ATVB Named Lecture Review

2016 Jeffrey M. Hoeg Award Lecture

Immune Checkpoints in Atherosclerosis: Toward Immunotherapy for Atheroprotection

Ellen Rouwet, Esther Lutgens

Abstract—Innate and adaptive immune effector mechanisms, in conjunction with hyperlipidemia, are important drivers of atherosclerosis. The interaction between the different immune cells and the secretion of cytokines and chemokines determine the progression of atherosclerosis. The activation or dampening of the immune response is tightly controlled by immune checkpoints. Costimulatory and coinhibitory immune checkpoints represent potential targets for immune modulatory therapies for atherosclerosis. This review will discuss the current knowledge on immune checkpoints in atherosclerosis and the clinical potential of immune checkpoint targeted therapy for atherosclerosis.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2018;38:1678-1688. DOI: 10.1161/ATVBAHA.118.307742.)**

Key Words: atherosclerosis ■ chemokines ■ cytokines ■ hyperlipidemia ■ mice

Atherosclerosis, the underlying cause of the majority of cardiovascular diseases (CVDs), is a lipid-driven, inflammatory disease of the large arteries. Both innate and adaptive immune effector mechanisms operate during atherogenesis. In conjunction with hyperlipidemia, the interaction between the different immune cells and the secretion of immune regulatory and activating cytokines and chemokines determine the progression of atherosclerosis.[1] The activation or dampening of the immune response is a delicate process that is tightly controlled by immune checkpoint proteins. This review will discuss the role of immune cell interactions in atherosclerosis and will highlight the current knowledge on how immune checkpoints are involved in this process. Furthermore, we will discuss clinical potential of immune checkpoint therapy for the treatment of atherosclerosis.

See Insight Into Esther Lutgens on page 1689

Immune Cell Interactions in Atherosclerosis

Atherosclerosis is initiated by the focal subendothelial accumulation of apoB (apolipoprotein B) containing lipoproteins in the intimal layer of the arterial wall, which trigger both a monocytes-macrophages dominated innate immune response and an adaptive immune response.[1–3]

In brief, (ox)LDL ([oxidized] low-density lipoprotein) is the main driver of the atherosclerotic process. Oxidation of LDL generates oxidation-specific epitopes that are recognized as damage-associated molecular patterns by pattern recognition receptors of the innate immune system, including scavenger receptors, Toll-like receptors, soluble pattern recognition receptors, and natural antibodies secreted by B-1 cells, that all cause macrophage activation and release of proatherogenic cytokines.[4]

Binding and internalization of oxLDL particles by pattern recognition receptors primes antigen-presenting cells (APCs) to initiate T-cell responses. The most important APCs for the activation of naive T cells are dendritic cells (DCs), whereas DCs, macrophages, and B cells serve as APCs for differentiated memory and effector T cells.[5]

After uptake of (ox)LDL, its apoB-derived peptides are presented on the APC cell surface in association with MHC (major histocompatibility complex) proteins.[6] After binding of the antigen-MHC complex to the T-cell receptor (TCR), naive T cells differentiate into various effector and regulatory subsets with distinct functions. Although not the most predominant cell type in the plaque, human and murine atherosclerotic lesions contain many T-cell subsets, including $CD4^+$, $FoxP3^+$, and $CD8^+$ T cells.[7,8]

On engagement of antigen-MHCII with the TCR, naive $CD4^+$ cells polarize into distinct subsets with characteristic cytokine profiles. T-helper 1 (Th1) cells which secrete proatherogenic cytokines such as IFN-γ (interferon γ) and TNF-α (tumor necrosis factor-α) are important drivers of atherosclerosis.[1] Other Th cell subtypes present in atherosclerosis are Th2 cells which secrete IL-4 (interleukin-4), IL-5, and IL-13, as well as Th17 cells with IL-17 and IL-22 as their signature cytokines. In contrast to the clear proatherogenic effects of Th1 cells, the impact of Th2 and Th17 cells in atherosclerosis is not clear.[7] Regulatory T cells, either derived from the thymus (natural Tregs [regulatory T cells]) or generated from naive $CD4^+$ cells (induced Tregs) exert immunoregulation through a variety of

Received on: February 21, 2018; final version accepted on: June 10, 2018.

From the Department of Surgery and Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands (E.R.); Department of Medical Biochemistry, Experimental Vascular Biology Laboratory, Academic Medical Center, Amsterdam, The Netherlands (E.L.); and Institute for Cardiovascular Prevention (IPEK), Ludwig Maximilian's University (LMU), Munich, Germany (E.L.).

The online-only Data Supplement is available with this article at https://www.ahajournals.org/journal/atvb/doi/suppl/10.1161/atvbaha.118.307742.

Correspondence to Esther Lutgens, MD, PhD, Department of Medical Biochemistry, Experimental Vascular Biology Laboratory, Academic Medical Center (AMC), Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands. E-mail E.Lutgens@amc.uva.nl

mechanisms, including suppression of T-cell proliferation and production of anti-inflammatory cytokines, such as IL-10 and TGF-β (transforming growth factor-β).[9] Tregs are especially present in early stages of atherosclerosis and decrease when lesions advance,[10] and exert strong atheroprotective effects.

On engagement of antigen-MHCI with the TCR, naive $CD8^+$ T cells differentiate into cytotoxic T cells, which have proatherogenic properties.[11]

Other lymphocyte subsets that promote atherosclerosis are $CD4^+CD28^{null}$ T cells,[12] natural killer T cells,[13] and B2 cells,[14] whereas the role of TCRγδ$^+$ T cells remains elusive.[15,16] Atheroprotective innate lymphocytes are innate lymphoid type II cells[17,18] and B1 cells.[14]

Immune Checkpoints

The activation or dampening of the immune response is a delicate process that is tightly controlled by immune checkpoint proteins. The family of immune checkpoint proteins, which mostly contains costimulatory and coinhibitory proteins of the B7 and TNF receptor families, was originally known to propagate or hamper T-cell proliferation and polarization after interaction between an APC and a T cell (signal 1). After recognition of its specific antigen via the TCR, signaling via costimulatory molecules induces T-cell activation (signal 2), which is further propagated via cytokines and chemokines (signal 3; Figure 1). Failure to costimulate results in T-cell anergy, where T cells proliferate, but do not fully mature and cannot mount a proper immune response, and can become tolerogenic.[19] In contrast to the immune enhancing effects of costimulatory receptors and ligands, coinhibitory immune checkpoint receptor-ligand pairs dampen the activation of T cells and are required to maintain tolerance to self-antigens.[20] The coinhibitory receptors on the T-cell surface bind ligands expressed on APCs and subsequently generate inhibitory signals that block T-cell activation. Some immune checkpoints such as CD27, inducible costimulatory molecule (ICOS), and CD40 play a role in Treg development.[21]

We now know that immune checkpoint proteins are not only expressed on T cells and APCs but also on a variety of other immune cells, such as neutrophils, macrophages, and mast cells, as well as nonimmune cells, including platelets, endothelial cells (ECs), vascular smooth muscle cells (VSMCs), adipocytes, hepatocytes, and epithelial cells. Immune checkpoints are also able to modulate inflammatory responses in these cell types.[22]

In the past decade, immune checkpoint modulation has yielded clinically successful cancer immunotherapy.[23] Not unexpected, immune checkpoint proteins also play an important role in the pathogenesis of atherosclerosis by driving or inhibiting multiple immunologic and inflammatory pathways in all stages of the disease.[24–26] Stimulatory and inhibitory immune checkpoints thus represent potential targets for immune modulatory and anti-inflammatory therapies for atherosclerosis.

Immune Checkpoints in Atherosclerosis

Concepts on the role of immune checkpoint regulation in atherogenesis are either inferred from hyperlipidemic mouse models, including LDL receptor-deficient ($LDLR^{-/-}$) or apoE-deficient ($ApoE^{-/-}$) mice, or—to a lesser extent—from observations in CVD patients or human atherosclerotic plaque specimens.

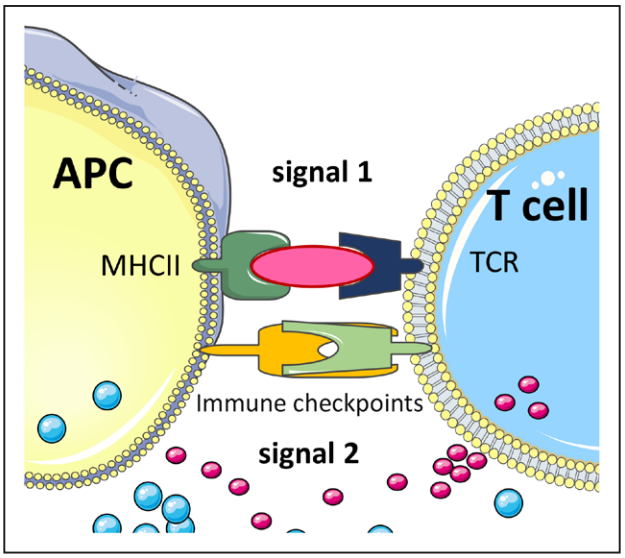

Figure 1. Antigen-presenting cell (APC)-T cell interactions in a nutshell. Signal 1: antigen presented by MHC (major histocompatibility complex) I (CD8 T cell) or MHCII (CD4 T cell) to the T-cell receptor (TCR) provides the first signal for T-cell activation. Signal 2: after activation by the TCR, T cells require secondary signaling via activating immune checkpoints (ie, costimulatory molecules). Inhibitory immune checkpoints, that is, coinhibitory molecules, can also exert inhibitory actions. Signal 3: the last signal that propagates immune responses is provided by chemokines and cytokines.

