ATVB Distinguished Scientist Award

How Costimulatory and Coinhibitory Pathways Shape Atherosclerosis

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Objective—Immune cells play a critical role in atherosclerosis. Costimulatory and coinhibitory molecules of the tumor necrosis factor receptor and CD28 immunoglobulin superfamilies not only shape T-cell and B-cell responses but also have a major effect on antigen-presenting cells and nonimmune cells.

Approach and Results—Pharmacological inhibition or activation of costimulatory and coinhibitory molecules and genetic deletion demonstrated their involvement in atherosclerosis. This review highlights recent advances in understanding how costimulatory and coinhibitory pathways shape the immune response in atherosclerosis.

Conclusions—Insights gained from costimulatory and coinhibitory molecule function in atherosclerosis may inform future therapeutic approaches.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2017;37:764-777. DOI: 10.1161/ATVBAHA.117.308611.)**

Key Words: antigen-presenting cells ■ atherosclerosis ■ B-lymphocytes ■ immunoglobulins ■ receptors, tumor necrosis factor

Brief Introduction to Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the artery wall. The resulting clinical events are the leading cause of death worldwide.[1] The factors contributing to atherosclerosis are multifaceted, encompassing environmental and genetic risk factors, perturbed cholesterol homeostasis, and a lingering immune response that all influence atherogenesis, plaque progression, vascular dysfunction, and ultimately plaque rupture or erosion, the proximal causes of major adverse cardiovascular events.[2–5] Dyslipidemia and endothelial dysfunction promote the increased influx and retention of lipoprotein particles including low-density lipoprotein (LDL).[6] Adhesion molecules are expressed by activated endothelial cells (EC) preferentially at sites of disturbed blood flow.[7,8] Furthermore, the modification of retained lipoproteins (eg, oxidation) can induce a low-grade immune response involving activation of ECs.[5,6] Adhering leukocytes, predominately inflammatory monocytes, transmigrate into the subendothelial space, and contribute to the proinflammatory micromilieu by secretion of chemokines that further increase recruitment of monocytes, neutrophils, and lymphocytes from the circulation.[9] Some monocytes differentiate into macrophages that scavenge the trapped lipoprotein particles and transform into foam cells. In addition to their recruitment, macrophages can also undergo local proliferation. Yet, their egress may be prevented by retention signals, thus also contributing to growth of the atherosclerotic lesion.[10,11] As the capacity to clear or store lipids is exceeded in these cells, they can undergo apoptosis. When the uptake of apoptotic cells (efferocytosis) fails these cells undergo secondary necrosis and form the acellular necrotic core of atherosclerotic lesions. Retained lipoprotein particles such as LDL undergo oxidation and other modifications. This makes them ligands for scavenger receptors like CD36, scavenger receptor-A, scavenger receptor-B, and toll-like receptors, which activate various proinflammatory signaling pathways that induce costimulatory molecules. Modified LDL presents lipid neoepitopes, the role of which in atherogenesis is poorly understood.[12,13]

See Insight Into Klaus Ley on page 778

Dendritic cells (DC) are key players in bridging innate and adaptive immunity. Lymph node and spleen DCs are most capable in presenting antigen to naive T cells. A network of vascular DCs is found in the arterial intima of healthy individuals and the frequency of DCs in arteries increases further during the course of atherosclerosis.[6,14] DCs can also take up lipids and contribute to foam cell formation.[15,16] Although foam cells seem not to leave the progressive atherosclerotic lesion, monocyte-derived DCs are able to leave atherosclerotic lesions in regression.[17] Most monocyte-derived cells in atherosclerotic lesions express high levels of major histocompatibility complex class II (MHC-II), which is required for presentation of peptide antigens to $CD4^+$ T cells. These mechanisms provide the basis for activation of T cells in atherosclerotic lesions and recall responses to both, model antigens and atherosclerosis antigens, which have

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Nonstandard Abbreviations and Acronyms

APC	antigen-presenting cell
CTLA-4	cytotoxic T-lymphocyte–associated protein 4
DC	dendritic cell
EC	endothelial cell
GITR	glucocorticoid-induced TNFR-related protein
HVEM	herpes virus entry mediator
IFNγ	interferon gamma
Ig	immunoglobulin
IL	interleukin
LDL	low-density lipoprotein
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
NK	natural killer
SR	scavenger receptor
TCR	T-cell receptor
TNFRSF	tumor necrosis receptor superfamily
TNFSF	tumor necrosis factor superfamily
Treg	regulatory T cell

been demonstrated in mouse arteries using multiphoton imaging.[18,19] B cells found in the adventitia also express MHC-II.[20,21] In general, B cells can process soluble and membrane-associated antigen after their antigenic activation via the B-cell receptor. In vivo imaging demonstrated CCR7-dependent migration of antigen-specific B cells[22] to the B-cell T-cell boundary in lymph nodes after antigen-encounter where these cells engaged in interactions with antigen-specific T cells for up to 60 minutes.[23] B cells are important antigen-presenting cells, but less able to present antigen to naive T cells than DCs.[24] The reconstitution of $\mu mt^{-/-}$ mice lacking B cells with B cells derived from $MhcII^{-/-}$ mice resulted in an impaired antigen-specific T-cell response, demonstrating that antigen presentation by B cells contributes to T-cell activation.[25] The B-cell–specific deletion of MHC-II did not alter numbers of T cells, but reduced the frequency of activated $CD4^+$ and $CD8^+$ T cells in a mouse model of lupus, which was accompanied by amelioration of disease and improved kidney function.[26] Similarly, mice were protected from experimental autoimmune encephalitis and displayed reduced Th1 and Th17 responses when B cells were devoid of MHC-II expression.[27] However, the role of antigen presentation by B cells in atherosclerosis is unknown.

