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How the immune system shapes atherosclerosis: roles of innate and adaptive immunity

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Abstract

Atherosclerosis is the root cause of many cardiovascular diseases. Extensive research in preclinical models and emerging evidence in humans have established the crucial roles of the innate and adaptive immune systems in driving atherosclerosis-associated chronic inflammation in arterial blood vessels. New techniques have highlighted the enormous heterogeneity of leukocyte subsets in the arterial wall that have pro-inflammatory or regulatory roles in atherogenesis. Understanding the homing and activation pathways of these immune cells, their disease-associated dynamics and their regulation by microbial and metabolic factors will be crucial for the development of clinical interventions for atherosclerosis, including potentially vaccinationbased therapeutic strategies. Here, we review key molecular mechanisms of immune cell activation implicated in modulating atherogenesis and provide an update on the contributions of innate and adaptive immune cell subsets in atherosclerosis.

The major cardiovascular diseases, including coronary artery disease, myocardial infarction, stroke and peripheral artery disease, dominate death and disability statistics globally. Atherosclerosis, which is a common pathology underlying many cardiovascular diseases, is characterized by the accumulation of lipid-laden, immune cell-rich plaques known as atheromata in large and medium-sized arteries. Advanced plaques can rupture or erode, causing thromboses that occlude arteries and obstruct blood flow, leading to an array of life-threatening clinical manifestations known as major adverse cardiovascular events (MACEs). Disease pathogenesis is, in part, linked to dyslipidaemia and hypercholesterolaemia, which can be triggered by genetic factors, diet and lifestyle choices, and to metabolic disorders such as obesity and type 2 diabetes. Current therapies for atherosclerosis, such as statins or inhibitors of the serine protease PCSK9, aim to control levels of low-density lipoprotein (LDL) cholesterol in the blood[1]. However, despite the effectiveness of these therapies in reducing LDL cholesterol levels to guideline recommendations, MACEs are reduced by less than 50%[2]. Some of the residual risk is thought to be inflammatory in nature[3]. The

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Author contributions

All authors contributed to all aspects of the article.

Competing interests

K.L. is a co-founder of Atherovax. M.O. and K.L. are named as co-inventors on patents applied for by La Jolla Institute for Immunology relating to cardiovascular diagnostics and therapeutics, and might have the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics. P.R. declares no competing interests.

Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS), which tested the effects of immunomodulation through IL-1β inhibition, led to successful reduction of MACEs and provided the first major evidence for the feasibility of treating the inflammatory component of atherothrombosis[4]. Colchicine, as given in the Colchicine Cardiovascular Outcomes Trial (COLCOT)[5], also reduced MACEs. In both studies, however, systemic immune suppression resulted in a higher incidence of infections in the treatment arm than in the placebo group, which led to increased serious adverse events and even fatality. This underlines the need to develop immune-targeted interventions that can modulate atherosclerosis more precisely, and are safe, durable and efficacious. Selectively targeting specific components of the immune network that promote atherogenesis will require an in-depth mechanistic knowledge of the cellular and molecular mechanisms that drive atherosclerosis.

Role of immune cells in atherogenesis

The complex and multifactorial aetiology of atherosclerosis involves contributions from both metabolic and immune mediators. Atherogenesis begins with endothelial dysfunction in susceptible regions of arteries that are characterized by disturbed blood flow (predilection sites). This allows the entry of cholesterol-rich, apolipoprotein B (APOB)-containing lipoproteins into the subendothelial space, where they are retained through interactions with intramural extracellular matrix components. Oxidative, enzymatic and chemical modifications of the trapped lipoproteins activate endothelial cells and vascular smooth muscle cells (VSMCs). Chemokine signalling and expression of adhesion molecules trigger a cascade of immune cell influx, leading to the nucleation of a lesion that can develop to form an atherosclerotic plaque. Both innate and adaptive immune cells, particularly dendritic cells (DCs), macrophages and T cells, dominate the cellular landscape of the evolving plaque.

