Lymphocytic tumor necrosis factor receptor superfamily co-stimulatory molecules in the pathogenesis of atherosclerosis

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Purpose of review
The role of lymphocytes in the chronic inflammatory disease atherosclerosis has emerged over the past decade. Co-stimulatory molecules of the heterogeneous tumor necrosis factor receptor superfamily play a pivotal role in lymphocyte activation, proliferation and differentiation. Here we describe the immune modulatory properties and mechanisms of four tumor necrosis factor receptor superfamily members in atherosclerosis.

Recent findings
CD40/CD40L, OX40L/OX40, CD70/CD27 and CD137/CD137L are present in human atherosclerotic plaques and have shown strong immune modulatory functions in atherosclerosis, resulting in either atherogenic or atheroprotective effects in mouse models of atherosclerosis.

Summary
Insight into the immune modulatory mechanisms of co-stimulatory interactions in atherosclerosis can contribute to clinical exploitation of these interactions in the treatment of cardiovascular disease.

Keywords
adaptive immune system, atherosclerosis, co-stimulatory molecules, lymphocytes, tumor necrosis factor receptor superfamily

INTRODUCTION
Atherosclerosis is a chronic inflammatory disease of the arterial wall. Injurious stimuli including shear stress and modification of LDL and its subendothelial deposition activate the endothelium [1,2]. Subsequently, inflammatory cells of both the innate and adaptive immune system are attracted and infiltrate into the subendothelial space where atherosclerotic plaques arise via intertwined immunological interactions between immune cells, endothelial cells, platelets and smooth muscle cells [3].

THE ADAPTIVE IMMUNE SYSTEM IN ATHEROSCLEROSIS
The role of the innate immune system in atherosclerosis has long been established. In the early 1990s, the contribution of the adaptive immune system was considered as T-lymphocytes and antigen presenting cells (APCs) were found in close proximity within the atherosclerotic plaque [1,2]. Studies in animal models indeed proved lymphocytes to play a major role in atherosclerosis. For example, Cd4+/ Apoe−/− mice show less atherosclerotic plaques in the aortic root and adoptive transfer of CD4+ T cells into atherosclerosis-prone SCID Apoe−/− mice aggravates atherogenesis in an interferon (IFN)γ-associated manner [4,5]. T lymphocytes recognize epitopes on the apoB100 unit of native LDL, which supports the production of IgG to oxLDL by B cells [6]. Lymphocytes are also present in aortic tertiary lymphoid organs in very advanced atherosclerosis, in which they are considered to serve as a site for local responses against plaque antigens [7].

The divergent effects of B cells on atherosclerosis are attributed to subset-specific effects. B1a B cells, mainly present in peritoneal cavities,
KEY POINTS

- The adaptive immune systems impacts atherogenesis.
- TNFRSF family of co-stimulators regulates immune cell activation in the context dependent manner.
- Various members of the TNFRSF family are known atherogenic or atheroprotective factors.
- Targeting TNFRSF members may enable specific immunomodulation in atherosclerosis.

TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY

In atherosclerosis, several members of the TNF (receptor) superfamily (such as CD40, OX40, CD137, GITR, CD30, TNF-related apoptosis-inducing ligand and CD27) have been studied in the context of atherosclerosis (Table 1) + indicated refs. [17,18–35,36**,37–41]. In general, the expression of these molecules is enhanced upon engagement of an antigen with the TCR or BCR.

Most TNFRSF members lack intrinsic activity and require downstream adaptor proteins to induce intracellular signaling, TNFR-associated factors (TRAFs). TRAFs are recruited to the cytoplasmic tail of the trimeric TNFRs and engage with the conserved TRAF domain [40]. Until today, seven distinct TRAF molecules have been identified that interact with TNFRSF, of which five are implicated in atherosclerosis in various cellular environments: TRAF1, 2, 3, 5 and 6 [40]. Depending on cell type, environmental conditions and TRAF-binding site different downstream signaling pathways are initiated [41].

Several studies have revealed the effects of the different TRAF molecules in atherosclerosis, but the role of the different individual TNFRSF–TRAF interactions is still under investigation. Endothelial specific Traf6−/−/Apoel−/− mice showed reduced atherosclerosis, whereas myeloid cell-specific TRAF6-deficient Apoe−/− mice developed accelerated atherosclerosis [42]. Traf1−/−/Ldlr−/− mice developed smaller atherosclerotic lesions because of impaired monocyte migration and adhesion to the endothelium [45]. TRAF5 is atheroprotective, as shown by Traf5−/−/Ldlr−/− mice [46]. TRAF3 serves as an inhibitor for TRAF2/5 nuclear factor (NF)κB activation upon stimulation of various TNFRSF members [47]. TRAF2 and TRAF3 upregulation is associated with shear stress [48,49].