A plethora of immune checkpoints is involved in atherogenesis, each via their unique mechanisms as detailed below. The balance between the cell type or subset specific actions of these ligand-receptor pairs, for example, effector or regulatory T cell, macrophage, DC, or B cell within the network can determine whether the immune checkpoint protein promotes or limits atherosclerosis. In Table I in the online-only Data Supplement, the function of the immune checkpoint proteins discussed below on atherosclerosis-relevant cell types is indicated in detail.

Costimulatory Immune Checkpoints

CD28-CD80/86

The best-characterized costimulatory dyad of the B7 family is CD28-CD80/86. CD28 is constitutively expressed on naive T cells and on B cells, thymocytes, and macrophages and induces costimulation via interaction with its ligands CD80 (B7-1) and CD86 (B7-2) on APCs. CD80 and CD86 are also considered markers of classically activated macrophages and are present in human atherosclerotic plaques.[27] Hyperlipidemic mice deficient in both CD80 and CD86 ($CD80^{-/-}CD86^{-/-}LDLR^{-/-}$ mice) on a high-fat diet developed less atherosclerosis and the effector T cells in their spleen and lymph nodes produced less IFN-γ, suggesting that the CD28-CD80/86 dyad regulates atherosclerosis by priming T cells.[28] Pharmacological inhibition of CD28-CD80/CD86 interactions by the CTLA-4 (cytotoxic T-lymphocyte–associated protein 4) immunoglobulin fusion protein abatacept reduced atherosclerosis in $ApoE^{-/-}$ mice.[29] Likewise, genetic deficiency of CD80 and CD86 as well as systemic treatment with abatacept prevented intimal thickening in a mouse model for postinterventional remodeling.[30]

In line with this, human atherosclerotic plaques contained CD80/86$^+$ macrophages, and CD28$^+$ T cells, with higher expression levels of CD80 and CD86 in vulnerable compared with stable plaques.[31] Furthermore, ex vivo blocking of CD80 in human carotid endarterectomy specimens reduced cytokine secretion.[32] Patients experiencing coronary artery disease and those at risk of stroke displayed an increased expression of CD80 and CD86 on monocyte-derived DCs and B cells.[33,34] CD80 was recently identified as an imaging marker to detect vulnerable plaques, both ex vivo in human atherosclerotic plaques and in vivo in atherosclerotic arteries of ApoE$^{-/-}$ mice.[35,36] CD80-specific noninvasive imaging may thus be a promising tool to discriminate between stable and vulnerable atherosclerotic plaques and the CD28-CD80/86 dyad a potential therapeutic target for plaque stabilization.

ICOS-ICOSL

ICOS and its ligand ICOSL, members of the B7 family, have a dual role in atherosclerosis. LDLR$^{-/-}$ mice deficient in ICOS developed more atherosclerotic plaques containing more CD4$^+$ T cells and macrophages. CD4$^+$ T cells from ICOS-deficient mice proliferated more and secreted more proinflammatory cytokines, with a concomitant reduction in the number and suppressive function of Tregs. On the basis of these findings, it was suggested that ICOS modulates atherosclerosis through its effect on Treg responses.[37] ICOS is also important for regulation of proatherogenic B-cell responses, a process that involves follicular helper T cells, CD8$^+$ Tregs, and ICOS-ICOSL signaling. Blocking ICOS-ICOSL signaling reduced the development of atherosclerosis in ApoE$^{-/-}$ mice by restoring control of the follicular helper T-germinal center B-cell axis.[38] The role of the ICOS-ICOSL dyad in human atherosclerosis remains to be determined.

CD40-CD40L

CD40 ligand (CD40L, TNFSF5 [tumor necrosis factor super family 5], CD154) and its receptor CD40 (TNFRSF5 [tumor necrosis factor receptor super family 5]) is a well-characterized costimulatory ligand-receptor pair of the TNF(R) family that is involved in atherosclerosis.[24,39,40] CD40L is primarily induced on T cells and platelets after activation, whereas its receptor CD40 is constitutively expressed by DCs, macrophages, B cells, and by other cell types such as ECs, VSMCs, platelets, and epithelial cells. This costimulatory dyad generates cell-appropriate inflammatory responses and upregulates other costimulatory molecules such as CD80/86. CD40-CD40L interaction not only enhances T-cell stimulation and mediates T-cell help for cytotoxic T-cell priming but also induces B-cell activation and proliferation and is crucial for Ig isotype switching.[41,42] Genetic or antibody-mediated disruption of CD40L in ApoE$^{-/-}$ mice and LDLR$^{-/-}$ mice decreased atherosclerotic plaque burden and induced collagen-rich plaques containing few immune cells, that is, the murine equivalent of a stable plaque.[43–46] Likewise, inhibition of the CD40 receptor in ApoE$^{-/-}$ and LDLR$^{-/-}$ mice resulted in less atherosclerosis with a more stable plaque phenotype, which was attributed to hematopoietic CD40 inhibition.[47] Moreover, deficiency of CD40L prevented the occurrence of angiotensin II–induced aneurysm formation.[48]

Although the CD40L expressing T cell is an important driver of atherosclerosis, the results of T-cell–specific deletion or overexpression of CD40L are yet awaited. CD40L is also highly expressed on platelets, and platelet CD40L is also involved in the initiation and progression of atherosclerosis, independent of its role in platelet aggregation.[49,50] CD40L-deficient platelets halted the platelet-induced aggravation of atherosclerosis by hampering platelet-leukocyte aggregation and leukocyte recruitment to the endothelium.[51] Similarly, ApoE$^{-/-}$ mice receiving CD40-deficient platelets displayed reduced atherosclerosis with less advanced plaques in a model of platelet-induced aggravated atherosclerosis.[52] A recent study showed that activation of CD40 in DCs indirectly attenuated atherosclerosis, by impairing lipid uptake resulting from inflammatory bowel disease.[53] CD40 is also expressed by classically activated (M1) macrophages, and stimulation of CD40 on cultured monocytes and macrophages induced the release of proinflammatory mediators and matrix metalloproteinases.[54] In contrast, CD40-deficient mice exhibited reduced macrophage activation in atherosclerotic plaques.[47] Further roles for the CD40-CD40L dyad in the innate immune system remain to be elucidated.

In human atherosclerotic plaques, CD40 and CD40L are expressed on a wide variety of cell types, including T cells, DCs, B cells, ECs, and VSMCs.[55] The expression of the CD40L-CD40 dyad was associated with features of plaque vulnerability. The soluble form of CD40L, sCD40L (soluble CD40 ligand), which is cleaved predominantly from platelets after activation, is a useful biomarker for CVD severity. Elevated sCD40L levels are associated with hypercholesterolemia, stroke, diabetes mellitus, and acute coronary syndrome and can predict recurrent CVD.[56–60]

CD40-TRAF Signaling

CD40 has limited direct signaling capacity and needs TRAFs (TNF receptor–associated factors) to propel its signaling. CD40 has 2 distinct binding sites for TRAFs: a proximal site for TRAF6 and a distal site for TRAF1/2/3/5 binding, which enables CD40 to activate different signaling pathways.[40] TRAF1 acts as a regulator rather than an activator of CD40 signaling. Multiple cell-type–specific CD40-TRAF signal transduction cascades are involved in atherosclerosis, including IKK/NFκB (inhibitor of kappa B kinase/nuclear factor kappa B), STAT3 (signal transducer and activation of transcription 3), PI3K/Akt (phosphatidyl inositol 3 kinase/Ak thymoma), p38/MAPK (phospo 38/mitogen-activated protein kinase), ERK1/2 (extracellular signal-regulated kinase 1/2), and JNK (c-Jun N-terminal kinase) pathways.[40] CD40-TRAF signaling drives expression of cytokines, chemokines, adhesion molecules, other costimulatory molecules, matrix metalloproteinases, immunoglobulin class switching, and cell survival. The activation and functional consequences of a specific signaling cascade depend on the cell type involved. For example, in monocytes and macrophages, CD40-TRAF6 interaction, but not CD40-TRAF2/3/5, activated NFκB proinflammatory pathways, whereas CD40-TRAF2 signaling mediated activation of proinflammatory pathways in ECs and SMCs.[61]

In hyperlipidemic mice with site-directed mutagenesis at the CD40-TRAF6 or CD40-TRAF2/3/5 binding site in MHCII$^+$ cells, CD40-TRAF6, but not CD40-TRAF2/3/5 signaling, was proatherogenic.[47,62] Mice lacking CD40-TRAF6

interactions exhibited impaired arterial leukocyte recruitment and hardly contained plaque macrophages. The majority of CD40-TRAF6$^{-/-}$ monocytes in blood and spleen were of the patrolling Ly6C^{low} subtype, and CD40-TRAF6$^{-/-}$ macrophages were anti-inflammatory.[47] These data stress the importance of macrophage CD40-TRAF6 signaling in atherosclerosis.