Early lesions known as fatty streaks are characterized by cholesteryl ester-laden ‘foam cells’, which accumulate owing to unregulated uptake of native and modified lipoproteins, predominantly by macrophages and VSMCs. More-advanced lesions contain a core region of activated immune cells, cholesterol crystals and extracellular lipids, surrounded by a fibrous cap of VSMCs and collagen. Recent studies using lineage-tracing and transcriptomic analyses have revealed that the role of VSMCs in atherogenesis is not limited to their ability to produce extracellular matrix for cap formation[6,7]. Cholesterol accumulation in VSMCs has been demonstrated in sections of human coronary arteries and mouse aortas[6]. Single-cell transcriptomic studies in mouse models suggest that VSMC-derived foam cells constitute about half of all foam cells[8]. VSMCs exhibit enormous phenotypic plasticity[9,10] and can acquire lesion-promoting macrophage-like features[11], as well as plaque-stabilizing fibroblast-like features[10].

Over time, excessive death of immune cells in the lesion core puts strain on their removal by efferocytosis. Insufficient efferocytosis results in secondary necrosis and the formation of a necrotic core, in addition to primary necrosis[12]. Pro-inflammatory forms of programmed cell death such as necroptosis and pyroptosis also occur in the vessel wall[12] and may contribute to atherosclerosis progression. Both growth and stability of the progressing plaques can

differentially affect disease outcome. Plaque growth is driven by a net positive balance between continued recruitment of immune cells and macrophage proliferation versus apoptosis and efflux. Plaques are rendered unstable by increased inflammatory activity, a growing necrotic core and thinning of the fibrous cap, owing to decreased collagen synthesis and increased secretion of matrix metalloproteinases. Calcifications in the fibrous cap concentrate mechanical stresses[13], promoting rupture or erosion of these plaques. Exposure of the thrombogenic material in the plaque to blood platelets and coagulation factors leads to the formation of a thrombus that can immediately block blood flow, resulting in end organ damage.

Flow cytometry, mass cytometry and sequencingbased multidimensional single-cell molecular profiling approaches have resolved the cellular compositions of atherosclerotic plaques and of the arterial wall at unprecedented resolution[8,14,15]. An anti-inflammatory network of immune cells and resolving factors attempts to mitigate the collateral damage associated with immune cell activation[16,17], but chronic inflammation prevails during plaque progression. Thus, interventions that can selectively inhibit atherosclerosis-associated maladaptive inflammation or boost anti-atherogenic regulatory processes have the potential to improve patient outcome through decreased progression or enhanced resolution of atherosclerosis. For example, pioneering work in mouse models of atherosclerosis has established the prophylactic potential of peptide vaccines to boost regulatory T (T_{reg}) cell responses and prevent atherosclerosis[18]. Tolerogenic vaccination holds promise as an efficient, durable and relatively inexpensive approach to induce protective adaptive immunity in patients with atherosclerosis[19]. If successful, it could save the lives of millions of at-risk individuals around the world.

Here, we provide a succinct overview of the key innate and adaptive immune components that orchestrate inflammation in atherosclerosis, and discuss their activation by endogenous danger signals and microbial triggers. The effects of the immune system or immune mediators on non-immune cells in atherosclerotic plaques are outside the scope of this Review. As much of our current mechanistic understanding of the immune-dependent processes in the vessel wall is based on preclinical data, we focus primarily on recent studies in mouse models of atherosclerosis, but note that these have limitations with respect to human disease (BOX 1).

Innate immune cells

A wide range of innate immune cells, including macrophages, DCs, monocytes, mast cells and neutrophils, are relevant to atherosclerosis progression[20]. Recent studies have also highlighted the involvement of natural killer cells and non-cytotoxic innate lymphoid cells (BOX 2).

Monocytes and macrophages.

In healthy arteries, monocytes are rare. Hypercholesterolaemia promotes the proliferation of haematopoietic stem and progenitor cells (BOX 3), leading to systemic monocytosis[21]. Subendothelial accumulation of lipoproteins and chemokines triggers an influx of monocytes into the vessel wall[22] (FIG. 1). The chemokine receptor CCR2 and its

main ligand CCL2 regulate the recruitment of classical monocytes[23,24], which dominate monocyte influx in atherosclerosis[21,25]. Plaque monocytes differentiate into macrophages and monocyte-derived DCs that can present antigens to T cells[26]. Most non-classical monocytes do not transmigrate, instead having a characteristic 'patrolling' behaviour and a role in maintaining vascular endothelial homeostasis[27,28]. Although some non-classical monocytes can enter the atheroma, their extravascular function remains unclear. In mouse models of atherosclerosis, non-classical monocytes have been shown to have an atheroprotective role[29]. However, in humans, mass cytometry analysis of blood monocyte subtypes showed that a non-classical monocyte subset expressing the carbohydrate marker 6-sulfo LacNAc was enriched in the blood of patients with cardiovascular disease[30].