The most dominant outcome of TNFRSF–TRAF signaling is NFκB and mitogen-activated protein kinase (MAPK) activity, which mediates activation, proliferation and the production of proinflammatory cytokines and adhesion molecules [41,42].

In this review, we will discuss the contribution of CD40/CD40L, OX40/OX40L and CD27/CD27L to atherogenesis in detail, as these TNFRSF members are well studied and are considered promising candidates for therapy in atherosclerosis.

CD40/CD40L

The CD40/CD40L axis is one of the best-studied co-stimulatory pathways. CD40L, also known as CD154 or TNFSF5, is expressed on various cell types, both immune cells and nonhematopoietic cells, particularly CD4+ T cells and platelets [21]. CD40-CD40L interactions among T cells, B cells and dendritic cells and their subsequent immune cell activation or modulation. Three signals are required for activation of naive T or B cells (and other APCs such as dendritic cells and macrophages): the first signal is the binding of the T-cell receptor (TCR) or B-cell receptor (BCR) to an antigen, the second signal is provided through co-stimulatory molecules and the third signal through cytokines secreted by cells carrying the co-stimulatory molecules [15].

The engagement of co-stimulatory molecules on the surface of mature APCs to their counter receptors on T cells is required for proliferation and escape from anergy. Primed T cells are a heterogeneous group of cells differentiated into one of the T-cell subtypes based on encountered APC-secreted cytokine profile: T-helper CD4+ (with Th1, Th2, Th17 subprofiles), cytotoxic CD8+ cells and regulatory T cells [16].

Although the effects of the different co-stimulatory molecules are well established, the role of the different co-stimulatory pathways during the pathogenesis of atherosclerosis still needs to be fully elucidated. In this review, we will summarize the role of the tumor necrosis factor receptor superfamily (TNFRSF) members in atherosclerosis, and highlight the most important findings.
interactions mediate T-cell activation, proliferation and cytokine secretion required for B-cell activation. CD40 is constitutively expressed on APCs, including dendritic cells and B cells on which its expression is required for humoral immune responses such as proliferation and isotype switching [16,50]. CD40 has two distinct binding sites for TRAF: a proximal site for TRAF1/2/3/5 and a distal site for TRAF6 binding [51]. TRAF6 activation is linked to NF\(\kappa\)B, Signal Transducer and Activators of Transcription 3 and protein kinase B activation [52]. TRAF1/2/3/5 activates the NF\(\kappa\)B, MAPK/p38 and c-Jun N-terminal kinases (JNK) pathways [43].

It is important to recall that CD40/CD40L is expressed on numerous plaque-related cells, such as macrophages, platelets and dendritic cells, in addition to lymphocytes. Various studies showed that \(\text{Apoe}^{-/-}/\text{Cd40}^{-/-}\) mice, as well as anti-CD40L antibody-treated \(\text{Ldlr}^{-/-}\) or \(\text{Apoe}^{-/-}\) develop reduced atherosclerosis and/or a plaque phenotype that shows a reduction in inflammation, reminiscent of stable atherosclerotic plaques in humans [18–30]. The effect of CD40L on atherosclerosis appeared hematopoietic cell-independent, as shown by \(\text{Cd40l}^{-/-}/\text{Ldlr}^{-/-}\) chimeras. No difference regarding plaque size and phenotype was observed in these mice [53]. However, the contribution of the different CD40L-expressing hematopoietic cell-types still needs to be confirmed using conditional knock-out mice. Platelet CD40L is atherogenic, and plays a major role in platelet-leukocyte aggregation and leukocyte recruitment to the endothelium [16].