OX40-OX40L

OX40 (TNFRSF4, CD134) is primarily expressed on activated CD4$^+$ and CD8$^+$ T cells, whereas its ligand OX40L (TNFSF4, CD252) is expressed by DCs, macrophages, ECs, mast cells, and B cells. OX40-OX40L interactions promote sustained T-cell responses by enhancing the survival and expansion of effector and memory T-cell populations as well as isotype switching of B cells. Interruption of the OX40-OX40L signaling pathway in C57Bl6 or LDLR$^{-/-}$ mice attenuated atherogenesis and induced regression of atherosclerotic lesions, in part by increasing the levels of protective anti-oxLDL IgM antibodies and lowering Th2 responses.[63–65] Moreover, the less common allele of SNP rs3850641 of Ox40 was associated with myocardial infarction.[65]

Patients with an acute coronary syndrome had increased expression of OX40L in coronary plaques, increased expression of OX40 and OX40L in circulating T cells, and higher serum levels of soluble OX40L compared with those with stable angina.[66,67] OX40L was also expressed by macrophages in carotid endarterectomy plaques.[68]

CD30-CD30L

CD30 (TNFRSF8) and its ligand CD30L (TNFSF8, CD153) are expressed on T and B cells. Interruption of CD30-CD30L signaling in LDLR$^{-/-}$ mice inhibited T-cell proliferation and activation and reduced atherosclerosis.[69] Except for the identification of CD30$^+$ macrophages and T cells in coronary plaques, the role of this costimulatory dyad in human atherosclerosis is unknown.[70]

CD137-CD137L

CD137 (TNFRSF9, 4-1BB) is expressed on activated CD4$^+$ and CD8$^+$ T cells, natural killer cells, monocytes, and DCs, whereas its ligand CD137L (TNFSF9, 4-1BBL) is expressed on monocytes, macrophages, DCs, and activated B cells. Activation of CD137 increased T-cell infiltration and proinflammatory cytokine release in atherosclerotic lesions of hyperlipidemic mice and promoted leukocyte recruitment by inducing adhesion molecule expression on ECs.[71,72] Conversely, CD137 deficiency was associated with reduced proinflammatory cytokine production and a reduction in atherosclerosis. CD137 signaling also induced plaque instability with concurrent plaque necrosis, decreased collagen content, increased VSMC apoptosis, and increased macrophage and effector T-cell infiltration.[73] CD137 is expressed in human atherosclerotic lesions on CD8$^+$ T cells and ECs.[71] Elevated plasma levels and monocyte-associated expression of CD137 and CD137L were detected in patients with acute stroke or acute coronary syndrome.[74,75]

GITR-GITRL

GITR (glucocorticoid-induced TNFRSF18 [TNF receptor family related protein]) is highly expressed on Tregs but also on CD4$^+$ effector memory T cells as well as on several other immune and nonimmune cells. GITR ligand (GITRL, TNFSF18) is expressed on ECs and APCs. In LDLR$^{-/-}$ mice, activation of GITR reduced atherosclerosis by limiting inflammation through increasing the number of Tregs in the vasculature and in lymphoid organs. These data suggested that the GITR-GITRL dyad is a costimulatory signal that limits atherosclerosis by enhancing Treg responses.[76] In contrast, in human atherosclerosis, GITR and GITRL were mainly expressed on plaque macrophages, and GITR-GITRL interaction stimulated the production of the proinflammatory cytokine TNF-α and the matrix-degrading enzyme MMP-9 (matrix metalloproteinase-9).[77] Thus, the exact role of the GITR-GITRL dyad is not clear yet.

CD27-CD70

CD27 (TNFRSF7) is found on naive T cells, B cells, and natural killer cells, whereas its ligand CD70 (TNFSF7) is expressed on activated T cells, B cells, macrophages, and DCs. CD27-CD70 interactions enhance the expansion of effector and memory T cells and are indispensable for Treg development. Deficiency of either CD70 or CD27 resulted in an impairment of Treg development in experimental models of atherosclerosis. However, the mechanisms how CD70 and CD27 drive atherogenesis differed between the 2 models. CD70-deficient ApoE$^{-/-}$ mice displayed larger plaques with a more advanced plaque phenotype characterized by an increased necrotic core. Lack of CD70 impaired the inflammatory capacity and cholesterol in- and efflux capacity of macrophages and rendered macrophages metabolically inactive and prone to apoptosis, resulting in increased necrotic core formation and progression of atherosclerosis. The amount of aortic Treg levels, as well as the total number of T cells and Tregs in the plaque were not affected, suggesting that although CD70-deficient mice have a reduction in Tregs, CD70 predominantly mediates its effects via macrophages.[78] CD27-deficient ApoE$^{-/-}$ mice also showed exacerbated lesion development and increased inflammation, which was associated with a decrease in Tregs.[79] Thymic Tregs of CD27$^{-/-}$ApoE$^{-/-}$ mice were more prone to apoptosis, resulting in a decrease amount of Tregs in atherosclerotic plaques and the aorta. Consequently, CD27$^{-/-}$ApoE$^{-/-}$ mice had increased aortic inflammation and more atherosclerosis.[79] Patients with an acute myocardial infarction displayed a reduced expression of CD27 on circulating mononuclear cells.[80] Conversely, CD70 and CD27 expression were higher in ruptured than in stable human carotid artery plaques, most likely reflecting a compensatory immune regulatory response to plaque rupture.[78,79]

Herpes Virus Entry Mediator/LTbR-LIGHT

The costimulatory lymphotoxin-related inducible ligand LIGHT (TNF superfamily member 14 [TNFSF14]) and its 2 receptors herpes virus entry mediator (TNFRSF14, HVEM [herpes virus entry mediator]) and lymphotoxin β-receptor (LTbR [lymphotoxin beta receptor], TNFRSF3) are expressed by a multitude of immune and nonimmune cells. LIGHT herpes virus entry mediator interaction promotes atherogenesis and plaque instability, by inducing proatherogenic cytokines and matrix metalloproteinase activity.[81] LTbR-deficient ApoE$^{-/-}$ mice exhibited lower plaque burden with reduced

plaque macrophage content and inflammation.[82] Inhibition of LIGHT-LTbR interactions attenuated dyslipidemia in LDLR$^{-/-}$ mice.[83] Patients with atherosclerosis displayed high plasma levels of LIGHT, associated with inflammation and lipid accumulation in macrophages.[84,85–88] High expression levels of herpes virus entry mediator have been detected in human carotid plaques, mainly in relation to macrophages.[81] Patients with atherosclerosis displayed upregulation of the LIGHT-LTbR axis.[86–88]

Coinhibitory Immune Checkpoints

CTLA-4-CD80/86

The coinhibitory receptor CTLA-4 (CD152) is primarily expressed on activated T cells and negatively regulates the CD80/86-CD28 pathway. Interaction of the ligands CD80 and CD86 on APCs with CTLA-4 limits effector T-cell activation and induces Treg responses, resulting in reduced inflammation. CTLA-4 is also constitutively expressed on Tregs and is required for exerting their suppressive functions. Moreover, Tregs require CTLA-4 to downregulate CD80/86 expression on APCs, thereby impairing antigen presentation and activation of immune responses.[89]

Overexpression of CTLA-4 by T cells in ApoE$^{-/-}$ mice reduced atherosclerotic lesion formation. This was associated with decreased numbers of effector T cells, decreased production of proinflammatory cytokines, and decreased expression of the costimulatory molecules CD80 and CD86 on APCs.[90] Conversely, antibody-mediated inhibition of CTLA-4-CD80/86 interaction with CTLA-4 blocking antibodies in ApoE3Leiden mice resulted in a vast increase in atherosclerosis.[30] Interestingly, the CTLA-4-CD80/86 axis initiates the immune-suppressive tryptophan catabolism in DCs, resulting in increased expression of indoleamine-2,3-dioxygenase in DCs.[91,92] In atherosclerosis, it could well be that CTLA-4 mediates its protective effects partly via indoleamine-2,3-dioxygenase. Inhibition of indoleamine-2,3-dioxygenase has been shown to result in increased atherosclerosis by increasing macrophage, VSMC activation, and EC activation.[93] The role of the CTLA-4-CD80/86 inhibitory checkpoint in human atherosclerosis remains to be resolved.

PD-1-PD-L1/2

The other well-defined coinhibitory dyad is the PD-1 (programmed cell death 1, CD279) receptor which binds PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273). Both PD-1 and PD-L1/2 are expressed on several cell types, including T cells, B cells, DCs, monocytes, and macrophages. Binding of PD-L1/2 to PD-1 inhibits T-cell activation. Deficiency of the ligands PD-L1 and PD-L2 increased the atherosclerotic burden in hypercholesterolemic LDLR$^{-/-}$ mice, which was associated with increased numbers of activated CD4^{+} T cells, higher serum levels of the proinflammatory cytokine TNF-α, and increased responsiveness of T cells to APCs.[94] Similarly, LDLR$^{-/-}$ mice deficient in the receptor PD-1 or treated with a blocking anti-PD1 antibody displayed larger atherosclerotic lesions with more CD4^{+} and CD8^{+} T cells and macrophages, higher circulating TNF-α levels, and higher cytotoxic activity of CD8^{+} T cells.[95] Although PD-1 deficiency was also associated with expansion of antiatherogenic Treg cells, proatherogenic T-cell activation prevailed.[96] These experimental findings indicate that the PD-1-PD-L1/2 pathway exerts an atheroprotective effect by suppressing APC-dependent T-cell activation. Patients with coronary atherosclerosis exhibited decreased expression of PD-1 and PD-L1 on circulating T cells and myeloid DCs.[97] Furthermore, patients with an acute coronary syndrome had a lower expression of PD-L1 on peripheral blood Tregs when compared with those with stable coronary artery disease.[98]

TIGIT

Binding of the coinhibitory molecule TIGIT (T-cell immunoglobulin and ITIM domain) with its ligands CD112, CD113, or CD155 inhibits T-cell proliferation and activation. In hypercholesterolemic LDLR$^{-/-}$ mice, stimulation of TIGIT limited proatherogenic T cell responses, but did not affect atherosclerotic lesion development.[99]

TIM Proteins

The TIM (T-cell immunoglobulin and mucin domain) proteins are expressed by numerous immune cells, recognize apoptotic, phosphatidylserine expressing cells, and function both as costimulators and coinhibitors.[100] TIM-1 exerts coinhibitory functions on Th2 cells, and was recently associated with carotid atherosclerosis in humans.[87] Antibody-mediated blocking of TIM-1 in LDLR$^{-/-}$ mice resulted in enhanced Th2 responses and a decreased number of circulating Tregs, thereby aggravating atherosclerosis.[101] Inhibition of TIM-3, a coinhibitor, but also an activator in innate immune responses increased the number of circulating monocytes and plaque macrophages as well as activated CD4^{+} T cells, while decreasing Tregs, and increased atherosclerosis in LDLR$^{-/-}$ mice.[102] Inhibition of TIM-4, which is expressed as coinhibitor on DCs and immune modulator on marginal zone macrophages increased Th1 and Th2 responses, reduced the clearance of apoptotic cells and also promoted atherosclerosis in mice.[101] An SNP in TIMD4, the gene encoding for TIM-4 is associated with protection against CVD.[103,104]