Monocyte-derived macrophages initiate the uptake and clearance of lipoproteins, resulting in the formation of lipid-rich foam cells[25]. Recent studies involving lipidomic and transcriptomic analyses of lipid-loaded macrophages have revealed an enrichment of anti-inflammatory genes in cells with foamy characteristics, suggesting that foam cell formation by itself is not pro-inflammatory[15,31,32]. However, increasing oxidative stress in the artery wall and an accumulation of modified lipoproteins may overwhelm or rewire macrophage metabolism[25,33] (BOX 4). This triggers an inflammatory cytokine and chemokine cascade that leads to pro-atherogenic immune cell infiltration and activation. Lipid-engorged arterial macrophages that undergo cell death are initially cleared by other macrophages through efferocytosis[34]. When efferocytes become overwhelmed, they release pro-inflammatory cellular and lipid contents[34]. Plaque-resident macrophages cannot migrate much[35,36] and undergo local proliferation[37]. The retention of macrophages in the artery wall is promoted by a combination of local and systemic signals that include adhesion molecules (such as vascular cell adhesion molecule 1 (VCAM1) and platelet endothelial cell adhesion molecule 1)[35] and neural guidance factors such as netrin 1 and semaphorin 3E that block the chemotaxis of macrophages towards CCL19 and CCL21 (REFS[38,39]). Intriguingly, high-density lipoprotein has been reported to promote the egress of macrophages from plaques through a process mediated by CCR7, the receptor for CCL19 and CCL21 (REFS[40,41]). It is controversial whether the egress of plaque macrophages to the lumen or lymphatic system can affect atherosclerosis progression[21,42,43].

In general, plaque macrophages can have pro-inflammatory or anti-inflammatory features, but do not follow a classical M1 or M2 classification[44]. Certain subsets of foam cells and resident macrophages can promote the resolution of inflammation and healing[31,32]. By contrast, inflammatory macrophages have increased levels of mRNA encoding MHC class II molecules, Fcγ receptor I (also known as CD64), the co-stimulatory molecules CD80 and CD86, nitric oxide synthase 2 (NOS2) and pro-inflammatory cytokines such as IL-6, tumour necrosis factor (TNF) and IL-1β. Single-cell studies, involving mass cytometry and single-cell RNA sequencing (scRNA-seq), have defined at least five distinct subsets of macrophages in mouse aortas[8]. The inflammatory macrophage subset, which is derived from circulating monocytes, is the main population involved in atherogenesis and is not found in healthy arteries. Inflammatory macrophages are characterized by increased expression of cytokine and chemokine transcripts as well as inflammasome components[45,46]. A second subset, known as type I interferon-inducible cells[47], is also derived from monocytes, and is defined by increased expression of numerous interferon-