\(\text{Cd40}^{-/-}/\text{Apoe}^{-/-}\) mice, as well as \(\text{Cd40}^{-/-}/\text{Ldlr}^{-/-}\) chimeras show reduced atherosclerosis and develop
atherosclerotic plaques that contain less inflammatory cells and more fibrosis [54]. In contrast, in another report, Cda40⁻/⁻/Ldlr⁻/⁻ mice fed with a high-cholesterol diet for 8 and 16 weeks develop atherosclerotic lesions comparable in size with Ldlr⁻/⁻ mice [55]. The main pathway mediating the proatherogenic role of CD40 in atherosclerosis is the major histocompatibility complex (MHC)II-related CD40-TRAF6 pathway. Mice with deficient CD40-TRAF6 signaling in MHCI⁺ cells developed little atherosclerosis, owing to reduced leukocyte recruitment and polarization towards Ly6c⁺ monocytes and M2 macrophages. In contrast, mice with deficient CD40-TRAF2/3/5 signaling in MHCI⁺ cells showed no reduction in atherosclerosis [54].

Polymorphisms in the CD40 gene (-1C/T) correlate with unstable coronary plaques, whereas the C allele frequency increased risk of coronary plaque disruption in 699 patients [24]. Moreover, several studies showed that sCD40L in the circulation is highly correlated with cardiovascular risk in humans, but its biological function remains to be elucidated until today [23,56,57].

CD27/CD70

CD27/TNFRSF7 is constitutively expressed on T cells and its expression increases upon cell activation. CD27 has also been identified on B cells, hematopoietic progenitors and natural killer cells. CD27 ligand, known as CD70 or TNFSF7, is transiently expressed on activated T cells, activated B cells and dendritic cells. The downstream signaling of CD27 is mediated by TRAF2 and TRAF5, activating the NFκB and JNK signaling pathways; CD70 ligation triggers PI3K/Akt and MEK/extracellular-signal-regulated kinases (ERK)/MAPK activation [17*,58].

Ligation of T cell CD27 to B cell CD70 has a bidirectional effect. On the one hand, it increases long-lasting T-cell survival in both primary and memory responses, and the expansion of Th1 IFNγ-secreting effector T cells [17*,59]. On the other hand, CD70 triggering on B cells impairs the formation of IgG antibodies, but not of IgM [17*]. CD27 ligation on B cells promotes cell proliferation and cell cycle entry [60]. It is further upregulated during germinal centre formation but does not elevate isotype switching to atherogenic IgG [60]. In addition, CD27 on B cells ligates to CD70 on T cells to stimulate CD8⁺ T cells proliferation [61]. The complex interactions indicate a fine balance between CD27 and CD70 expression on B and T cells for optimal immune responses. CD27/CD70 rescues atheroprotective regulatory Foxp3⁺ T cells from apoptosis in the thymus [62,63**]. Moreover, antiatherogenic regulatory Foxp3⁺ T cells are rescued from apoptosis in the thymus by CD27/CD70 [64**]. The co-stimulatory molecules promote inflammation by impairment of the development of Th17 helper T cells, partly via JNK pathway activation by CD27.

van Olffen et al. recently showed that B cell-specific Cda70-transgenic mice were protected against atherosclerotic lesion development despite the increase in IFNγ producing effector T cells. However, this transgenic model exhibits B-cell loss and exhaustion of the naïve T-cell pool, and in atherosclerosis, develops massive monocytosis. The authors explain the observed phenotype by the increased susceptibility of monocytes from CD70-transgenic mice to apoptosis [28]. CD27 and CD70 have also been identified on lymphocytes in human plaques, suggesting a role in atherogenesis [29].

OX40/OX40L

OX40, also known as CD134 and TNFRSF4, is transiently upregulated on activated CD4⁺ T cells, CD8⁺ T cells and memory T cells, and plays a role in sustained clonal expansion, cytokine secretion by effector T cells and homeostasis of effector and memory T cells [17*]. OX40 on regulatory T cells either directly inhibits their suppressive capacity or desensitizes effector T cells to the regulatory signal [65**]. As is true for many co-stimulatory molecules, expression of OX40 is not restricted to lymphocytes, but is also present on other cells. OX40 engages with OX40L on plaque-related cells such as mature APCs, smooth muscle cells and activated endothelial cells. OX40L can be expressed on activated T cells simultaneously with OX40, creating an amplification of the effector T cell responses. The intracellular signaling upon OX40 ligation has been partly elucidated in CD4⁺ T cells [17*]. Antigen recognition-independent activation depends on TRAF1, 2, 3 and 5-mediated IkBa degradation, and subsequent NFκB1 activation, hence migration of transcription factors p50 and RelA to the nucleus where it delivers signals for survival, proliferation and cytokine secretion, through NFATc1 activation [33,66]. Antigen recognition-independent activation is triggered by B cells and activated T cells. Moreover, in the antigen and TCR-dependent pathway, OX40 activates a synergizing pathway involving Akt [67].