LAG3

LAG3 (lymphocyte activation gene 3, CD223) is an inhibitory immune checkpoint protein that is in clinical development as an anticancer drug and considered very potent.[105] Although mechanistic data on LAG3 on the development of atherosclerosis are lacking, decreased LAG3 protein levels are associated with increased CVD risk, suggesting a role for LAG3 in atherosclerosis.[106]

Immunotherapy for Atherosclerosis

In spite of clinical and experimental data supporting a critical role for lipid-driven inflammation in atherosclerosis, pharmaceutical interventions to slow the progression of atherosclerosis in patients thus far predominantly focused on reducing lipid levels. Only recently, the first trial that treated patients with coronary artery disease with an anti-inflammatory drug was published, the CANTOS trial (Canakinumab Anti-inflammatory Thrombosis Outcome Study).[107] In CANTOS,

patients with a history of myocardial infarction and an elevated level of high-sensitivity C-reactive protein were randomly assigned to a human monoclonal antibody targeting IL-1β, canakinumab, or to placebo. IL-1β inhibition reduced high-sensitivity C-reactive protein levels from baseline, when compared with placebo, and lowered the incidence of recurrent cardiovascular events. CANTOS is the first study in humans showing that anti-inflammatory therapy lowers the rate of cardiovascular events, independent of lipid levels.

Ongoing clinical trials on anti-inflammatory drugs in atherosclerosis include the CIRT (Cardiovascular Inflammation Reduction Trial), which investigates whether inhibition of nonspecific T-cell inflammation with methotrexate will reduce further cardiovascular events in patients with coronary artery disease and diabetes mellitus or metabolic syndrome.[108] The CANTOS study has helped moving the inflammatory hypothesis of atherosclerosis forward. However, despite the beneficial effects of IL-1β inhibition on cardiovascular outcomes, the incidence of fatal infection was almost 2-fold higher in the canakinumab than in the placebo group. This underlines the need to find other, better tailored anti-inflammatory agents that do not increase the risk of death from infection.[107]

On the basis of experimental data, inhibition of costimulatory molecules or stimulation of coinhibitory molecules are alternative anti-inflammatory interventions that might serve as targets for atheroprotection. A vast amount of knowledge on the role of immune checkpoint therapy is derived from cancer research because malignant neoplastic growth and metastasis require immunosuppression. Coinhibitory receptors are highly expressed in tumors and interactions with their ligands within the tumor microenvironment are crucial to the inhibition of antitumor immune responses. In cancer immunotherapy, antibody-mediated blockade of immune checkpoint inhibitors strongly promotes immune-mediated tumor destruction. Currently, 6 immune checkpoint inhibitors are approved by the Food and Drug Administration for the treatment (of advanced stages) of several malignancies (https://www.fda.gov). Ipilimumab, an anti–CTLA-4 antibody, is approved for treatment of melanoma as well as renal cell carcinoma. Pembrolizumab and/or nivolumab, both anti-PD1 antibodies, are approved for treatment of melanoma, nonsmall cell lung cancer, metastatic nonsquamous cell lung cancer, head and neck squamous cell carcinoma, refractory classical Hodgkin lymphoma, metastatic urothelial cell carcinoma, metastatic gastric and gastrointestinal junction adenocarcinoma, renal cell carcinoma, and hepatocellular carcinoma. The 3 anti-PDL1 antibodies Atezolizumab, Avelumab, and Durvalumab are indicated for treatment of urothelial cell carcinoma, nonsmall cell lung cancer, and/or metastatic Merkel cell carcinoma. The list of FDA approved immune checkpoint inhibitors, as well as treatment indications, and therapy regimens, including combination therapy, is expected to grow exponentially during the next years.[23,109]

Although immune checkpoint inhibitors have substantially improved the clinical outcomes of numerous cancers, the release of restrained antitumor immune responses is associated with immune-related adverse events. The risk of immune-related adverse events for treatment with anti–CTLA-4, anti-PD1, or anti–PD-L1 antibodies is 70% to 90% and is even higher with combination regimens. The immune-related adverse events occur as a result of immune activation and consequent autoimmune-like diseases in different organ systems, including dermatitis, colitis, hepatitis, pneumonitis, and endocrine disorders.[110] The adverse cardiovascular effects of these newly emerging targeted anticancer drugs are unclear.[111] The incidence of immune-related cardiac toxicity is rare, with reported cardiac events in <1% patients, but is probably underreported, and will increase as the list of therapeutic indications for these agents keeps growing. Immune myocarditis and associated heart failure or conduction abnormalities are potentially fatal.[112–114] In terms of vascular side effects, patients treated with anti–CTLA-4 antibodies have been reported to develop giant cell arteritis.[115] Furthermore, inefficiency of the inhibitory PD-1-PD-L1 immune checkpoint was recently associated with inflammatory vessel disease.[116] As long-term survival of cancer patients treated with checkpoint inhibitors becomes more common, chronic manifestation on atherosclerosis may become more evident.

In contrast to the paradigm shift in oncology where new therapeutic agents target immune cells rather than cancer cells, and the promising clinical benefits of manipulating T cell cosignaling to induce tolerance in the fields of rheumatoid arthritis and transplantation, immune checkpoint modulation for the treatment of CVD is still in its infancy.[117,118] Although modulation of immune checkpoints has the potential to limit malignant and inflammatory diseases, the prominent role of these proteins in tumorigenesis, infection, autoimmunity, and atherosclerosis requires balanced and highly specific immunomodulation. Immune checkpoint therapy may induce off-target effects, of which immune suppression is the highest risk. However, short-term treatment with costimulatory antagonists or coinhibitory agonists on top of conventional therapy might be able to prevent atherosclerotic plaque rupture or reduce the increase inflammatory burden that is often seen after an acute cardiovascular event.[1]

As coinhibitory proteins are key in preventing autoimmune disease, combatting atherosclerosis, which shows features of autoimmune disease, by coinhibitory agonists has potential beneficial effects. Experimental studies in hyperlipidemic mice have already proven a beneficial role for CTLA-4 overexpression in limiting atherosclerotic disease,[90] and agonistic antibodies against the immune checkpoint TIM-1, a strong inducer of immune tolerance,[100] would also have high potential to reduce atherosclerosis. However, data on the role of agonizing coinhibitory immune checkpoints in CVD are only limited, and additional studies, including monitoring of potential side effects such as tumorigenesis, and the potential of combination therapies, need to be performed.

As studies in hyperlipidemic mice have shown, blocking costimulation strongly reduces atherosclerosis. However, one of the risks of long-term treatment is immune suppression. To circumvent this problem, we have designed small molecule inhibitors that selectively block the interaction between CD40 and TRAF6 (TRAF-STOPs), without affecting CD40-TRAF2/3/5 interactions, thereby keeping CD40-mediated immunity such as Ig-isotype switching and T-cell proliferation intact.[119–121] Blocking CD40-TRAF6 interactions by small molecule inhibitor 6860766 or 6877002 reduced the accumulation of $CD4^+$ and $CD8^+$ T cells and macrophages in adipose tissue, decreased hepatosteatosis, and improved insulin sensitivity in