Antibodies specific for plaque-restricted antigens such as oxidized LDL were detected in human atherosclerotic plaques.[13] Further antigens detected by antibodies in atherosclerosis are HSP60 and HSP65.[28] Lipid peroxidation-derived neoepitopes are found on the surface of oxidized LDL.[29] Spectratyping analysis of the T-cell receptor (TCR) repertoire in atherosclerotic lesions revealed a limited variety of TCRs, which is the hallmark of an oligoclonal T-cell response.[30] Unfortunately, TCR spectratyping allows no conclusions about the nature of the antigenic epitopes. The presence of oligoclonal T cells in atherosclerotic lesions indicates the presence of an adaptive immune response mounted against atherosclerosis-relevant antigens. Such a response usually requires the migration of antigen-presenting cells (APC) such as DCs carrying plaque-derived antigens to lymph nodes although direct proof for such activity in atherosclerosis is still missing. DCs can foster the development of atherosclerosis by modulating the differentiation of effector T cells.[18] Furthermore, ex vivo aorta cultures demonstrated substantial interactions between antigen-loaded DC and antigen-specific T cells within the aortic lesion.[19] Recent evidence suggests peptide moieties of $apoB_{100}$ as major atherosclerosis-specific antigens that are MHC-II restricted.[31–33] To produce an effective immune response, $CD4^+$ T cells must recognize antigens in the presence of costimulatory signals, which are the subject of this review.

$CD4^+$ T Cells in Atherosclerosis

Most lymphocytes in murine atherosclerotic lesions are $CD4^+$ T cells, whereas human atherosclerotic lesions have equal numbers of $CD4^+$ and $CD8^+$ T cells.[34,35] Many lesional $CD4^+$ T cells secrete interferon γ, and mice deficient for the Th1 lineage transcription factor T-bet or the Th1 cytokine interferon γ display reduced atherosclerotic development.[36,37] Many of these cells express both T-bet and low levels of the transcription factor Foxp3, suggesting that these cells may derive from regulatory T cells (Tregs).[38,39] The contribution of Th2 cells in atherogenesis is unclear. The key cytokines produced by Th2 cells are interleukin (IL)-4, IL-5, IL-10, and IL-13, all of which can contribute to atheroprotection.[40,41] However, depending on the atherosclerotic mouse model studied, IL-4 either enhanced or decreased atherosclerotic lesion formation.[40,42] Similarly, the role of Th17 cells in atherosclerosis is controversial, as some studies ascribe Th17 cells a proatherogenic function, whereas others suggest an atheroprotective role for IL-17, the hallmark cytokine of Th17 cells.[43–46]

Tregs, a subset of $CD4^+$ T cells, suppress the proliferation of effector $CD4^+$ T cells in response to antigen presentation. Tregs are subdivided into natural Tregs and induced Tregs. Natural Tregs are generated in the thymus by selection against self-antigens if the signal strength provided by the TCR is low to intermediate,[47,48] whereas induced Tregs are generated in response to transforming growth factor β in the periphery.[49] Tregs are potent antiatherogenic cells and exert their function by multiple effector mechanisms,[50] most prominently by secretion of the anti-inflammatory cytokines IL-10 and transforming growth factor β. Deficiency of these cytokines either systemically or in T cells was shown to be proatherogenic.[51,52] The adoptive transfer of Tregs ameliorated atherosclerosis,[53] whereas depletion of Tregs exacerbated atherosclerosis.[54] However, this was accompanied by altered liver lipid metabolism, which makes the data difficult to interpret.[54] In vitro, both differentiation of Th17 cells and Tregs from naive T cells require the cytokine transforming growth factor β. Atherosclerotic lesions of hyperlipidemic $Ldlr^{-/-}$ mice demonstrated a significant loss of Tregs at advanced stages of atherosclerosis.[55] Additionally, emerging data suggest that Tregs may convert to Th17 cells in mice and humans as atherosclerosis progresses.[38,39,56–58] Tregs in atherosclerotic lesions also can acquire a Th1-like phenotype associated with interferon γ production and loss of suppressive capacity.[31,32] However, more study is needed to provide conclusive evidence and underlying mechanisms of Treg plasticity in atherosclerosis.

Costimulatory Pathways in Atherosclerosis

A functional T-cell response requires not only recognition of MHC–antigen complexes via the antigen-specific TCR

but also the integration of costimulatory signals exceeding a certain threshold.[59] These signals are important during different stages of a T-cell response including clonal expansion, skewing toward T-cell effector phenotypes and enhancing T-cell survival in primary and secondary immune reactions. Two main families of costimulatory molecules are instrumental for these processes: the immunoglobulin (Ig)-like CD28 family and the tumor necrosis factor receptor superfamily (TNFRSF; Figure). In general, the expression and functionality of costimulatory and coinhibitory is conserved between mice and humans. Important examples of functional differences between species will be given below.[60]

CD28–CD80/CD86

CD28 on naive T cells binds to CD80 and CD86 on APCs. Although CD80 is constitutively expressed on many APCs, CD86 is strongly induced by inflammatory stimuli such as Toll-like receptor agonists derived from pathogens (eg, lipopolysaccharide).[61] Interactions of CD28 with CD80/CD86 are essential to induce a T-cell response including proliferation of effector cells and memory formation. Of note, almost all murine $CD4^+$ and $CD8^+$ T cells express CD28, whereas only 80% of human $CD4^+$ T cells and 50% of $CD8^+$ T cells express CD28.[62] The absence of CD28 stimulation during an antigen-specific stimulus renders T cells anergic, leading to irreversible unresponsiveness to their cognate antigen.[63] The activation of T cells occurs physiologically only when the T cell receives a TCR and costimulatory signal. Superagonistic CD28 antibodies were thought to have beneficial effects and advanced to clinical trials, but they caused a severe cytokine storm in healthy volunteers, probably by the activation of tissue memory $CD4^+$ T cells.[64] This cytotoxic effect was not apparent in rodents where a Treg response was favored after transient lymphocytosis.[65] The expression of CD80 and CD86 is increased on monocyte-derived DC of patients with cardiovascular disease.[66] Expression of CD80 and CD86 strongly correlates with lesional inflammation and plaque vulnerability.[67,68] Similarly, atherosclerotic lesions of hypercholesterolemic mice exhibited T cells, DC, and macrophages expressing CD28 and CD80/CD86, respectively.[69]