inducible genes, including *Ifit3*, *Irf7* and *Isg15* (REF.[48]). This subset is found only during atherosclerosis and has a pro-inflammatory role through type I interferon production[31,45]. Foam cells expressing TREM2 (triggering receptor expressed on myeloid cells 2)[45] have been identified in mice with both early and advanced atherosclerotic lesions but not healthy arteries. TREM2^{hi} macrophages can be derived either from circulating monocytes or from embryonic precursors[8]. In addition to *Trem2*, they express *Cd9*, *Ctsd*, *Fabp4* and *Abcg1*, which have roles in lipid metabolism, cholesterol efflux and oxidative phosphorylation. Labelling with the neutral lipid dye BODIPY showed that lipid-filled macrophage-derived foam cells are TREM2^{+} cells[31]. Although these macrophages are prevalent in atherosclerotic arteries, they are characterized by low levels of expression of inflammatory genes and are postulated to have a role in mitigating vascular inflammation[31,32]. This subset of TREM2^{hi} macrophages resembles the CD11c–YFP^{+} macrophages identified in intact mouse aortas by intravital microscopy[36]. Recently, an aortic intima-resident macrophage (Mac^{AIR}) subset was identified[49]. These macrophages are derived from blood monocytes, which are mostly seeded shortly after birth and then maintained by local proliferation. Under the inflammatory conditions associated with plaque progression, Mac^{AIR} cells also differentiate from recruited monocytes. Fate-mapping studies with Mac^{AIR} depletion models have shown a pro-atherogenic role of these cells in early lesion development. Mac^{AIR} cells constitutively express *Il1b* mRNA but seem to lack other inflammasome-associated genes. They have similarities with the transcriptomic profile of cavity macrophages, a subset that was found in a single-cell meta-analysis study of atherosclerotic aortas[8]. As both Mac^{AIR} cells and cavity macrophages are defined by the expression of *Itgax*, genes encoding MHC class II, *Cd226*, *Ccr2* and *Retnla*[8,49], we suspect that they represent the same macrophage subset. In both atherosclerotic and healthy arteries, resident macrophages[8] derived embryonically from CX3CR1^{+} precursor cells are found[50]. They proliferate locally in the adventitia[37,50] and are characterized by the expression of lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), MHC class II and macrophage mannose receptor C type 1 (MRC1)[50]. Depletion of arterial LYVE1^{+} macrophages in animal models suggested that they inhibit collagen production by VSMCs and thereby limit arterial stiffness[51]. Most resident macrophages originate from embryonic progenitors, whereas atherosclerotic aortas have a prevalence of monocyte-derived macrophages[8,49,50]. Although these single-cell transcriptomic analyses of mouse aortas have revealed that plaque macrophages are more heterogeneous than was shown by traditional immunophenotyping (FIG. 1), most of these new macrophage populations have been defined by their gene expression profiles and lack comprehensive characterization of protein markers and functional studies.

Efforts to define immune and non-immune cell populations in human atherosclerotic plaques are also under way[52]. Transcriptomes of human macrophage subsets isolated from carotid endarterectomy specimens showed significant overlap with the macrophage clusters defined in mouse aortas, particularly with respect to the inflammatory macrophages and the TREM2^{+} foam cell-like macrophages[15]. Another study suggests that some human macrophages acquire wound-healing properties after plaque rupture[14]. Identification of the precise role of each of the aortic macrophage subsets in modulating atherosclerosis, together with the characterization of their functions and crosstalk with other immune cells such

as T cells and DCs, will be crucial for understanding their relevance to atherosclerosis progression.

Dendritic cells.

Both conventional DCs (cDCs) and plasmacytoid DCs (pDCs) have been detected in mouse and human arteries and atherosclerotic lesions[53]. $CD103^+$ type 1 cDCs are derived from $FLT3^+$ migratory pre-cDCs[54] and are similar to the lymphoid-resident $CD8\alpha^+$ DCs[55]. $CD11b^+$ type 2 cDCs are the most abundant aortic DC subset and also include monocyte-derived DCs. An inflammatory $CD11c^+CD11b^+CD8\alpha^-$ CCL17-secreting DC subset, expressing high levels of the co-stimulatory molecules CD40, CD80 and CD86, has been described specifically in atherosclerotic lesions but not in healthy vessels[56].