Mice with mutation of the Ox40l gene developed smaller atherosclerotic lesions compared with control animals, whereas mice overexpressing the gene develop larger lesions, indicating an atherogenic role for OX40L [30]. Nakano et al. [31] recently demonstrated that Apoe⁻⁻/Ox40l⁻⁻ mice show reduced aortic atheroma compared with Apoe⁻⁻ mice because of OX40/OX40L-dependent
neovascularization in vasa vasorum. Moreover, similar to CD40L, the importance of vascular rather than bone marrow OX40L is shown by transplantation of OX40L+/− and wild-type bone marrow into Apoe−/− mice [31]. Ldlr−/− mice treated with an anti-OX40L antibody developed reduced atherosclerotic lesions because of inhibition of the interleukin-4-dependent T cell infiltration into the subendothelial space and therefore progression of atherosclerosis [71]. Studies to acute coronary syndrome patients showed elevated soluble CD137 levels in their circulation, as well as an increased expression of CD137 on peripheral monocytes [26,27].

**FUTURE PERSPECTIVES**

Besides lipid lowering, the importance of immune responses in atherosclerosis has emerged. The pivotal role of TNFRSF members in activation, differentiation and function of immune cells including lymphocytes allow modulation of the immune system in a highly refined manner. Targeting co-stimulatory molecules of the TNFRSF in atherosclerosis therefore will enable specific immune modulation of immune cells rather than commonly used systemic therapies, such as statins, β-blockers and lipid-lowering drugs. However, exact mechanisms of TNFRSF are still being unravelled.

So far, studies in humans and animal models confirm the immune modulatory role for several TNFRSF members in atherosclerosis. Especially CD40/CD40L, OX40/OX40L, CD27/CD70 and CD137/CD137L interactions have been studied as potential targets for immune modulation in vascular disease. Interestingly, each co-stimulatory dyad modulates the immune system during atherogenesis in its own way. CD40/CD40L predominantly affects macrophage and T cell recruitment, activation and polarization, with the CD40-TRAF6 pathway as the dominant pathway in atherosclerosis. OX40 augments T cell-mediated isotype switching, resulting in increased IgG1 and decreased IgM antibodies against oxLDL in hyperlipidemic conditions. The CD27/CD70 axis fine-tunes the optimal B and T-cell responses, and affects T helper cell development, whereas CD137 activation results in increased CD8+ cell infiltration in atherosclerotic plaques.

On the contrary, many key issues remain to be elucidated. In which stage of the disease the individual TNF(R)SF members exert their function, and how they interact with another, is not clear. The widespread expression of TNFRSF members beyond lymphocytes complicates clarification of the cell-type-specific TNFRSF members and their intercellular interactions in atherosclerosis. Studies in
cell-type-specific (conditional) knock-out animals are therefore eagerly awaited. Moreover, downstream signaling pathways have not been fully elucidated. Initial data regarding CD40-TRA2 signaling have shown that signaling pathways are highly dependent on cell type and type of disease.

On the contrary, once the cell and disease-type specific (inter)actions of the different TNFRSF members have been unravelled, and their signaling pathways have been elucidated, therapeutic applications in atherosclerosis will be feasible and have a high possibility of being successful. In addition, TNFRSF can be utilized as reliable biomarkers for risk estimation of cardiovascular events, as soluble factors correlate with cardiovascular risk.

CONCLUSION

In this review, we have described the diverse actions of the different co-stimulatory molecules in atherosclerosis. Co-stimulatory molecules have a great potential to become therapeutic targets to treat or halt the progression of cardiovascular diseases, as they are strong modulators of the immune system, a key feature they also show in atherosclerosis. By mediating the immune system toward a modulatory function by either blocking complete or cell-type specific co-stimulatory pathways, or signaling intermediates in the co-stimulatory pathways, atherosclerosis development or its progression could be halted. However, caution must be applied to prevent immunosuppressive complications.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

This article describes the role of the co-stimulatory CD30-CD40L dyad in atherosclerosis for the first time.


This is an excellent paper describing the link between the CD27-CD70 dyad and Tn17 responses.


43. This is an outstanding article on the link between Tregs and hyperlipidemia.


This is an excellent article on the link between the CD27-CD70 pathway and Treg development.