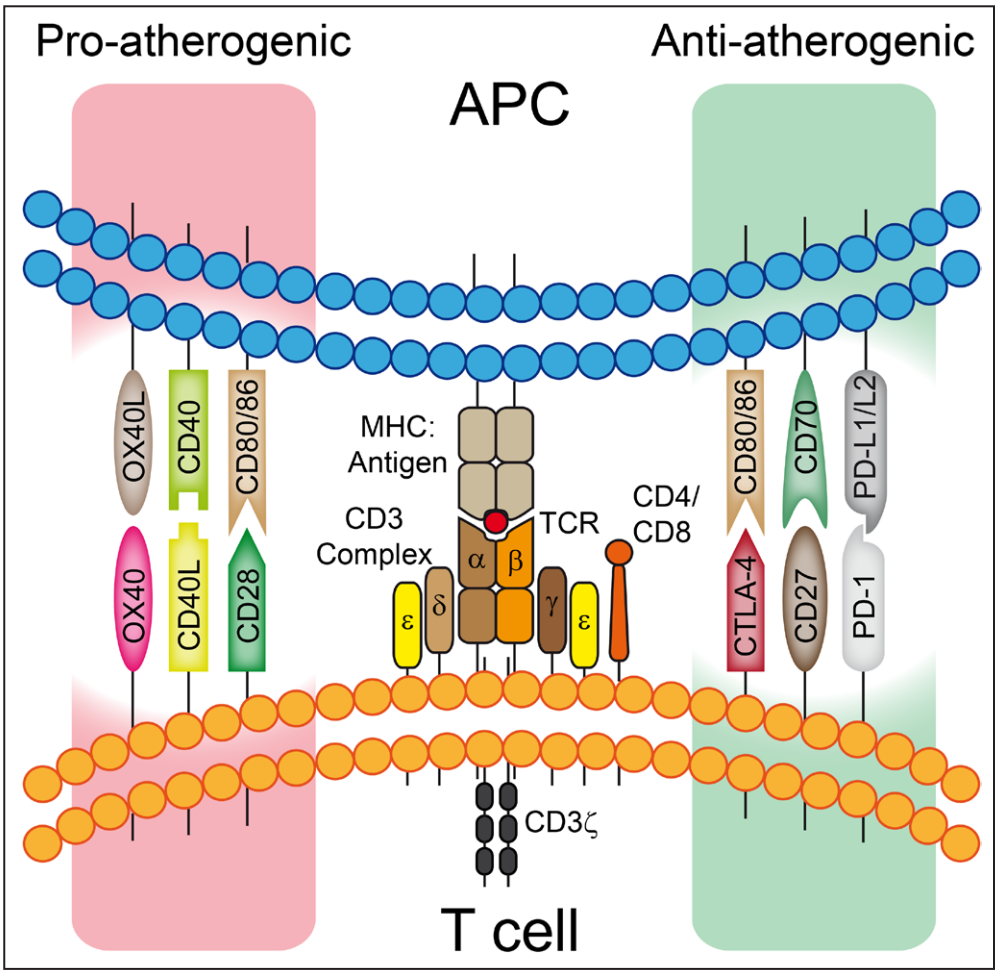

Figure. Antigen presenting cell (APC; top) interacting with a T cell (bottom). Antigenic peptide (red) is presented in the major histocompatibility complex (MHC; tan) to the T-cell receptor (TCR; α and β chains in shades of brown). The signal is transduced through the CD3 complex (γ, δ, ε, and ξ subunits). This can result either in T-cell activation, leading to differentiation to an effector T cell (Th1, Th2, Th17, TFH) or inhibition, leading to a regulatory T cell (Treg or Tr1), or tolerance. The accessory molecule (red) CD4 is required for interaction with MHC-II, CD8 for MHC-I. In general, activating costimulators tend to be proatherogenic (left; red rectangle), and inhibitory signals tend to be antiatherogenic (right; green rectangle).

Combined deficiency of CD80 and CD86 in atherosclerotic *Ldlr*$^{-/-}$ mice reduced atherosclerotic burden, which was accompanied by decreased abundance of MHC-II–expressing APC in atherosclerotic lesions and a reduced Th1 response.[69] However, lethally-irradiated *Ldlr*$^{-/-}$ mice transplanted with *Cd28*$^{-/-}$ or *Cd80*$^{-/-}$*Cd86*$^{-/-}$ bone marrow developed 2-fold larger lesions compared with control mice transplanted with wild-type bone marrow. This accelerated lesion development was likely caused by impaired Treg development and uncontrolled T-cell effector response.[53] Another costimulatory molecule of the CD28 superfamily is CD83. DC in human atherosclerotic lesions express CD83 and the content of CD83^{+} mature DC increased significantly in unstable plaques as compared with stable ones.[70,71] CD83 regulates B-cell activation and germinal center responses,[72] as well as CD4^{+} T-cell development.[73,74] Although expressed by activated APCs, the costimulatory function of CD83 seems to be dispensable for murine T-cell activation while CD83-stimulated human monocytes suppressed T cell responses.[75,76] However, the role and function of CD83 in cardiovascular disease is to date unidentified.