cDCs take up antigens in the vessel wall and activate antigen-specific naive T cells in draining lymph nodes or the spleen. Although DCs are present in the intimal layers of normal mouse and human arteries, their numbers are greatly increased and their phenotypes are markedly altered under atherosclerotic conditions[53]. Multiphoton live-cell imaging of aortic explants from atherosclerotic mice that were incubated with fluorescently labelled transgenic T cells showed significantly increased productive interactions between aortic CD11c–YFP^+ antigen-presenting cells (APCs) and T cells in the vessel wall only in the presence of the cognate antigen[57]. Polyclonal antigen-experienced $CD44^{hi}CD4^+$ T cells isolated from spleens of atherosclerotic mice, but not control mice, vigorously interacted with APCs in explanted aortas of atherosclerotic mice in the absence of any external antigen stimulation, which suggests that the T cells have a productive recall response to unidentified atherosclerosis epitopes[57]. Incubating ex vivo-isolated CD11c–$EYFP^+$ aortic APCs with ovalbumin-specific $CD8^+$ T cells and $CD4^+$ T cells triggered antigen-specific proliferation of both T cell subsets in the presence of ovalbumin, which indicates that aortic DCs can cross-present MHC class I-restricted antigens to $CD8^+$ T cells[58]. It is likely that under non-inflammatory conditions, DCs from healthy arteries present self-antigens in the absence of adequate co-stimulation, leading to T cell tolerance. However, in atherosclerotic lesions, the maturation of plaque DCs in the presence of Toll-like receptor (TLR) agonists, danger-associated ligands and pro-inflammatory cytokines probably favours a pro-atherogenic activation status. In lesions with necrotic cores, CLEC9A-dependent sensing of necrotic cells by $CD8\alpha^+$ DCs promotes atherosclerosis by restraining anti-inflammatory IL-10 production[59]. Accumulation of plaque $CD11b^-CD103^+IRF8^{hi}$ DCs derived from a $CLEC9A^+$ precursor contributes to atherogenesis by promoting pro-inflammatory T cell activity[60].

T cell activation by DCs is dependent on TLR-mediated maturation of DCs. Bone marrow transplantation from *Cd11c*–Cre^+*Myd88*$^{fl/fl}$ mice — which have $CD11c^+$ DCs deficient in the TLR adaptor MYD88 — into atherosclerotic mice led to decreased recruitment of both effector T cells and T_{reg} cells to atherosclerotic lesions[61]. The overall effect was larger lesion size, which was attributed to increased production of the monocyte-recruiting chemokine CCL2 as a result of the loss of T_{reg} cells[61]. Different cDC subtypes disparately modulate T_{reg} cell homeostasis. Increased generation of pro-inflammatory cytokine transcripts and increased average lesion size in the aortas of atherosclerotic mice

depleted of $CD103^+$ DCs were associated with a decrease in both the number of aortic T_{reg} cells and IL-10 secretion, suggesting that $CD103^+$ DCs may be potent inducers of T_{reg} cells[54]. With use of bone marrow transplants and RNA sequencing, a tolerogenic role of $CD11b^+CD11c^+$ DCs in transforming growth factor-β (TGFβ)-mediated induction of antigen-specific T_{reg} cells was demonstrated in atherosclerotic mice in which autophagy was disrupted through DC-specific abrogation of *Atg16l1* (REF.[62]). Unlike $CD11b^+$ DCs, autophagy disruptions in $CD8\alpha^+$ cDCs or $CD103^+$ cDCs did not induce a tolerogenic phenotype in these subsets. This showed that the previously reported atheroprotective role of $CD103^+$ DCs is not autophagy dependent, highlighting mechanistic differences in the regulatory network that modulates the tolerogenic properties of different DC subsets[62]. Inflammatory $CD11c^+CD11b^+CD8\alpha^-CCL17^+$ DCs inhibit T_{reg} cell development[56].

Atherosclerotic lesions also contain pDCs, albeit in low numbers. Plaque pDCs respond not only to CpG oligonucleotides of microbial origin but also to extracellular self-DNA complexed with cathelicidin-related antimicrobial peptide (CRAMP; encoded by *Cnlp*, also known as *Camp*), which is the mouse orthologue of human cathelicidin antimicrobial peptide (CAMP; also known as LL-37)[63]. Breaching immune tolerance to self-DNA complexed with CRAMP in these atherosclerotic mice increased antibody titres to double-stranded DNA and promoted atherogenesis through interferon-α (IFNα) production. Abrogation of antigen presentation in pDCs through MHC class II deficiency resulted in atheroprotection, which was independent of IFNα and was associated with inhibition of T helper 1 (T_H1) cell responses and T cell infiltration into lesions[64]. Other studies have reported an anti-atherogenic role of pDCs, associated with indoleamine 2,3-dioxygenase 1 (IDO1)-dependent induction of aortic T_{reg} cells[65,66]. These conflicting results are probably owing to differences in pDC identification, depletion strategies and mouse models of atherosclerosis used.

