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 atherop progression by controlling the inflammatory milieu. The subject of the present work concerns the role of natural killer (NK) cells in atherosclerosis progression.

Article, see p 47

NK cells are found at an average of 1 to 2 cells per lesion section. 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 immunoregulatory 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. Until now, the role of NK cells in atherosclerosis had remained unclear and controversial.

In this issue of Circulation Research, Nour-Eldine et al. 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 might affect other immune cells and thus atherosclerosis. However, beige mice have additional defects (Table) such as lysosomal storage impairments, which might affect other immune cells and thus atherosclerosis.

Further studies delineating NK cell activity involved transgenic mice expressing Ly49A under control of the granzyme γ-interferon (IFN-γ) promoter. Ly49A is an MHC-I–binding receptor that inhibits NK cell function and survival. These mice have fewer NK cells. The transplantation of bone marrow from these transgenic mice into Ldlr−/− mice reduced atherosclerosis, suggesting that NK cells may be proatherogenic. However, granzyme A is also expressed by NKT (natural killer T cell) cells and CD8 T cells, which have been both identified as proatherogenic. 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. Thus, 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 atherop progression.

Nour-Eldine et al. 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 (Ncr1Cre). 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 (R26lsl-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. transplanted bone marrow from Ncr1Cre R26lsl-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, the authors performed bone marrow transplantations of wild-type or Ncr1Cre R26lsl-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. 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. transplanted bone marrow from Noe mice on a C57BL6/J background into Ldlr−/− mice. After 8 weeks of HFD,
transgenes were crossed into the Ldlr−/− cells are not involved in atherosclerosis in the mouse models used. Whereas CD25+ type 2 ILCs curb the development of atherosclerosis in mice lacking NK cells or NKp46 and thus in small intestine and in the liver also express NKp46 and thus having hyperresponsive NK cells.

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 atherosclerosis. The TLR (Toll-like receptor) agonist poly(I:C) injections as a model of chronic viral infection would reveal a role of hyperresponsive NK cell function contributing to atherosclerosis.

The study by Nour-Eldine et al10 elegantly shows that NK cells are not involved in 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

Key Words: Editorsials | atherosclerosis | immune system | inflammation | killer cells, natural | macrophages

Table. Overview of Models Used to Assess NK Cell Function in Atherosclerosis.

<table>
<thead>
<tr>
<th>Model</th>
<th>NK Cells</th>
<th>Atherosclerosis</th>
<th>Other Cells Targeted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-asialo-GM1 serum&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80% depleted</td>
<td>Reduced</td>
<td>Myeloid cells, epithelial cells, CD8 T cells 60% depleted, NK T cells 60% depleted</td>
</tr>
<tr>
<td>Granzyme A-Ly49A transgenic mice&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Depleted</td>
<td>Reduced</td>
<td>Some CD, CD8 T cells, NK T cells</td>
</tr>
<tr>
<td>Beige mice&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Function impaired</td>
<td>Reduced</td>
<td>Neutrophils, smooth muscle, macrophages</td>
</tr>
<tr>
<td>Ncr1&lt;sup&gt;c&lt;/sup&gt; R&lt;sub&gt;26lsl-DTA&lt;/sub&gt; mice&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;90% depleted</td>
<td>Unaffected</td>
<td>ILC1 cells in liver, ILC3 cells in small intestine</td>
</tr>
<tr>
<td>Noc&lt;sup&gt;e&lt;/sup&gt; mice&lt;sup&gt;f&lt;/sup&gt; (gain-of-function point mutation in Ncr1&lt;sup&gt;γ&lt;/sup&gt;)</td>
<td>Hyperreactive (more IFN&lt;sub&gt;γ&lt;/sub&gt;)</td>
<td>Unaffected</td>
<td>None known, maybe ILC1 and ILC3</td>
</tr>
</tbody>
</table>

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 Noc transplanted Ldlr<sup>−/−</sup> 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.

In conclusion, Nour-Eldine et al<sup>11</sup> find no effect of NK cell depletion or hyperactivation on atherosclerosis in the Ldlr<sup>−/−</sup> mouse model under HFD conditions. This resolves a long-standing controversy in the field.
Natural Killer Cells at Ease: Atherosclerosis Is Not Affected by Genetic Depletion or Hyperactivation of Natural Killer Cells
Holger Winkels and Klaus Ley

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