Tumor Necrosis Factor Superfamily and TNFRSF Members

CD40–CD40L

CD40 (TNFRSF5) expression was first discovered on APC, especially B cells.[77] However, a plethora of immune and nonimmune cells expresses CD40 with different functions exerted on activation by its ligand CD40L (CD154, tumor necrosis factor superfamily [TNFSF] 5).[78,79] The costimulatory CD40–CD40L axis is an important master regulator of immune processes. Among others, germinal center formation and especially Ig class switching in B cells is highly dependent on CD40L expression by T cells. DCs receiving CD40L-mediated signals are more potent in antigen presentation and inducing a T-cell response..[80–83] CD40 is mediating T-cell memory formation and induces the expression of inflammatory cytokines (TNFα, IL-1α, IL-1β, IL-6, IL-8, and IL-12), chemokines (CCL2, CCL3, and CCL5), and matrix-degrading enzymes by monocytes and macrophages.[84–88] CD40–CD40L interactions also drive the expression of costimulatory molecules such as CD80, CD86, and CD70. Moreover, CD40–CD40L promotes CD8^{+} T-cell activation even without the need for CD4^{+} T-cell help.[84,89]

A genetic polymorphism in the 5′ untranslated region of CD40 was enriched in a case–control study of Chinese patients with acute coronary syndrome and ischemic stroke.[90,91] Furthermore, the rs1535045-T allele of the CD40 locus positively correlated with cardiovascular disease and plasma cholesterol levels in a Chinese Han population.[92]

Pharmacological inhibition of CD40–CD40L interactions or global deficiency reduced atherosclerotic burden accompanied by stable lesion formation in murine models of atherosclerosis.[93–96] CD40L expressed by activated thrombocytes fosters recruitment of monocytes to the inflamed endothelium and is important for platelet–leukocyte aggregate formation, which can contribute to atheroprogression.[97,98] Furthermore, platelet CD40L expression reduced the abundance of atheroprotective Tregs, further contributing to inflammation.[98] CD40 expression by platelets sustained atherosclerosis by increasing adhesion molecule expression of EC and subsequent increased adhesion of leukocytes.[99]

Pharmacological inhibition of CD40–CD40L interactions is an attractive target, but clinical trials were discontinued because of severe thromboembolic complications as discussed below.

On a cautionary note, transplantation of bone marrow deficient for costimulatory or inflammatory molecules into *Ldlr*$^{-/-}$ mice often yields results opposing studies using the corresponding compound deficient mice or respective blocking antibodies.[53,69,93,100,101] In particular, lesion size between *Ldlr*$^{-/-}$ mice *Ldlr*$^{-/-}$ *Cd40*$^{-/-}$ mice was comparable,[102] whereas transplantation of *Cd40*$^{-/-}$ bone marrow into *Ldlr*$^{-/-}$ mice reduced lesion formation.[103] *Apoe*$^{-/-}$ mice deficient for CD40 also harbored smaller lesions as compared with *Apoe*$^{-/-}$ control mice, which is likely based on defective tumor necrosis factor receptor-associated factor 6 signaling.[103] The underlying cause for the observed discrepancies is not clear and might be based on the varying kinetics of atherosclerosis between the mouse models involved. *Apoe*$^{-/-}$ mice develop spontaneous atherosclerosis, whereas *Ldlr*$^{-/-}$ mice need to receive a cholesterol-enriched diet. The comparison of mouse models of atherosclerosis is beyond the scope of this review and has been reviewed elsewhere.[104,105]

CD27–CD70

The costimulatory molecules CD27 (TNFRSF7) and CD70 (TNFSF7) play important roles during the establishment of long-term T-cell immunity.[106] Naive T cell express CD27, which is increased after TCR engagement with cognate peptide MHC complexes. CD27 is lost from the cell surface by proteolytic shedding, but long-lived central memory cells express CD27 again.[107] Apart from T cells, CD27 is found on natural killer (NK) cells, activated B cells, and hematopoietic stem cells.[106,108] CD70, the ligand for CD27, is expressed on T cells, B cells, and activated DC.[109] Whereas antigen-stimulated T cells and B cells transiently increase CD70 expression, APCs in the intestine and medullary thymic epithelial cells constitutively express CD70.[110] The development of effector T cells seems independent of the presence of CD27, whereas thymic output of natural Tregs is reduced in *Cd27*$^{-/-}$ mice.[110,111] CD27 and CD70 interactions play an important role in mounting fulminant CD4^{+} and CD8^{+} T-cell responses including memory formation at effector and priming sites.[110,112]

Proper CD27/CD70 signaling is needed for B-cell proliferation and plays an important role during the process of Ig synthesis.[113] Insufficient CD70 triggering on B cells leads to an impaired germinal center formation, thereby affecting the humoral immune response.[114] However, B cells from *Cd27*$^{-/-}$ mice still undergo class switching and Ig maturation in aged mice, thus other factors contribute and compensate for CD27 defects that are only present during early phases. In contrast, human CD27^{+} B cells produced a higher amount of Ig, IL-10, and displayed enhanced survival.[115–117] In accordance, humans carrying mutations in the CD27 gene suffer from a severe immunodeficiency characterized by hypogammaglobulinemia, dysregulated lymphoproliferation, and increased

susceptibility for infections with Epstein–Barr virus.[118–120] Costimulation of CD70 via CD27 induced B cell proliferation but impaired terminal differentiation and Ig secretion of human and murine B cells although stimulation of CD70 via soluble CD27 resulted in increased Ig secretion.[113,121,122] Thus, soluble and membrane-bound CD27 interacting with CD70 exert species-specific effects and seem to contribute to a germinal center reaction in mice. In humans, CD27 promotes terminal B-cell differentiation and CD70 might downregulate humoral immunity.