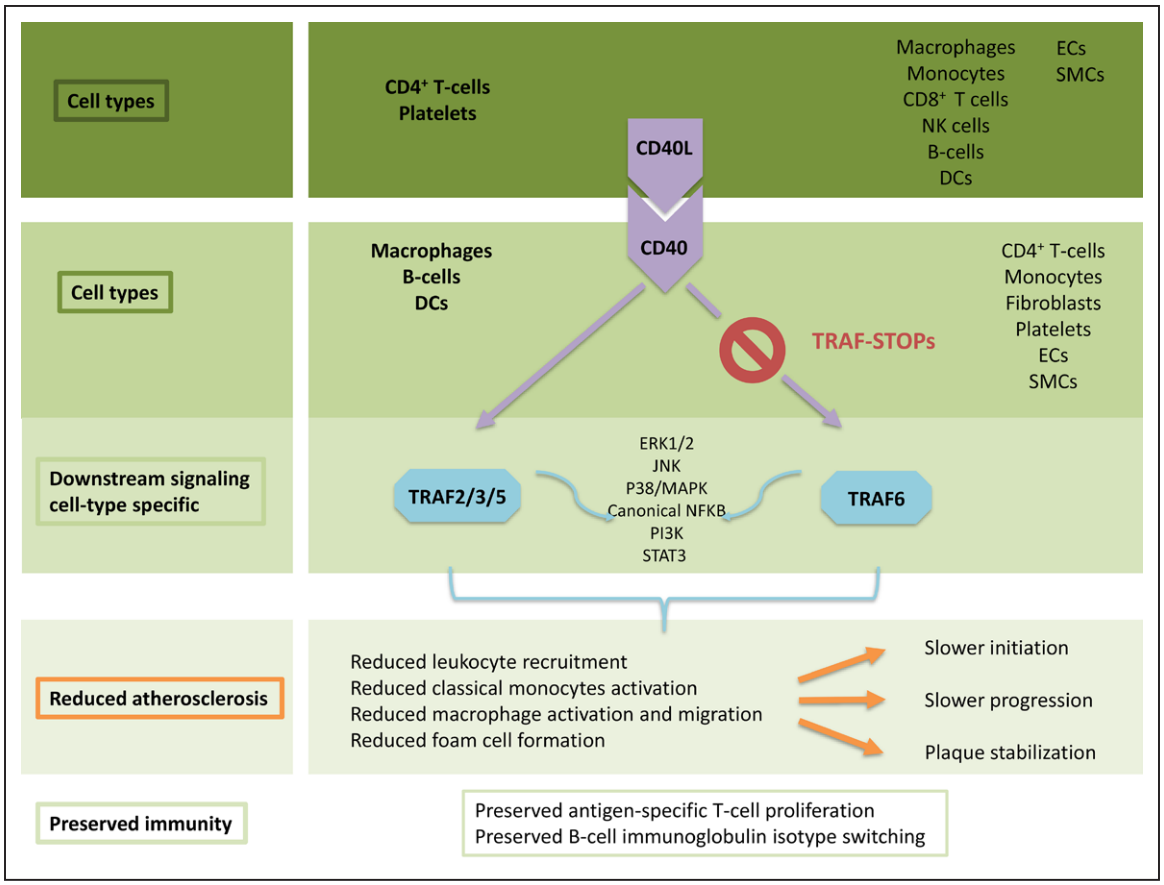

Figure 2. The costimulatory immune checkpoint proteins CD40L and CD40 are expressed on a plethora of cell types, that all exert their own CD40-signal transduction cascades. In monocytes and macrophages, CD40 signals via the adaptor molecule TRAF (TNF receptor–associated factor) 6. Inhibition of CD40-TRAF6 signaling via small molecule inhibitors directed against the CD40 binding grove at TRAF6 reduces canonical NFκB (nuclear factor kappa B) activation in myeloid cells, resulting in reduced myeloid cell recruitment and activation, thereby reducing atherosclerosis. As CD40-TRAF2/3/5 signaling remains intact, classical CD40-mediated immunity such as T-cell proliferation and immunoglobulin isotype switching are not affected. DC indicates dendritic cell; EC, dendritic cell; and SMC, smooth muscle cell.

a mouse model of diet-induced obesity.[119,122] Pharmacological inhibition of the CD40-TRAF6 pathway by compound 6877002 reduced monocyte recruitment and macrophage activation in an experimental model of neuroinflammation.[123] We recently demonstrated that TRAF-STOP treatment with either small molecule inhibitor 6860766 or small molecule inhibitor 6877002 reduced early atherosclerosis development and halted the progression of established atherosclerosis in ApoE$^{-/-}$ mice.[121] Moreover, TRAF-STOP treatment prevented classical monocyte activation, leukocyte recruitment and macrophage activation and migration in the arterial wall and induced a stable plaque phenotype (Figure 2). Nanomedicinal delivery of TRAF-STOPs specifically to macrophages, by incorporating compound 6877002 into rHDL (recombinant high-density lipoprotein) nanoparticles, reduced atherosclerotic plaque formation when given as prevention therapy, and reduced macrophage accumulation in existing plaques.[122] Moreover, nanotherapeutic delivery of rHDL-6877002 was safe and had a favorable biodistribution in nonhuman primates.[124]

Conclusions

Advances in immunology have accelerated our understanding of the complex immune network involved in the initiation and progression of atherosclerosis. Immune checkpoint therapy is a powerful therapeutic strategy to treat atherosclerosis. However, off-target effects might include immune suppression and highly selective immune checkpoint modulation, such as blocking CD40-TRAF6 signaling in macrophages, or enhancing coinhibition is a promising new (nano)therapeutic strategy to prevent atherosclerotic events in high-risk patients on top of optimal treatment of conventional cardiovascular risk factors, while preserving immune defense mechanisms.

Sources of Funding

We acknowledge the support from the Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development, and the Royal Netherlands Academy of Sciences for the GENIUS-II (Generating the Best Evidence-Based Pharmaceutical Targets for Atherosclerosis) project Generating the best evidence-based pharmaceutical targets for atherosclerosis part II. This work was supported by the Netherlands Organization for Scientific Research (NWO; VICI grant to E. Lutgens), the European Research Council (ERC consolidator grant to E. Lutgens), the European Union (horizon 2020, REPROGRAM to E. Lutgens), and the DFG (German Research Foundation; CRC 1123, project A5 to E. Lutgens).

Disclosures

None.

References

1. Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity*. 2013;38:1092–1104. doi: 10.1016/j.immuni.2013.06.009.
2. Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. *Immunity*. 2017;47:621–634. doi: 10.1016/j.immuni.2017.09.008.
3. Mohanta SK, Yin C, Peng L, Srikakulapu P, Bontha V, Hu D, Weih F, Weber C, Gerdes N, Habenicht AJ. Artery tertiary lymphoid organs contribute to innate and adaptive immune responses in advanced mouse atherosclerosis. *Circ Res*. 2014;114:1772–1787. doi: 10.1161/CIRCRESAHA.114.301137.
4. Binder CJ, Papac-Milicevic N, Witztum JL. Innate sensing of oxidation-specific epitopes in health and disease. *Nat Rev Immunol*. 2016;16:485–497. doi: 10.1038/nri.2016.63.
5. Ait-Oufella H, Sage AP, Mallat Z, Tedgui A. Adaptive (T and B cells) immunity and control by dendritic cells in atherosclerosis. *Circ Res*. 2014;114:1640–1660. doi: 10.1161/CIRCRESAHA.114.302761.
6. Nilsson J, Björkbacka H, Fredrikson GN. Apolipoprotein B100 autoimmunity and atherosclerosis - disease mechanisms and therapeutic potential. *Curr Opin Lipidol*. 2012;23:422–428. doi: 10.1097/MOL.0b013e328356ec7c.
7. Ketelhuth DF, Hansson GK. Adaptive response of T and B Cells in atherosclerosis. *Circ Res*. 2016;118:668–678. doi: 10.1161/CIRCRESAHA.115.306427.
8. Winkels H, Ehinger E, Vassallo M, et al. Atlas of the immune cell repertoire in mouse atherosclerosis defined by single-cell RNA-sequencing and mass cytometry. *Circ Res*. 2018;122:1675–1688. doi: 10.1161/CIRCRESAHA.117.312513.
9. Spitz C, Winkels H, Bürger C, Weber C, Lutgens E, Hansson GK, Gerdes N. Regulatory T cells in atherosclerosis: critical immune regulatory function and therapeutic potential. *Cell Mol Life Sci*. 2016;73:901–922. doi: 10.1007/s00018-015-2080-2.
10. Maganto-García E, Tarrio ML, Grabie N, Bu DX, Lichtman AH. Dynamic changes in regulatory T cells are linked to levels of diet-induced hypercholesterolemia. *Circulation*. 2011;124:185–195. doi: 10.1161/CIRCULATIONAHA.110.006411.
11. 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.
12. Kovalcsik E, Antunes RF, Baruah P, Kaski JC, Dumitriu IE. Proteasome-mediated reduction in proapoptotic molecule Bim renders $CD4^+CD28$null T cells resistant to apoptosis in acute coronary syndrome. *Circulation*. 2015;131:709–720. doi: 10.1161/CIRCULATIONAHA.114.013710.
13. Getz GS, Reardon CA. Natural killer T cells in atherosclerosis. *Nat Rev Cardiol*. 2017;14:304–314. doi: 10.1038/nrcardio.2017.2.
14. Tsiantoulas D, Diehl CJ, Witztum JL, Binder CJ. B cells and humoral immunity in atherosclerosis. *Circ Res*. 2014;114:1743–1756. doi: 10.1161/CIRCRESAHA.113.301145.
15. Vu DM, Tai A, Tatro JB, Karas RH, Huber BT, Beasley D. γδT cells are prevalent in the proximal aorta and drive nascent atherosclerotic lesion progression and neutrophilia in hypercholesterolemic mice. *PLoS One*. 2014;9:e109416. doi: 10.1371/journal.pone.0109416.
16. Cheng HY, Wu R, Hedrick CC. Gammadelta (γδ) T lymphocytes do not impact the development of early atherosclerosis. *Atherosclerosis*. 2014;234:265–269. doi: 10.1016/j.atherosclerosis.2014.03.007.
17. Newland SA, Mohanta S, Clément M, et al. Type-2 innate lymphoid cells control the development of atherosclerosis in mice. *Nat Commun*. 2017;8:15781. doi: 10.1038/ncomms15781.
18. 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.
19. Valdor R, Macian F. Induction and stability of the anergic phenotype in T cells. *Semin Immunol*. 2013;25:313–320. doi: 10.1016/j.smim.2013.10.010.
20. Baumeister SH, Freeman GJ, Dranoff G, Sharpe AH. Coinhibitory pathways in immunotherapy for Cancer. *Annu Rev Immunol*. 2016;34:539–573. doi: 10.1146/annurev-immunol-032414-112049.
21. Mahmud SA, Manlove LS, Schmitz HM, Xing Y, Wang Y, Owen DL, Schenkel JM, Boomer JS, Green JM, Yagita H, Chi H, Hogquist KA, Farrar MA. Costimulation via the tumor-necrosis factor receptor superfamily couples TCR signal strength to the thymic differentiation of regulatory T cells. *Nat Immunol*. 2014;15:473–481. doi: 10.1038/ni.2849.
22. Kusters PJH, Lutgens E, Seijkens TTP. Exploring immune checkpoints as potential therapeutic targets in atherosclerosis. *Cardiovasc Res*. 2018;114:368–377. doi: 10.1093/cvr/cvx248.
23. Gotwals P, Cameron S, Cipolletta D, Cremasco V, Crystal A, Hewes B, Mueller B, Quaratino S, Sabatos-Peyton C, Petruzzelli L, Engelman JA, Dranoff G. Prospects for combining targeted and conventional cancer therapy with immunotherapy. *Nat Rev Cancer*. 2017;116:180–301.
24. Bäck M, Weber C, Lutgens E. Regulation of atherosclerotic plaque inflammation. *J Intern Med*. 2015;278:462–482. doi: 10.1111/joim.12367.
25. Lichtman AH. T cell costimulatory and coinhibitory pathways in vascular inflammatory diseases. *Front Physiol*. 2012;3:18.
26. Ley K, Gerdes N, Winkels H. ATVB Distinguished Scientist Award: how costimulatory and coinhibitory pathways shape atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2017;37:764–777. doi: 10.1161/ATVBAHA.117.308611.
27. Stöger JL, Gijbels MJ, van der Velden S, Manca M, van der Loos CM, Biessen EA, Daemen MJ, Lutgens E, de Winther MP. Distribution of macrophage polarization markers in human atherosclerosis. *Atherosclerosis*. 2012;225:461–468. doi: 10.1016/j.atherosclerosis.2012.09.013.
28. 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.
29. Ma K, Lv S, Liu B, Liu Z, Luo Y, Kong W, Xu Q, Feng J, Wang X. CTLA4-IgG ameliorates homocysteine-accelerated atherosclerosis by inhibiting T-cell overactivation in apoE(-/-) mice. *Cardiovasc Res*. 2013;97:349–359. doi: 10.1093/cvr/cvs330.
30. Ewing MM, Karper JC, Abdul S, de Jong RC, Peters HA, de Vries MR, Redeker A, Kuiper J, Toes RE, Arens R, Jukema JW, Quax PH. T-cell co-stimulation by CD28-CD80/86 and its negative regulator CTLA-4 strongly influence accelerated atherosclerosis development. *Int J Cardiol*. 2013;168:1965–1974. doi: 10.1016/j.ijcard.2012.12.085.
31. de Boer OJ, Hirsch F, van der Wal AC, van der Loos CM, Das PK, Becker AE. Costimulatory molecules in human atherosclerotic plaques: an indication of antigen specific T lymphocyte activation. *Atherosclerosis*. 1997;133:227–234.
32. Doesch AO, Zhao L, Gleissner CA, Akhavanpoor M, Rohde D, Okuyucu D, Hakimi M, Dengler TJ, Katus HA, Erbel C. Inhibition of B7-1 (CD80) by RhuDex® reduces lipopolysaccharide-mediated inflammation in human atherosclerotic lesions. *Drug Des Devel Ther*. 2014;8:447–457. doi: 10.2147/DDDT.S59594.
33. Dopheide JF, Sester U, Schlitt A, Horstick G, Rupprecht HJ, Münzel T, Blankenberg S. Monocyte-derived dendritic cells of patients with coronary artery disease show an increased expression of costimulatory molecules CD40, CD80 and CD86 in vitro. *Coron Artery Dis*. 2007;18:523–531. doi: 10.1097/MCA.0b013e3282eff1ad.
34. Mantani PT, Ljungcrantz I, Andersson L, Alm R, Hedblad B, Björkbacka H, Nilsson J, Fredrikson GN. Circulating CD40+ and CD86+ B cell subsets demonstrate opposing associations with risk of stroke. *Arterioscler Thromb Vasc Biol*. 2014;34:211–218. doi: 10.1161/ATVBAHA.113.302667.
35. Müller A, Mu L, Meletta R, Beck K, Rancic Z, Drandarov K, Kaufmann PA, Ametamey SM, Schibli R, Borel N, Krämer SD. Towards non-invasive imaging of vulnerable atherosclerotic plaques by targeting co-stimulatory molecules. *Int J Cardiol*. 2014;174:503–515. doi: 10.1016/j.ijcard.2014.04.071.
36. Meletta R, Steier L, Borel N, Mu L, Keller C, Chiotellis A, Russo E, Halin C, Ametamey SM, Schibli R, Krämer SD, Müller Herde A. CD80 Is upregulated in a mouse model with shear stress-induced atherosclerosis and allows for evaluating CD80-targeting PET tracers. *Mol Imaging Biol*. 2017;19:90–99. doi: 10.1007/s11307-016-0987-0.
37. Gotsman I, Grabie N, Gupta R, Dacosta R, MacConmara M, Lederer J, Sukhova G, Witztum JL, Sharpe AH, Lichtman AH. Impaired regulatory T-cell response and enhanced atherosclerosis in the absence of inducible costimulatory molecule. *Circulation*. 2006;114:2047–2055. doi: 10.1161/CIRCULATIONAHA.106.633263.
38. Clement M, Guedj K, Andreata F, et al. Control of the T follicular helper-germinal center B-cell axis by $CD8^+$ regulatory T cells limits atherosclerosis and tertiary lymphoid organ development. *Circulation*. 2015;131:560–570. doi: 10.1161/CIRCULATIONAHA.114.010988.
39. Michel NA, Zirlik A, Wolf D. CD40L and its receptors in atherothrombosis-an update. *Front Cardiovasc Med*. 2017;4:40. doi: 10.3389/fcvm.2017.00040.