We conclude that both cDCs and pDCs can modulate atherogenesis in mice, in part through regulation of T cell activation and adaptive immune responses, in an antigen-dependent manner. The exact mechanisms by which DCs exert such control require further elucidation. Studies of DCs in human atherosclerosis have been observational so far, and their functional role remains to be characterized.

Adaptive immune cells

Adaptive immunity is a key modulator of atherosclerosis (FIG. 2). Here, we focus on recent preclinical data that highlight the role of MHC class II-mediated activation of $CD4^+$ T cells by various APCs (BOX 5) and the relative contributions of the most widely studied subclasses of $CD4^+$ T_H cells. The roles of $CD8^+$ T cells and lipid antigen-specific natural killer T cells and γδ T cells in atherosclerosis are summarized in BOX 2. The roles of antibodies and B cells in atherosclerosis have been reviewed elsewhere[67].

T cells are present in the adventitia of healthy arteries of wild-type mice[68] and the arteries of mouse models that are genetically prone to develop atherosclerosis[46]. scRNA-seq studies have detected aortic T cells at all stages of atherosclerotic disease[8]. Chemokine receptors such as CCR5 and CXCR6 mediate the infiltration of T cells into the plaque[69,70]. CCL5,

the main ligand for CCR5, is found abundantly in atherosclerotic lesions[70]. Circulating activated platelets produce CCL5 and deliver it to the vessel wall through the formation of platelet–monocyte aggregates[71]. Blocking the binding of CCL5 to CCR5 with monoclonal antibodies to the ligand or receptor resulted in reduced infiltration of effector $CD4^+$ T cells into aorta explants[70]. CXCL16, which is expressed as both soluble and membrane-bound proteins in mouse and human lesions, is produced by several types of immune cell, including macrophages, DCs, aortic VSMCs and endothelial cells[72]. The CXCR6-dependent chemotaxis of T cells is likely to be mediated by soluble CXCL16 (REF.[69]).

$CD4^+$ T cell subpopulations can differentially impact atherosclerosis progression through immune activation or immune suppression, or by providing help to B cells for antibody production[73]. Co-stimulatory molecules and immune checkpoint proteins have been reported to have a pivotal role in modulating atherogenesis[74]. The prevailing notion is that regulatory T cells, such as $FOXP3^+$ T_{reg} cells and $IL\text{-}10^+$ type 1 regulatory T cells (Tr1 cells), dominate before disease onset but that pro-inflammatory $CD4^+$ effector T cells outcompete them after the onset of atherosclerosis.

T_H1 cells.

IFNγ-secreting T_H1 cells, which express the lineage-defining transcription factor T-bet (encoded by *Tbx21*), are the most prominent $CD4^+$ T cell subtype in atherosclerotic plaques. Several lines of evidence, including genetic deficiency of T-bet, IFNγ or its receptor, or administration of exogenous IFNγ, have established a pro-atherogenic role of T_H1 cells in promoting lesion development and plaque instability[75]. TNF, which is expressed by multiple effector T cell types, promotes lesion development[76], in part by facilitating leukocyte transmigration across vascular endothelial cells[77]. IFNγ secreted in the developing lesion induces uptake of oxidized LDL (oxLDL) and foam cell formation, but foam cells have little inflammatory potential[31,32]. IFNγ can also polarize macrophages towards a pro-inflammatory phenotype and promote VSMC proliferation[73,76].

T_{reg} cells.

Foxp3-expressing $CD25^+$ T_{reg} cells and the anti-inflammatory cytokines IL-10 and TGFβ correlate negatively with atherogenesis[17]. In atherosclerosis-prone *Foxp3*–GFP^+ reporter mice, fewer aortic T_{reg} cells were found after prolonged administration of a cholesterol-rich diet than a normal diet[78]. However, the number of GFP^+ cells reported in that study is higher than the entire leukocyte content of atherosclerotic mouse aortas, which suggests that there might have been contamination from adjacent adipose tissue. Defective recruitment of T_{reg} cells in atherosclerosis-prone mice fed a cholesterol-rich diet was associated with reduced selectin-mediated binding to the endothelial lining[78]. In transgenic mice, diphtheria toxin-mediated depletion of T_{reg} cells under hypercholesterolaemic settings led to increased lesion size[79]. Surprisingly, T_{reg} cell depletion also induced pro-atherogenic shifts in the plasma cholesterol profile through impaired lipoprotein catabolism in the liver, which was linked to reduced expression of the very low density lipoprotein-binding protein sortilin 1 (REF.[79]). This complicates interpretation of the results, and the direct effect of T_{reg} cell depletion on vascular inflammation remains to be investigated.

