Natural Killer Cells at Ease

Atherosclerosis Is Not Affected by Genetic Depletion or Hyperactivation of Natural Killer Cells

Holger Winkels, Klaus Ley

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 natural killer (NK) cells in atherosclerosis progression.

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NK cells are found at an average of 1 to 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 MHC-I (major histocompatibility complex 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-segment–elevation myocardial infarction 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 (HFD) 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 proatherogenic.[3] However, beige mice have additional defects (Table) 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 promoter. 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 proatherogenic.[6] However, granzyme A is also expressed by NKT (natural killer T cell) cells and CD8 T cells, which have been both identified as proatherogenic.[7–9] Thus, this model is not suitable to isolate the role of NK cell function.

A third set of studies applied rabbit anti-asialo-GM1 serum. Injection of this serum into apolipoprotein-deficient ($Apoe^{-/-}$) mice depleted NK cells and significantly reduced atherosclerosis formation, again ascribing NK cells a proatherogenic role.[10] However, the glycolipid asialo-GM1 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 cell–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 promoter (Ncr^{iCre}). The *Ncr1* gene encodes the NK cell–specific inhibitory receptor NKp46 (natural cytotoxicity triggering receptor 1). 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 with control mice after 8, 12, or 15 weeks of HFD.

To test whether anti-asialo-GM1 treatment is specific for NK cells,[10] the authors performed bone marrow transplantations of wild-type 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 proinflammatory cytokine interferon-γ 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,

From the Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, CA (H.W., K.L.); and Department of Bioengineering, University of California San Diego, La Jolla (K.L.).

Correspondence to Klaus Ley, MD, Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle Dr, La Jolla, CA 92037. E-mail klaus@lji.org

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Table. 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
Ncr1^{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

IFN indicates interferon; ILC, innate lymphoid cell; NK, natural killer; and NKT, natural killer T cell.

no difference in lesion formation was observed between mice harboring hyperresponsive NK cells and controls. As expected, the production of interferon-γ 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 (Toll-like receptor) agonist poly-I:C enhances perforin, granzyme B, and interferon-γ. Indeed, poly(I:C)-treated mice lacking NK cells were protected from elevated atherosclerosis. Thus, NK cells are proatherogenic under conditions of chronic viral infections, which might have an implication on the cardiovascular health status of patients experiencing 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 modeled 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 3 time points were studied (feeding HFD for 8, 12, or 15 weeks). A limitation is that type 1 and type 3 innate lymphoid cells (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 using 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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Disclosures

None.

References

1. Nour-Eldine W, Joffre J, Zibara K, Esposito B, Giraud A, Zeboudj L, Vilar J, Terada M, Bruneval P, Vivier E, Ait-Oufella H, Mallat Z, Ugolini S, Tedgui A. Genetic depletion or hyperresponsiveness of natural killer cells do not affect atherosclerosis development. *Circ Res*. 2018;122:47–57. doi: 10.1161/CIRCRESAHA.117.311743.
2. Backteman K, Ernerudh J, Jonasson L. Natural killer (NK) cell deficit in coronary artery disease: no aberrations in phenotype but sustained reduction of NK cells is associated with low-grade inflammation. *Clin Exp Immunol*. 2014;175:104–112. doi: 10.1111/cei.12210.
3. Linton MF, Major AS, Fazio S. Proatherogenic role for NK cells revealed. *Arterioscler Thromb Vasc Biol*. 2004;24:992–994. doi: 10.1161/01.ATV.0000128896.45976.f0.
4. Ward DM, Griffiths GM, Stinchcombe JC, Kaplan J. Analysis of the lysosomal storage disease Chediak-Higashi syndrome. *Traffic*. 2000;1:816–822.
5. Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM. In vivo natural killer cell activities revealed by natural killer cell-deficient mice. *Proc Natl Acad Sci USA*. 2000;97:2731–2736. doi: 10.1073/pnas.050588297.
6. Whitman SC, Rateri DL, Szilvassy SJ, Yokoyama W, Daugherty A. Depletion of natural killer cell function decreases atherosclerosis in low-density lipoprotein receptor null mice. *Arterioscler Thromb Vasc Biol*. 2004;24:1049–1054. doi: 10.1161/01.ATV.0000124923.95545.2c.
7. Getz GS, Reardon CA. Natural killer T cells in atherosclerosis. *Nat Rev Cardiol*. 2017;14:304–314. doi: 10.1038/nrcardio.2017.2.
8. Kyaw T, Winship A, Tay C, Kanellakis P, Hosseini H, Cao A, Li P, Tipping P, Bobik A, Toh BH. Cytotoxic and proinflammatory CD8+ T lymphocytes promote development of vulnerable atherosclerotic plaques in apoE-deficient mice. *Circulation*. 2013;127:1028–1039. doi: 10.1161/CIRCULATIONAHA.112.001347.
9. Cochain C, Koch M, Chaudhari SM, Busch M, Pelisek J, Boon L, Zernecke A. CD8+ T cells regulate monopoiesis and circulating Ly6C-high monocyte levels in atherosclerosis in mice. *Circ Res*. 2015;117:244–253. doi: 10.1161/CIRCRESAHA.117.304611.
10. Selathurai A, Deswaerte V, Kanellakis P, Tipping P, Toh BH, Bobik A, Kyaw T. Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms. *Cardiovasc Res*. 2014;102:128–137. doi: 10.1093/cvr/cvu016.
11. Narni-Mancinelli E, Jaeger BN, Bernat C, et al. Tuning of natural killer cell reactivity by NKp46 and Helios calibrates T cell responses. *Science*. 2012;335:344–348. doi: 10.1126/science.1215621.
12. Engelbertsen D, Foks AC, Alberts-Grill N, Kuperwaser F, Chen T, Lederer JA, Jarolim P, Grabie N, Lichtman AH. Expansion of CD25+ innate lymphoid cells reduces atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2015;35:2526–2535. doi: 10.1161/ATVBAHA.115.306048.
13. Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, Merval R, Esposito B, Cohen JL, Fisson S, Flavell RA, Hansson GK, Klatzmann D, Tedgui A, Mallat Z. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med*. 2006;12:178–180. doi: 10.1038/nm1343.
14. Buono C, Pang H, Uchida Y, Libby P, Sharpe AH, Lichtman AH. B7-1/B7-2 costimulation regulates plaque antigen-specific T-cell responses and atherogenesis in low-density lipoprotein receptor-deficient mice. *Circulation*. 2004;109:2009–2015. doi: 10.1161/01.CIR.0000127121.16815.F1.

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