40. Engel D, Seijkens T, Poggi M, Sanati M, Thevissen L, Beckers L, Wijnands E, Lievens D, Lutgens E. The immunobiology of CD154-CD40-TRAF interactions in atherosclerosis. *Semin Immunol*. 2009;21:308–312. doi: 10.1016/j.smim.2009.06.004.
41. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev*. 2009;229:152–172. doi: 10.1111/j.1600-065X.2009.00782.x.
42. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature*. 1998;393:480–483. doi: 10.1038/31002.
43. Lutgens E, Gorelik L, Daemen MJ, de Muinck ED, Grewal IS, Koteliansky VE, Flavell RA. Requirement for CD154 in the progression of atherosclerosis. *Nat Med*. 1999;5:1313–1316.
44. Lutgens E, Cleutjens KB, Heeneman S, Koteliansky VE, Burkly LC, Daemen MJ. Both early and delayed anti-CD40L antibody treatment induces a stable plaque phenotype. *Proc Natl Acad Sci USA*. 2000;97:7464–7469.
45. Mach F, Schönbeck U, Sukhova GK, Atkinson E, Libby P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. *Nature*. 1998;394:200–203. doi: 10.1038/28204.
46. Schönbeck U, Sukhova GK, Shimizu K, Mach F, Libby P. Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. *Proc Natl Acad Sci USA*. 2000;97:7458–7463.
47. Lutgens E, Lievens D, Beckers L, et al. Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. *J Exp Med*. 2010;207:391–404. doi: 10.1084/jem.20091293.
48. Kusters PJH, Seijkens TTP, Beckers L, Lievens D, Winkels H, de Waard V, Duijvestijn A, Lindquist Liljeqvist M, Roy J, Daugherty A, Newby A, Gerdes N, Lutgens E. CD40L deficiency protects against aneurysm formation. *Arterioscler Thromb Vasc Biol*. 2018;38:1076–1085. doi: 10.1161/ATVBAHA.117.310640.
49. André P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR, Wagner DD. CD40L stabilizes arterial thrombi by a beta3 integrin–dependent mechanism. *Nat Med*. 2002;8:247–252. doi: 10.1038/nm0302-247.
50. Kuijpers MJ, Mattheij NJ, Cipolla L, van Geffen JP, Lawrence T, Donners MM, Boon L, Lievens D, Torti M, Noels H, Gerdes N, Cosemans JM, Lutgens E, Heemskerk JW. Platelet CD40L modulates thrombus growth via phosphatidylinositol 3-kinase β, and not via CD40 and IκB kinase α. *Arterioscler Thromb Vasc Biol*. 2015;35:1374–1381. doi: 10.1161/ATVBAHA.114.305127.
51. Lievens D, Zernecke A, Seijkens T, et al. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. *Blood*. 2010;116:4317–4327. doi: 10.1182/blood-2010-01-261206.
52. Gerdes N, Seijkens T, Lievens D, Kuijpers MJ, Winkels H, Projahn D, Hartwig H, Beckers L, Megens RT, Boon L, Noelle RJ, Soehnlein O, Heemskerk JW, Weber C, Lutgens E. Platelet CD40 exacerbates atherosclerosis by transcellular activation of endothelial cells and leukocytes. *Arterioscler Thromb Vasc Biol*. 2016;36:482–490. doi: 10.1161/ATVBAHA.115.307074.
53. Kusters P, Seijkens T, Bürger C, Legein B, Winkels H, Gijbels M, Barthels C, Bennett R, Beckers L, Atzler D, Biessen E, Brocker T, Weber C, Gerdes N, Lutgens E. Constitutive CD40 signaling in dendritic cells limits atherosclerosis by provoking inflammatory bowel disease and ensuing cholesterol malabsorption. *Am J Pathol*. 2017;187:2912–2919. doi: 10.1016/j.ajpath.2017.08.016.
54. Vogel DY, Glim JE, Stavenuiter AW, Breur M, Heijnen P, Amor S, Dijkstra CD, Beelen RH. Human macrophage polarization *in vitro*: maturation and activation methods compared. *Immunobiology*. 2014;219:695–703. doi: 10.1016/j.imbio.2014.05.002.
55. Mach F, Schönbeck U, Sukhova GK, Bourcier T, Bonnefoy JY, Pober JS, Libby P. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. *Proc Natl Acad Sci USA*. 1997;94:1931–1936.
56. Garlichs CD, John S, Schmeisser A, Eskafi S, Stumpf C, Karl M, Goppelt-Struebe M, Schmieder R, Daniel WG. Upregulation of CD40 and CD40 ligand (CD154) in patients with moderate hypercholesterolemia. *Circulation*. 2001;104:2395–2400.
57. Kopp CW, Steiner S, Nasel C, Seidinger D, Mlekusch I, Lang W, Bartok A, Ahmadi R, Minar E. Abciximab reduces monocyte tissue factor in carotid angioplasty and stenting. *Stroke*. 2003;34:2560–2567. doi: 10.1161/01.STR.0000094425.06242.64.
58. Sanguigni V, Ferro D, Pignatelli P, Del Ben M, Nadia T, Saliola M, Sorge R, Violi F. CD40 ligand enhances monocyte tissue factor expression and thrombin generation via oxidative stress in patients with hypercholesterolemia. *J Am Coll Cardiol*. 2005;45:35–42. doi: 10.1016/j.jacc.2004.09.047.
59. Cipollone F, Ferri C, Desideri G, Paloscia L, Materazzo G, Mascellanti M, Fazia M, Iezzi A, Cuccurullo C, Pini B, Bucci M, Santucci A, Cuccurullo F, Mezzetti A. Preprocedural level of soluble CD40L is predictive of enhanced inflammatory response and restenosis after coronary angioplasty. *Circulation*. 2003;108:2776–2782. doi: 10.1161/01.CIR.0000103700.05109.0D.
60. Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, Simoons ML; CAPTURE Study Investigators. Soluble CD40 ligand in acute coronary syndromes. *N Engl J Med*. 2003;348:1104–1111. doi: 10.1056/NEJMoa022600.
61. Mukundan L, Bishop GA, Head KZ, Zhang L, Wahl LM, Suttles J. TNF receptor-associated factor 6 is an essential mediator of CD40-activated proinflammatory pathways in monocytes and macrophages. *J Immunol*. 2005;174:1081–1090.
62. Donners MM, Beckers L, Lievens D, et al. The CD40-TRAF6 axis is the key regulator of the CD40/CD40L system in neointima formation and arterial remodeling. *Blood*. 2008;111:4596–4604. doi: 10.1182/blood-2007-05-088906.
63. van Wanrooij EJ, van Puijvelde GH, de Vos P, Yagita H, van Berkel TJ, Kuiper J. Interruption of the Tnfrsf4/Tnfsf4 (OX40/OX40L) pathway attenuates atherogenesis in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2007;27:204–210. doi: 10.1161/01.ATV.0000251007.07648.81.
64. Foks AC, van Puijvelde GH, Bot I, ter Borg MN, Habets KL, Johnson JL, Yagita H, van Berkel TJ, Kuiper J. Interruption of the OX40-OX40 ligand pathway in LDL receptor-deficient mice causes regression of atherosclerosis. *J Immunol*. 2013;191:4573–4580. doi: 10.4049/jimmunol.1200708.
65. Wang X, Ria M, Kelmenson PM, et al. Positional identification of TNFSF4, encoding OX40 ligand, as a gene that influences atherosclerosis susceptibility. *Nat Genet*. 2005;37:365–372. doi: 10.1038/ng1524.
66. Yan J, Chen G, Gong J, Wang C, Du R. Upregulation of OX40-OX40 ligand system on T lymphocytes in patients with acute coronary syndromes. *J Cardiovasc Pharmacol*. 2009;54:451–455. doi: 10.1097/FJC.0b013e3181be7578.
67. Shi JZ, Wang LY, Zhu Y, Zhao CY, Liu W, Jiang SL, You HW, Wang Y, Xu FY, Tang YS, Cui LQ. OX40 ligand levels and high-sensitivity C-reactive protein levels in blood from local coronary plaque and the femoral artery in patients with acute coronary syndrome or stable angina. *J Int Med Res*. 2011;39:1275–1283. doi: 10.1177/147323001103900415.
68. Olofsson PS, Söderström LA, Jern C, Sirsjö A, Ria M, Sundler E, de Faire U, Wiklund PG, Ohrvik J, Hedin U, Paulsson-Berne G, Hamsten A, Eriksson P, Hansson GK. Genetic variants of TNFSF4 and risk for carotid artery disease and stroke. *J Mol Med (Berl)*. 2009;87:337–346. doi: 10.1007/s00109-008-0412-5.
69. Foks AC, Bot I, Frodermann V, de Jager SC, Ter Borg M, van Santbrink PJ, Yagita H, Kuiper J, van Puijvelde GH. Interference of the CD30-CD30L pathway reduces atherosclerosis development. *Arterioscler Thromb Vasc Biol*. 2012;32:2862–2868. doi: 10.1161/ATVBAHA.112.300509.
70. Boyle JJ. Association of coronary plaque rupture and atherosclerotic inflammation. *J Pathol*. 1997;181:93–99. doi: 10.1002/(SICI)1096-9896(199701)181:1<93::AID-PATH696>3.0.CO;2-H.
71. Olofsson PS, Söderström LA, Wågsäter D, Sheikine Y, Ocaya P, Lang F, Rabu C, Chen L, Rudling M, Aukrust P, Hedin U, Paulsson-Berne G, Sirsjö A, Hansson GK. CD137 is expressed in human atherosclerosis and promotes development of plaque inflammation in hypercholesterolemic mice. *Circulation*. 2008;117:1292–1301. doi: 10.1161/CIRCULATIONAHA.107.699173.
72. Jeon HJ, Choi JH, Jung IH, et al. CD137 (4-1BB) deficiency reduces atherosclerosis in hyperlipidemic mice. *Circulation*. 2010;121:1124–1133. doi: 10.1161/CIRCULATIONAHA.109.882704.
73. Jung IH, Choi JH, Jin J, et al. CD137-inducing factors from T cells and macrophages accelerate the destabilization of atherosclerotic plaques in hyperlipidemic mice. *FASEB J*. 2014;28:4779–4791. doi: 10.1096/fj.14-253732.
74. Yu Y, He Y, Yang TT, Jiang H, Xiang YJ, Fang LB, Hjelmstrom P, Gao XG, Liu GZ. Elevated plasma levels and monocyte-associated expression of CD137 ligand in patients with acute atherothrombotic stroke. *Eur Rev Med Pharmacol Sci*. 2014;18:1525–1532.
75. Dongming L, Zuxun L, Liangjie X, Biao W, Ping Y. Enhanced levels of soluble and membrane-bound CD137 levels in patients with acute coronary syndromes. *Clin Chim Acta*. 2010;411:406–410. doi: 10.1016/j.cca.2009.12.011.
76. Meiler S, Smeets E, Winkels H, Shami A, Pascutti MF, Nolte MA, Beckers L, Weber C, Gerdes N, Lutgens E. Constitutive GITR activation