Three different mouse models of atherosclerosis regression — delivery of antagonistic *Apob*-antisense oligonucleotide[80], inducible genetic deletion of microsomal triglyceride transfer protein ('Reversa' mice)[41] and surgical transplantation of atherosclerotic aortic arches[81] — suggested that the regression of established plaques is associated with a restoration of plaque T_{reg} cell numbers, mostly peripherally induced T_{reg} cells that do not express neuropilin 1 (NRP1)[82]. It is not clear whether these $NRP1^-$ peripherally induced T_{reg} cells are derived from local activation of naive T cells in the vessel wall or are generated in secondary lymphoid organs upon encountering aorta-derived tolerogenic DCs. However, these data indicate that plaque regression is associated with an anti-inflammatory microenvironment that can support the local expansion of tolerogenic immune cells[82]. T_{reg} cell enrichment in regressing plaques, but not lipid lowering alone, led to macrophage-mediated dampening of inflammation and tissue repair through improved efferocytosis and enhanced synthesis and sensing of pro-resolving lipid mediators such as lipoxin A4 and the resolvins RvD1 and RvD6 (REF.[82]).

T_H2 cells.

Transcripts of T_H2 cell-related cytokines, such as IL-4, IL-5 and IL-13, are expressed in mouse plaques. IL-4 seems to have variable effects on atherogenesis[83,84], whereas IL-5 and IL-13 are atheroprotective[73]. IL-5 can induce the production of oxLDL-targeting IgM antibodies by B1 cells[85]. Antibody-dependent clearance of apoptotic cells dampens inflammation and prevents necrotic core formation[86]. An inverse relationship between IL-5 and atherosclerosis is evident particularly in those areas of the artery where blood flow is oscillatory and shear stress is low[87]. IL-13 deficiency, under conditions of hypercholesterolaemia, resulted in larger lesions and accelerated atherosclerosis progression[88]. Conversely, low-dose IL-13 stabilized existing plaques through increased collagen formation, inhibition of VCAM1-dependent monocyte infiltration and induction of M2-type macrophages that clear oxLDL more efficiently than pro-inflammatory M1-type macrophages[88]. Recently, group 2 innate lymphoid cells, which are an innate cell source of IL-5 and IL-13, have also been reported in atherosclerosis[8,89] (BOX 2). Further studies involving cell-type-specific deficiency of T_H2 cell-related cytokines are needed to clarify the relative contributions of innate lymphoid cells and T cells.

T_H17 cells.

The role of T_H17 cells in atherosclerosis remains controversial, partly owing to the context-dependent plasticity of T_H17 cell function. The numbers of T cells expressing IL-17 or the T_H17 cell lineage-defining transcription factor RORγt (encoded by *Rorc*) were positively correlated with atherosclerosis in mice[90]. Furthermore, ablation of IL-17A–IL-17 receptor A (IL-17RA) signalling in atherosclerotic mice using neutralizing antibodies to IL-17A, bone marrow transplants from *Il17a*$^{-/-}$ mice or adenovirus-mediated in vivo expression of soluble IL-17RA inhibited pro-inflammatory cytokine and chemokine production, leukocyte infiltration and plaque formation[69,90–92]. However, a plaque-stabilizing role of IL-17A has also been documented[93,94]. Deficiency of TRIM21, a ubiquitin E3 ligase involved in inflammation, leads to an increased T_H17 cell response in hypercholesterolaemic settings[95]. These TRIM21-deficient T_H17 cells were associated with a non-pathogenic gene expression profile and led to the formation of larger but stabler plaques[95].

T follicular helper cells.