Mice deficient for CD27 demonstrated a reduction in the proliferative capacity of antigen-specific T cells, which is not dependent on cell cycle entry.[123] CD27 acts antiapoptotic in various manners, thus contributing to T-cell survival and memory formation. T cells stimulated in vitro by CD27 signaling increase expression of the antiapoptotic molecule B-cell lymphoma-extra large.[124] Moreover, CD27 acts indirect on antigen-experienced $CD8^+$ T cells as deficiency for CD27 reduced production of the autocrine growth factor IL-2, thus limiting survival and proliferation of all T cells in nonlymphoid tissue.[125] In addition, the pharmacological inhibition of CD27/CD70 interactions with a blocking antibody increased FasL expression on $CD4^+$ T cells, which in turn induced apoptosis of virus-specific $CD8^+$ T cells.[126] Early work addressing the role of CD27 and CD70 on atherosclerosis demonstrated an atheroprotective role for stimulation of CD70.[127] Chronic CD70 overexpression in B cells continuously induced CD27 signaling on T cells, leading to a predominant, presumably proatherogenic Th1 response. However, this was accompanied by increased rate of apoptosis among proatherogenic $Ly6C^+$ monocytes, thus reducing macrophage abundance in atherosclerotic lesions. Furthermore, constitutive CD27/CD70 signaling led to immunopathology characterized by the conversion of naïve T cells into interferon γ–producing effector T cells leading to the progressive loss of B cells.[127,128] Thus, the model of B-cell–restricted CD70 overexpression is of limited use to determine the precise roles of CD27 and CD70 in atherosclerosis.

Macrophages are the main lesional CD70-expressing cells.[129] Macrophages deficient for CD70 displayed a unique phenotype characterized by enhanced M1 and M2 marker expression, yet were less viable and incompetent in mounting a proper inflammatory response.[129] Furthermore, *Cd70*$^{-/-}$ macrophages were less efficient in scavenging and cholesterol efflux, thereby contributing pathogenically to atherosclerosis progression. Accordingly, deficiency of CD70 resulted in exacerbated atherosclerosis.[129] Of note, CD70 and ApoE compound mutant mice were protected from hypertension and renal damage because of reduced accumulation of $CD4^+$ and $CD8^+$ effector memory T cells.[130]

GITR–GITRL

Tregs express significant levels of GITR (glucocorticoid-induced TNFR-related protein; TNFRSF18). Stimulation with its ligand (TNFSF18, GITRL) reduces their suppressive capacity.[131] However, naive T cells also express GITR at low levels, which is increased on activation.[132] Moreover, mast cells, APCs, NK cells, and granulocytes express GITR.[133] GITR expression on human immune cells is more restricted, and expression has been described in Tregs, NK cells, and macrophages.[134] The latter cells also demonstrated GITRL expression on Toll-like receptor signaling, whereas antigen recognition drives GITRL expression in T cells.[133] Functional differences between species-specific GITR–GITRL interactions have been reviewed elsewhere.[134] Furthermore, EC express GITRL, which is enhanced on lipopolysaccharide treatment, suggesting potential interaction of T cells and EC via this costimulatory axis during inflammation.[135,136] In human atherosclerotic lesions, plaque-resident macrophages demonstrate GITR and GITRL expression.[137] Interestingly, GITR stimulation of macrophages increased expression of matrix metalloproteinase (MMP)-9, which colocalized with GITR expression in atherosclerotic lesions, suggesting that GITR–GITRL interactions exert plaque destabilizing effects.[137] In mice, chronic GITR stimulation is atheroprotective.[138] *Ldlr*$^{-/-}$ mice transplanted with bone marrow from B-cell–restricted GITRL overexpressing mice showed enhanced thymic generation and lesional abundance of Tregs. Although deficiency for GITR is protective in murine models of asthma, experimental colitis, and collagen-induced arthritis, the exact role of GITR in atherosclerosis needs to be clarified.[139]

OX40–OX40L

OX40 (CD134/TNFRSRF4) and its ligand (OX40L/CD252/TNFSF4) are predominantly expressed on activated $CD4^+$ and $CD8^+$ T cells, whereas naive and resting memory T cells express neither OX40 nor OX40L.[140] Besides other immune cells such as neutrophils and NK cells express OX40 constitutively, whereas OX40L is inducible among others on APC, EC, and smooth muscle cells.[140] OX40 and OX40L expression is important for expansion of antigen-specific T cells to mount a functional T-cell response and to form memory.[141,142] OX40 also functions as a negative regulator of Tregs, thus further increasing the proinflammatory response.[143] In addition, OX40 interactions with OX40L drive B-cell activation and Ig production and play a role in macrophage activation.[144,145] Plaque-resident macrophages express OX40L in mice and humans, whereas lesional T cells were positive for OX40.[146,147] Of note, a single nucleotide polymorphism of the *OX40* gene in intron 5 was significantly associated with myocardial infarction.[148] Spontaneous mutations in the *Tnfsf4* locus of healthy C57BL/6 mice are associated with susceptibility for atherosclerosis.[146] The minor allele of the single nucleotid polymorphism rs3850641 of *TNFSF4* is associated with an increased risk for women to develop myocardioal infarction.[29] However, another group could not confirm associations between the *TNFSF4* genotype and an increased risk to develop carotid artery disease or stroke.[147] Genetic disruption or pharmacological inhibition of this costimulatory dyad attenuated atherosclerotic development and even caused regression of established atherosclerotic lesions, including a reduced neovascularization of the vasa vasorum.[29,149,150]

CD30–CD30L

CD30 (TNFRSF8) and CD30L (TNFSF8, CD153) are expressed by activated T cells and B cells. Furthermore, mature DC, macrophages, and mast cells demonstrate CD30L

expression.[151] Costimulation via CD30–CD30L induces T-cell proliferation and activation and is important for long-lived CD8+ T-cell memory formation.[152] The global absence of CD30 in mice reduced secondary humoral responses by an impaired induction of follicular germinal center responses accompanied by reduced antibody production.[153,154] Pharmacological CD30L blockage efficiently prevented development of spontaneous type I diabetes mellitus in nonobese diabetic mice.[80] Furthermore, CD30L blockage reduced atherosclerotic burden in *Ldlr*$^{-/-}$ mice presumably by reducing overall T-cell proliferation in the spleen and lymph nodes without affecting the humoral response.[155]

