe ILC1 and ILC3