reduces atherosclerosis by promoting regulatory CD4+ T-cell responses-brief report. *Arterioscler Thromb Vasc Biol*. 2016;36:1748–1752. doi: 10.1161/ATVBAHA.116.307354.
77. Kim WJ, Bae EM, Kang YJ, Bae HU, Hong SH, Lee JY, Park JE, Kwon BS, Suk K, Lee WH. Glucocorticoid-induced tumour necrosis factor receptor family related protein (GITR) mediates inflammatory activation of macrophages that can destabilize atherosclerotic plaques. *Immunology*. 2006;119:421–429. doi: 10.1111/j.1365-2567.2006.02453.x.
78. Winkels H, Meiler S, Smeets E, et al. CD70 limits atherosclerosis and promotes macrophage function. *Thromb Haemost*. 2017;117:164–175. doi: 10.1160/TH16-04-0318.
79. Winkels H, Meiler S, Lievens D, et al. CD27 co-stimulation increases the abundance of regulatory T cells and reduces atherosclerosis in hyperlipidaemic mice. *Eur Heart J*. 2017;38:3590–3599. doi: 10.1093/eurheartj/ehx517.
80. Yan W, Wen S, Wang L, Duan Q, Ding L. Comparison of cytokine expressions in acute myocardial infarction and stable angina stages of coronary artery disease. *Int J Clin Exp Med*. 2015;8:18082–18089.
81. Lee WH, Kim SH, Lee Y, Lee BB, Kwon B, Song H, Kwon BS, Park JE. Tumor necrosis factor receptor superfamily 14 is involved in atherogenesis by inducing proinflammatory cytokines and matrix metalloproteinases. *Arterioscler Thromb Vasc Biol*. 2001;21:2004–2010.
82. Grandoch M, Feldmann K, Göthert JR, et al. Deficiency in lymphotoxin β receptor protects from atherosclerosis in apoE-deficient mice. *Circ Res*. 2015;116:e57–e68. doi: 10.1161/CIRCRESAHA.116.305723.
83. Lo JC, Wang Y, Tumanov AV, Bamji M, Yao Z, Reardon CA, Getz GS, Fu YX. Lymphotoxin beta receptor-dependent control of lipid homeostasis. *Science*. 2007;316:285–288. doi: 10.1126/science.1137221.
84. Yuan X, Gu Y, Lai X, Gu Q. LIGHT is increased in patients with coronary disease and regulates inflammatory response and lipid metabolism in oxLDL-induced THP-1 macrophages. *Biochem Biophys Res Commun*. 2017;490:732–738. doi: 10.1016/j.bbrc.2017.06.110.
85. Scholz H, Sandberg W, Damås JK, Smith C, Andreassen AK, Gullestad L, Frøland SS, Yndestad A, Aukrust P, Halvorsen B. Enhanced plasma levels of LIGHT in unstable angina: possible pathogenic role in foam cell formation and thrombosis. *Circulation*. 2005;112:2121–2129. doi: 10.1161/CIRCULATIONAHA.105.544676.
86. Andrés-Blasco I, Vinué Á, Herrero-Cervera A, Martínez-Hervás S, Nuñez L, Piqueras L, Ascaso JF, Sanz MJ, Burks DJ, González-Navarro H. Hepatic lipase inactivation decreases atherosclerosis in insulin resistance by reducing LIGHT/Lymphotoxin β-Receptor pathway. *Thromb Haemost*. 2016;116:379–393. doi: 10.1160/TH15-10-0773.
87. Lind L, Ärnlöv J, Lindahl B, Siegbahn A, Sundström J, Ingelsson E. Use of a proximity extension assay proteomics chip to discover new biomarkers for human atherosclerosis. *Atherosclerosis*. 2015;242:205–210. doi: 10.1016/j.atherosclerosis.2015.07.023.
88. Owens AW, Matulevicius S, Rohatgi A, Ayers CR, Das SR, Khera A, McGuire DK, de Lemos JA. Circulating lymphotoxin β receptor and atherosclerosis: observations from the Dallas Heart Study. *Atherosclerosis*. 2010;212:601–606. doi: 10.1016/j.atherosclerosis.2010.06.003.
89. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008;322:271–275. doi: 10.1126/science.1160062.
90. Matsumoto T, Sasaki N, Yamashita T, Emoto T, Kasahara K, Mizoguchi T, Hayashi T, Yodoi K, Kitano N, Saito T, Yamaguchi T, Hirata K. Overexpression of cytotoxic T-lymphocyte-associated antigen-4 prevents atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2016;36:1141–1151. doi: 10.1161/ATVBAHA.115.306848.
91. Boasso A, Herbeuval JP, Hardy AW, Winkler C, Shearer GM. Regulation of indoleamine 2,3-dioxygenase and tryptophanyl-tRNA-synthetase by CTLA-4-Fc in human CD4+ T cells. *Blood*. 2005;105:1574–1581. doi: 10.1182/blood-2004-06-2089.
92. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol*. 2003;4:1206–1212. doi: 10.1038/ni1003.
93. Polyzos KA, Ovchinnikova O, Berg M, Baumgartner R, Agardh H, Pirault J, Gisterå A, Assinger A, Laguna-Fernandez A, Bäck M, Hansson GK, Ketelhuth DF. Inhibition of indoleamine 2,3-dioxygenase promotes vascular inflammation and increases atherosclerosis in Apoe-/- mice. *Cardiovasc Res*. 2015;106:295–302. doi: 10.1093/cvr/cvv100.
94. Gotsman I, Grabie N, Dacosta R, Sukhova G, Sharpe A, Lichtman AH. Proatherogenic immune responses are regulated by the PD-1/PD-L pathway in mice. *J Clin Invest*. 2007;117:2974–2982. doi: 10.1172/JCI31344.
95. Bu DX, Tarrio M, Maganto-Garcia E, Stavrakis G, Tajima G, Lederer J, Jarolim P, Freeman GJ, Sharpe AH, Lichtman AH. Impairment of the programmed cell death-1 pathway increases atherosclerotic lesion development and inflammation. *Arterioscler Thromb Vasc Biol*. 2011;31:1100–1107. doi: 10.1161/ATVBAHA.111.224709.
96. Cochain C, Chaudhari SM, Koch M, Wiendl H, Eckstein H-H, Zernecke A. Programmed cell death-1 deficiency exacerbates T cell activation and atherogenesis despite expansion of regulatory T cells in atherosclerosis-prone mice. *PLoS One*. 2014;9:e93280.
97. Lee J, Zhuang Y, Wei X, Shang F, Wang J, Zhang Y, Liu X, Yang Y, Liu L, Zheng Q. Contributions of PD-1/PD-L1 pathway to interactions of myeloid DCs with T cells in atherosclerosis. *J Mol Cell Cardiol*. 2009;46:169–176. doi: 10.1016/j.yjmcc.2008.10.028.
98. Li SH, Chen WJ, Yan M, Shu YW, Liao YH. Expression of coinhibitory PD-L1 on $CD4^+CD25^+FOXP3^+$ regulatory T cells is elevated in patients with acute coronary syndrome. *Coron Artery Dis*. 2015;26:598–603. doi: 10.1097/MCA.0000000000000282.
99. Foks AC, Ran IA, Frodermann V, Bot I, van Santbrink PJ, Kuiper J, van Puijvelde GHM. Agonistic anti-TIGIT treatment inhibits T cell responses in LDLr deficient mice without affecting atherosclerotic lesion development. *PLoS One*. 2013;8:e83134.
100. Freeman GJ, Casasnovas JM, Umetsu DT, DeKruyff RH. TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. *Immunol Rev*. 2010;235:172–189. doi: 10.1111/j.0105-2896.2010.00903.x.
101. Foks AC, Engelbertsen D, Kuperwaser F, Alberts-Grill N, Gonen A, Witztum JL, Lederer J, Jarolim P, DeKruyff RH, Freeman GJ, Lichtman AH. Blockade of Tim-1 and Tim-4 enhances atherosclerosis in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2016;36:456–465. doi: 10.1161/ATVBAHA.115.306860.
102. Foks AC, Ran IA, Wasserman L, Frodermann V, Ter Borg MN, de Jager SC, van Santbrink PJ, Yagita H, Akiba H, Bot I, Kuiper J, van Puijvelde GH. T-cell immunoglobulin and mucin domain 3 acts as a negative regulator of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2013;33:2558–2565. doi: 10.1161/ATVBAHA.113.301879.
103. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet*. 2009;41:56–65. doi: 10.1038/ng.291.
104. Do R, Willer CJ, Schmidt EM, et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet*. 2013;45:1345–1352. doi: 10.1038/ng.2795.
105. Andrews LP, Marciscano AE, Drake CG, Vignali DA. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev*. 2017;276:80–96. doi: 10.1111/imr.12519.
106. Golden D, Kolmakova A, Sura S, Vella AT, Manichaikul A, Wang XQ, Bielinski SJ, Taylor KD, Chen YI, Rich SS, Rodriguez A. Lymphocyte activation gene 3 and coronary artery disease. *JCI Insight*. 2016;1:e88628. doi: 10.1172/jci.insight.88628.
107. Ridker PM, Everett BM, Thuren T, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914.
108. Huet F, Akodad M, Fauconnier J, Lacampagne A, Roubille F. Anti-inflammatory drugs as promising cardiovascular treatments. *Expert Rev Cardiovasc Ther*. 2017;15:109–125. doi: 10.1080/14779072.2017.1273771.
109. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;348:56–61. doi: 10.1126/science.aaa8172.
110. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*. 2016;54:139–148. doi: 10.1016/j.ejca.2015.11.016.
111. Moslehi JJ. Cardiovascular toxic effects of targeted cancer therapies. *N Engl J Med*. 2016;375:1457–1467.
112. Johnson DB, Balko JM, Compton ML, et al. Fulminant myocarditis with combination immune checkpoint blockade. *N Engl J Med*. 2016;375:1749–1755. doi: 10.1056/NEJMoa1609214.
113. Escudier M, Cautela J, Malissen N, et al. Clinical features, management, and outcomes of immune checkpoint inhibitor-related cardiotoxicity. *Circulation*. 2017;136:2085–2087. doi: 10.1161/CIRCULATIONAHA.117.030571.
114. Varricchi G, Galdiero MR, Tocchetti CG. Cardiac toxicity of immune checkpoint inhibitors: cardio-oncology meets immunology. *Circulation*. 2017;136:1989–1992. doi: 10.1161/CIRCULATIONAHA.117.029626.
115. Goldstein BL, Gedmintas L, Todd DJ. Drug-associated polymyalgia rheumatica/giant cell arteritis occurring in two patients after