4-1BB–4-1BBL

The costimulatory molecule 4-1BB (TNFRSF9, CD137) is expressed on activated CD4+ T cells, CD8+ T cells, NK cells, resting monocytes, and DC, whereas its ligand is predominantly expressed on APC.[156] Mice deficient for 4-1BB have fewer NK and NKT cells, whereas T-cell development is not affected.[157,158] Furthermore, 4-1BB deficiency increased the number of myeloid progenitor cells and mature DCs, yet reduced the survival of the latter cell type.[159,160] Of note, although 4-1BBL reduced human NK cell activity in cocultures with tumor cell lines, it activated murine NK cells and led to enhanced killing activity.[161] In addition, *Tnfrsf9*$^{-/-}$ mice mounted a stronger antigen-specific T-cell response although DC functionality is impaired.[162] Presumably, these effects would contribute to progression of atherosclerosis. However, stimulation of 4-1BB with an agonistic antibody increased atherosclerotic burden accompanied by enhanced lesional inflammation whereas 4-1BB deficiency attenuates atherosclerosis in mice.[163,164] Advanced atherosclerotic lesions accumulated more macrophages and T cells when 4-1BB was lacking.[165] Lesions in these mice showed signs of vulnerability, accompanied by reduced smooth muscle cell survival and collagen production.[165] Interestingly, macrophage glucose metabolism is regulated by the interaction of 4-1BBL with its receptor, leading to increased metabolic activity and cell proliferation.[166] Thus, intervention in 4-1BB–4-BBL interactions might be a valuable therapeutic option in macrophage-driven diseases such as atherosclerosis.

LIGHT–Herpes Virus Entry Mediator

Herpes virus entry mediator (HVEM; TNFRSF14) is expressed by resting T cells and APCs, whereas its main ligand, LIGHT (TNFSF14), is expressed by activated T cells, monocytes, DCs, and NK cells.[167,168] HVEM can also interact with lymphotoxin-α, B and T lymphocyte attenuator, and CD160.[169] Interactions with LIGHT and lymphotoxin-α contribute to T-cell activation and cytokine production, whereas ligation of HVEM to B- and T-lymphocyte attenuator and CD160 promotes coinhibitory effects.[169] The contribution of HVEM and LIGHT to atherosclerosis is not fully understood. EC and macrophages in atherosclerotic lesions express HVEM and LIGHT, and both transcripts were elevated in aortas of atherosclerotic *Apoe*$^{-/-}$ mice.[170] HVEM signaling induced the production of MMP-1, MMP-9, and MMP-13 by monocytic cells in vitro and the staining of MMPs overlapped with HVEM in human atherosclerotic lesions, suggesting that HVEM signaling contributes to plaque destabilization and rupture.[171] Further proatherogenic features of this costimulatory axis involve the adhesion of platelets to EC and contribute to atheroprogression by guiding leukocyte adhesion to the inflamed endothelium.[172,173] Furthermore, LIGHT expressed by platelets induces signals in EC and monocytes that increase the expression of adhesion molecules and chemokines.[174] The increased expression of proatherogenic inflammatory mediators such as IL-8 and MCP-1 depends on a LIGHT-mediated induction of proteinase-activated receptor 2.[170] T-cell–restricted overexpression of LIGHT induced hyperlipidemia by a substantial reduction of hepatic lipase expression.[175] In the liver hepatic lipase is surface expressed and promotes uptake of lipoproteins containing cholesterol and triglycerides, hydrolyzing the latter. However, the mechanisms underlying how T-cell–mediated LIGHT expression alters liver metabolism is not understood.

Coinhibitory Pathways Shaping Atherosclerosis

PD-1–PD-L1/PD-L2

The CD28-superfamily also includes the coinhibitory molecule PD-1 (CD279), which binds to PD-L1 (CD274) and PD-L2 (CD273). Whereas PD-L1 expression is broadly found on APCs and tissue cells of nonhematopoietic origin, especially in the presence of innate inflammatory stimuli, PD-L2 expression is mainly restricted to APCs.[176] Furthermore, PD-L1 can be expressed in vitro by vascular smooth muscle cell and vascular EC in vitro and in vivo.[177–179] Interestingly, in vitro incubation of vascular ECs with oxidized LDL increased PD-L1 expression.[180] This led to a strong induction of anti-inflammatory cytokine production by cocultured Tregs displaying a potential atherosclerosis counterbalancing mechanism.[180] Furthermore, hypercholesterolemia promoted PD-L1 expression on splenic macrophages and DCs of *Ldlr*$^{-/-}$ mice.[181] On the contrary, circulating T cells and myeloid DC from patients with coronary artery disease demonstrated reduced PD-1 and PD-L1 expression compared to healthy individuals.[182] Deficiency of PD-1 or its ligands increased CD4+ and CD8+ T-cell activation and their influx into atherosclerotic lesions that accelerated atherosclerosis in *Ldlr*$^{-/-}$ mice.[181,183] Similarly, the administration of a PD-1–blocking antibody to *Ldlr*$^{-/-}$ mice exacerbated atherosclerosis and lesional T-cell infiltration.[183] The overall increased T-cell activation in atherosclerotic *Pd1*$^{-/-}$ mice did not favor a certain subtype, but enhanced abundance and response of pro- and antiatherogenic subsets, suggesting that the proinflammatory compartment outcompetes immunosuppression by Tregs.[184] PD-1 and Tim-3 expression defines highly exhausted CD8+ T cells. In vitro restimulation of PD-1+Tim-3+ CD8+ T cells isolated from human atherosclerotic lesions demonstrated skewing toward an anti-inflammatory cytokine profile, which was reverted by applying PD-1– and Tim-3–blocking antibodies, suggesting that these particular CD8+ T cells in lesions are of regulatory nature, whereas other reports attribute CD8+ T-cell proatherogenic function.[149,150,185] Monoclonal antibodies to PD1 and PD-L1 and PD-L2 are now widely used in immunotherapy of cancer patients.[186] It should be considered that these treatments may increase cardiovascular risk (Table).

