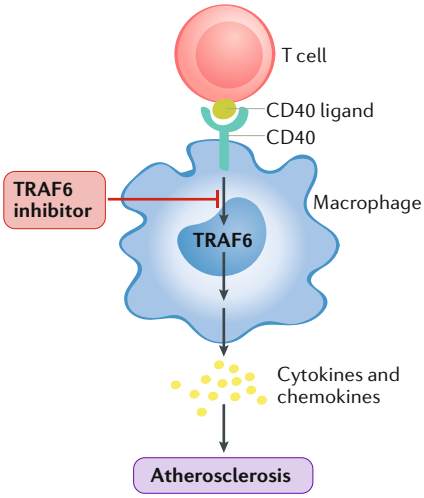
ATHEROSCLEROSIS

Nanoimmunotherapy targeting CD40–TRAF6 signalling to reduce atherosclerosis



Credit: V. Summersby/Macmillan Publishers Limited

> Treatment for 6 weeks with the nanoparticles attenuated the development of early atherosclerosis

Binding of the CD40 receptor on monocytes and macrophages to CD40 ligand on T cells is a well-known driver of atherosclerosis. Following activation, CD40 recruits TNF receptor-associated factor 6 (TRAF6) leading to a pro-inflammatory signalling cascade. Investigators now show that inhibiting CD40–TRAF6 signalling in macrophages can reduce atherosclerosis, and that a nanoimmunotherapy approach to delivering the TRAF6 inhibitor is safe in both mice and non-human primates.

The C-terminal tail of CD40 has a proximal binding site for TRAF6 as well as distal binding sites for TRAF2, TRAF3, and TRAF5. Whereas TRAF6 has been shown to be involved in pathways mediating atherosclerosis and restenosis, TRAF2, TRAF3, and TRAF5 are required for CD40-associated immunity. Long-term inhibition of CD40 or CD40 ligand has anti-atherosclerotic effects, but also results in immune suppression and increased risk of thromboembolism. Therefore, Seijkens and colleagues focused on TRAF6 as a means of targeting atherosclerotic pathways.

The investigators identified small-molecule inhibitors that block the interaction between CD40 and TRAF6, while leaving interactions with TRAF2, TRAF3, and TEAF5 intact, meaning that CD40-mediated immunity is preserved. Treatment of young *Apoe*$^{-/-}$ mice with a TRAF6 inhibitor reduced the initiation of atherosclerosis, and treatment in *Apoe*$^{-/-}$ mice with established atherosclerosis stopped plaque progression and improved characteristics of plaque stability (increased collagen content and smaller necrotic core).

The TRAF6 inhibitor reduced CD40 and integrin expression in classical monocytes and thereby impaired monocyte recruitment to the vessel wall. The investigators targeted the treatment specifically to macrophages by incorporating the TRAF6 inhibitor into recombinant HDL nanoparticles. Treatment for 6 weeks with the nanoparticles attenuated the development of early atherosclerosis in *Apoe*$^{-/-}$ mice.

Another paper, published in *Nature Biomedical Engineering*, describes the development and evaluation of a nanoimmunotherapy that blocks the CD40–TRAF6 interaction specifically in monocytes and macrophages. The nanoparticle was constructed from the lipophilic small-molecule TRAF6 inhibitor encapsulated in phospholipids and human apolipoprotein A-I. The researchers used *Apoe*$^{-/-}$ mice aged 20 weeks that had been fed a high-cholesterol diet for 12 weeks to induce the development of atherosclerotic lesions. Mice received four intravenous infusions of either control phosphate-buffered saline, empty recombinant HDL nanoparticles, or TRAF6 inhibitor–HDL nanoparticles over the course of 7 days and were then euthanized 24 h later.

No significant difference in plaque size or collagen content in the aortic sinus area was observed between the groups. Nevertheless, the lesions in mice that received the active nanoparticles had a significant reduction in macrophage content compared with either of the control groups, indicating a transition to a more stable plaque phenotype. Similarly, macrophage content in the aorta was significantly reduced with active nanoparticles compared with either control group. Furthermore, treatment with active nanoparticles was associated with a significant reduction in T cell content in the aorta compared with the control groups. The investigators went on to show that the reduction in macrophage content was as a result of a decrease in monocyte recruitment rather than a reduction in local macrophage proliferation, which was unchanged. The CD40–TRAF6 inhibitor probably impaired monocyte migratory capacity and also had effects on endothelial cells that led to reduced transendothelial migration of monocytes. Of note, no toxic effects of the active nanoparticles were observed in either *Apoe*$^{-/-}$ mice or non-human primates.

"We are committed to translating our … nanobiologic immunotherapy to patients," comments Raphaël Duivenvoorden, one of the lead investigators. Duivenvoorden and colleagues plan to test the therapy in a non-human primate model of atherosclerosis, which will hopefully provide the basis for human testing.

Gregory B. Lim

ORIGINAL ARTICLES Seijkens, T. T. P. et al. Targeting CD40-induced TRAF6 signaling in macrophages reduces atherosclerosis. *J. Am. Coll. Cardiol.* **71**, 527–542 (2018) | Lameijer, M. et al. Efficacy and safety assessment of a TRAF6-targeted nanoimmunotherapy in atherosclerotic mice and non-human primates. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-018-0221-2 (2018)