treatment with ipilimumab, an antagonist of ctla-4. *Arthritis Rheumatol*. 2014;66:768–769. doi: 10.1002/art.38282.
116. Zhang H, Watanabe R, Berry GJ, Vaglio A, Liao YJ, Warrington KJ, Goronzy JJ, Weyand CM. Immunoinhibitory checkpoint deficiency in medium and large vessel vasculitis. *Proc Natl Acad Sci USA*. 2017;114:E970–E979. doi: 10.1073/pnas.1616848114.
117. Blair HA, Deeks ED. Abatacept: a review in rheumatoid arthritis. *Drugs*. 2017;77:1221–1233.
118. Kean LS, Turka LA, Blazar BR. Advances in targeting co-inhibitory and co-stimulatory pathways in transplantation settings: the Yin to the Yang of cancer immunotherapy. *Immunol Rev*. 2017;276:192–212. doi: 10.1111/imr.12523.
119. Chatzigeorgiou A, Seijkens T, Zarzycka B, et al. Blocking CD40-TRAF6 signaling is a therapeutic target in obesity-associated insulin resistance. *Proc Natl Acad Sci USA*. 2014;111:2686–2691. doi: 10.1073/pnas.1400419111.
120. Zarzycka B, Seijkens T, Nabuurs SB, Ritschel T, Grommes J, Soehnlein O, Schrijver R, van Tiel CM, Hackeng TM, Weber C, Giehler F, Kieser A, Lutgens E, Vriend G, Nicolaes GA. Discovery of small molecule CD40-TRAF6 inhibitors. *J Chem Inf Model*. 2015;55:294–307. doi: 10.1021/ci500631e.
121. Seijkens TTP, van Tiel CM, Kusters PJH, et al. Targeting CD40-induced TRAF6 signaling in macrophages reduces atherosclerosis. *J Am Coll Cardiol*. 2018;71:527–542. doi: 10.1016/j.jacc.2017.11.055.
122. van den Berg SM, Seijkens TT, Kusters PJ, Zarzycka B, Beckers L, den Toom M, Gijbels MJ, Chatzigeorgiou A, Weber C, de Winther MP, Chavakis T, Nicolaes GA, Lutgens E. Blocking CD40-TRAF6 interactions by small-molecule inhibitor 6860766 ameliorates the complications of diet-induced obesity in mice. *Int J Obes (Lond)*. 2015;39:782–790. doi: 10.1038/ijo.2014.198.
123. Aarts SABM, Seijkens TTP, Kusters PJH, et al. Inhibition of CD40-TRAF6 interactions by the small molecule inhibitor 6877002 reduces neuroinflammation. *J Neuroinflammation*. 2017;14:105. doi: 10.1186/s12974-017-0875-9.
124. Lameijer M, Binderup T, van Leent MMT et al. Efficacy and safety assessment of a TRAF6- targeted nanoimmunotherapy in atherosclerotic mice and non-human primates. *Nat Biomed Eng*. 2018;2,279–292.