Table. Expression Profile and Effects of Costimulatory Molecules in Atherosclerosis

Costimulatory Molecule		Expression on Resting Cells	Expression on Activated Cells	Effect on Atherosclerosis
Protein	Gene			
CD28	*Cd28*	Naive T cells, eosinophils, basophils, Treg	T cells	Unclear: increased atherosclerosis in *Ldlr*$^{-/-}$ mice transplanted with *Cd28*$^{-/-}$, *Cd80*$^{-/-}$, or *Cd86*$^{-/-}$ BM[53]
CD80	*Cd80*	++: APC	+++: APC	Reduced atherosclerosis in *Cd80*$^{-/-}$ *Cd86*$^{-/-}$ *Ldlr*$^{-/-}$ mice[69]
CD86	*Cd86*	+: APC	+++: APC	
CTLA-4/CD152	*Ctla4*	Tregs	Tregs, effector T cells	Clear: CTLA-4–blocking antibodies[189] increased atherosclerosis CTLA-4–Ig fusion protein[190] reduced atherosclerosis
CD40	*Tnfrsf5*	+++: B cells, SMC; ++: platelets; +: macrophages, neutrophils, EC	+++: T cells, APCs, platelets, neutrophils, EC	Clear: pharmacological inhibition[94–96] or global deficiency[93] reduced atherosclerosis
CD40L	*Tnfsf5*	++: B cells; +: macrophages, DC, neutrophils, EC, SMC	+++: T cells, B cells, macrophages, platelets; +: DC, EC, neutrophils	
CD27	*Tnfrsf7*	Naive T cells, NK cells, murine HSC	Human memory B cells, murine centroblasts, memory T cells	Unclear: CD70 deficiency[129] and overexpression[127] reduced atherosclerosis by limiting macrophage function or survival of Ly6Chi monocytes, respectively
CD70	*Tnfsf7*	MTEC, APC subset in lamina propria	APC	
GITR	*Tnfrsf18*	+++: Treg; +: naive CD4 T cells, Mast cells, APC	++: Macrophages	Clear: chronic GITRL overexpression is atheroprotective by Treg expansion[138]
GITRL	*Tnfsf18*	+: EC	+++: EC, T cells, APC	
OX40/CD134	*Tnfrsf4*	Neutrophils, NK cells	Effector CD4 and CD8 T cells	Clear: Genetic ablation[150] or pharmacological inhibition[149] attenuates atherosclerosis
OX40L/CD252	*Tnfsf4*	APC, EC, SMC	Effector CD4 and CD8 T cells, macrophages	
CD30	*Tnfrsf8*		Activated T and B cells	Insufficient data: pharmacological CD30L blockage reduced atherosclerosis[155]
CD30L	*Tnfsf8*	B cells, MTEC	Activated T and B cells, DC, macrophages, mast cells, granulocytes	
4-1BB/CD137	*Tnfrsf9*	Monocytes, DC, B cells, FDC, NK cells, granulocytes	Activated CD4 and CD8 T cells, EC	Clear: Agonistic 4-1BB stimulation[163] increased atherosclerosis, whereas genetic deficiency attenuated atherosclerosis.[164] Later stage lesions in 4-1BB KO mice show vulnerable lesions[165]
4-1BBL/CD137L	*Tnfsf9*		APC	
HVEM/CD270	*Tnfrsrf14*	T cells, APC	EC, macrophages	Insufficient data: Not fully understood, signaling induces proinflammatory mediator expression and altered liver metabolism[170,171]
LIGHT/CD258	*Tnfsf14*	Monocytes, NK cells, DC	T cells, EC, macrophages	
PD-1/CD279	*Pdcd1*	Myeloid DC, pro-B cells, Treg	Myeloid DC, T cells	Clear: Genetic deficiency of *Pd1* or *Pd-l1/Pd-l2*[181] or pharmacological inhibition of *Pd-1*[183] increased atherosclerosis in *Ldlr*$^{-/-}$ mice
PD-L1/CD274	*Pdcd1lg1*	+: vascular EC, vascular SMC	T cells, NK cells, macrophages, myeloid DC, B cell, epithelial cells, and vascular EC	
PD-L2/CD273	*Pdcd1lg2*	DC	DC	

+ indicates mild expression; ++, intermediate expression; +++, strong expression; APC, antigen-presenting cell; BM, bone marrow; CTLA, cytotoxic T-lymphocyte–associated protein 4; DC, dendritic cell; EC, endothelial cell; FDC, follicular DC; HSC, hematopoietic stem cell; KO, knockout; NK cell, natural killer cell; and SMC, smooth muscle cell.

CTLA-4–CD80/CD86

CTLA-4, CD152 (cytotoxic T-lymphocyte–associated protein 4) competes with CD28 for binding to CD80 and CD86. However, CTLA-4 decreases immune responses and functions as an immune checkpoint regulator. The clinical targeting of this molecule in tumor malignancies is discussed below. Effector T cells increase surface abundance of CTLA-4 after activation, whereas Tregs express CTLA-4 constitutively, which probably represents one of their main immunosuppressive effector mechanisms.[187] The impact of a genetic CTLA-4 deficiency on atherosclerosis is unknown because mice deficient for CTLA-4 succumb to an autoimmune lymphoproliferative disorder.[188] Application of CTLA-4–blocking antibodies resulted in a dramatic increase of atherosclerotic burden in hypercholesterolemic mice.[189] Conversely, the application of a CTLA-4-Ig fusion protein, which mimics CTLA-4 function, prevented CD80/CD86–CD28 interactions accompanied by reduced T-cell activation. Such treatment also resulted in limited neointima formation and reduced homocysteine-accelerated atherosclerosis.[189,190] In line with these results, transgenic mice constitutively expressing CTLA-4 on T cells were protected from atherosclerosis.[191] Similar to the effects of blocking PD1/PDL1/PDL2 in patients with cancer, CTLA4 blockade may trigger proatherosclerotic effects.

Potential Therapeutic Applications in Atherosclerosis

Targeting CD40/CD40L Interactions

The modulation of CD40/CD40L interactions was clinically tested in a variety of chronic inflammatory diseases and cancer.[78] However, pharmacological interference with antagonistic or agonistic antibodies triggered severe side effects. Patients having lupus glomerulonephritis experienced a marked reduction of hematuria when treated with a blocking anti-CD40L antibody.[155] However, this trial was prematurely discontinued as anti-CD40L antibody treatment caused thromboembolic events and myocardial infarctions, likely by the destabilization of platelet aggregates.[192,193] Also, agonistic or antagonistic anti-CD40 antibodies failed in diverse clinical disorders as the treatment was not efficient, or side effects including thrombocytopenia, neutropenia, and pleural effusion led to the discontinuation of trials.[194–196] An alternative therapeutic strategy harnesses interactions of CD40L with Mac-1, also known as CD11b/CD18 integrin, which is abundantly expressed on neutrophils, NK cells, monocytes, and macrophages.[197] The intraperitoneal application of a small peptide prevented interaction of CD40L with Mac-1 and reduced atherosclerotic burden in *Ldlr*$^{-/-}$ mice, potentially by reducing leukocyte recruitment to the inflammatory site.[198] Disrupting CD40–Mac-1 interactions did not prevent functional CD40–CD40L interactions and spared thrombotic events.[198] A different therapeutic approach targets the interaction of CD40 with its downstream signaling interaction partner tumor necrosis factor receptor-associated factor 6. Ablation of CD40–tumor necrosis factor receptor-associated factor 6 interactions led to more stable and smaller atherosclerotic lesions, accompanied by reduced monocyte influx into the arterial wall.[103] Furthermore, a small compound designed to prevent CD40–tumor necrosis factor receptor-associated factor 6 interactions increased survival of mice with induced sepsis and improved insulin resistance in obese mice.[199,200] However, the efficacy of this compound in atherosclerosis has yet to be tested.

Anti-CD27/CD70 Antibodies in Cancer and Cardiovascular Implications

Hematologic malignancies and solid tumors feature high CD70 expression.[201–205] The constitutive activation of effector T cells by persistent antigen and constitutive signaling via CD27/CD70 interaction leads to exhaustion, demonstrated in patients having B-cell non-Hodgkin lymphoma.[206] Exhausted T cells are less cytotoxic and incapable of attacking the tumor. The pharmacological inhibition of CD27/CD70 interaction by blocking-CD70 antibodies represents a promising therapeutic strategy in the treatment of such malignancies.[207,208] Furthermore, the opsonization of CD70 expressing tumor cells by these antibodies could induce antibody-dependent cellular cytotoxicity and phagocytosis, thus directly attacking the tumor cells and contributing to tumor regression. Alternatively, agonistic CD27 modulation by the CDX-1127 (varlilumab) antibody is under clinical evaluation in patients having B-cell malignancies, melanoma, and renal cell carcinoma.[209,210] Such agonistic CD27 stimulation successfully reactivated exhausted effector T cells, leading to a profound antitumor immune response and tumor regression in mice with a transgene expressing human CD27.[211] As CD70 deletion exacerbates atherosclerosis, potential side effects on the cardiovascular system by pharmacological modulation of CD27/CD70 interactions require consideration.[129]

Anti–CTLA-4 and Anti–PD-1 Antibodies in Oncotherapy and Their Potential Cardiovascular Effects

The coinhibitory molecules CTLA-4 and PD-1 regulate T-cell activation, however, in different ways. The blockage of both immune checkpoint mediators is desirable in advanced tumor malignancies because cytotoxic antitumor T cells are reactivated and not suppressed anymore. Dual blockage with the monoclonal antibodies ipilimumab and nivolumab, targeting CTLA-4 and PD-1, respectively, has been tested in clinical trials.[212] Recently, the Checkmate-67 phase III study demonstrated a 55% response rate when both antibodies were used to treat patients having advanced melanoma.[213] Furthermore, patients receiving treatment with both antibodies showed a median progression free survival of almost 12 months, 2 to 4 times higher than treatment with one or the other antibody. Data on the overall survival of the dual-treated patients are not yet published. Although an overall enhanced antitumor T-cell response is highly desirable, uncontrolled T-cell activity could exacerbate atherosclerosis and cardiovascular disease. Indeed, as pointed out above, pharmacological blockage or deficiency of CTLA-4 or PD-1 increased atherosclerotic burden. Overall, it has to be considered whether a patient having advanced tumor malignancies can gain expanded lifetime when undergoing immune checkpoint modulating therapy at the potential cost of accepting a higher risk for cardiovascular complications.

Open Questions and Future Directions

Past research has identified costimulatory and coinhibitory molecules as important modulators of immune response mediating effects on various cell types. Functional studies by genetic deletions or pharmacological manipulation have shown that these molecules significantly contribute to atherosclerosis (Figure). More research is needed to evaluate expression patterns of all these molecules on immune and nonimmune cells in health and disease, especially during atherosclerosis progression. In addition, not much is known about how immune cells integrate signals received by various costimulatory and coinhibitory pathways. Understanding the integration of costimulatory pathways in chronic inflammatory conditions such as atherosclerosis will help to develop new, tailored therapeutic approaches. The clinical success of blocking PD1, PD-L1, PD-L2, and CTLA-4 in cancer support the general feasibility of manipulating coinhibitory and costimulatory pathways. Whether such approaches will succeed in curbing major adverse cardiovascular events is the subject of ongoing and future studies.

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Disclosures

None.

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