A largely pro-atherogenic role of T follicular helper (T_{FH}) cells has been reported in atherosclerosis-prone mice[73]. T_{FH} cell responses regulate humoral immunity, antibody isotype switching and antibody affinity maturation. Exaggerated T_{FH} cell activity can trigger autoimmunity[96]. In models of atherosclerosis and autoimmune lupus, dyslipidaemic conditions were shown to augment lupus-related autoantibody formation and the differentiation of pro-inflammatory $CXCR3^+$ T_{FH} cells through production of IL-27, possibly released from TLR4-activated $CD8\alpha^-CD11b^+$ DCs[97]. An immunomodulatory role of $CD8^+$ regulatory T cells[98] and marginal zone B cells[99] in restraining T_{FH} cell differentiation and germinal centre reactions has been reported, but the detailed roles of those cell types in atherosclerosis development remain unknown.

T cell plasticity.

Atherosclerosis-related T cells are not only heterogeneous but also plastic. This means that one $CD4^+$ T cell subtype can acquire the transcriptomic and phenotypic properties of another subtype. Recent studies, using in-depth gene expression analysis and fate-mapping transgenic mouse models, have convincingly illustrated that atherogenic conditions can trigger functional plasticity of *Foxp3*-expressing T_{reg} cells such that they begin to express effector T cell-specific lineage-defining transcription factors, cytokines and chemokine receptors[70,100,101]. In some of these studies, T_{reg} cells became unstable and lost *Foxp3* expression to become ex-T_{reg} cells[101]. The overall effect is a progressive loss of T_{reg} cell-mediated immune suppression, which skews the balance towards a state of unrestrained inflammation. T_{reg} cell plasticity and instability, their possible mechanisms of action and their roles in atherosclerosis were recently reviewed[102].

Antigen specificity.

B cells and T cells are activated by antigen presentation. DCs that have acquired atherosclerosis-related antigens can leave the atherosclerotic artery wall, most likely through the draining lymphatics[103], and present antigens to B cells and T cells in draining lymph nodes. Indeed, axillar and cervical lymph nodes draining the carotids and the aortic arch are enlarged and contain more cells in atherosclerotic mice[75]. In older (>1 year) mice, organized arterial tertiary lymphoid organs in the aortic adventitia contain germinal centres, in which affinity maturation and isotype switching of B cell responses can occur[104].

Although there is evidence for oligoclonal proliferation of and expression of activation markers in plaque-associated T cells[14,73], it has been technically challenging to identify their antigen specificity. Multiphoton live-cell imaging showed that antigen-specific and MHC class II-restricted productive interactions between $CD4^+$ T cells and APCs occur in the aortic walls of atherosclerotic mice[57]. This highlighted the presence of both endogenous autoantigen and antigen-experienced $CD4^+$ T cells in $Apoe^{-/-}$ mice. Numerous studies involving atherosclerotic mice and peptide-immunization models have established that APOB, the core protein component of LDL, is a major atherosclerosis antigen[105]. Tetramer staining and ex vivo peptide restimulation assays have detected APOB-specific T cells in mice and humans[106,107]. Analysis of lineage-defining transcription factors, cytokine secretion potential and scRNA-seq of APOB-reactive T cells revealed a prevalence of pro-

inflammatory T_H1 cell and T_H17 cell phenotypes under atherosclerotic conditions[106,107]. Further in-depth phenotypic profiling of APOB-specific T cells in the plaque and in the blood by scRNA-seq and identification of the immunodominant epitopes that drive this pro-inflammatory T cell response will be crucial for understanding and modulating the autoimmune circuit in atherosclerosis.

Microbial and sterile triggers

Inducers of immune cell activation can be broadly classified as microbial pathogen-associated molecular patterns or endogenous danger-associated molecular patterns[108]. Recognition of these molecules by pattern recognition receptors such as TLRs and scavenger receptors results in the activation of signalling pathways and expression of pro-inflammatory cytokines such as TNF, IL-6, IFNγ and type I interferons[109–111]. Signalling through several pattern recognition receptors also culminates in the priming and activation of the NLRP3-dependent inflammasome cascade, which triggers caspase 1 activation, generation of the pro-inflammatory cytokines IL-1β and IL-18, and pyroptosis[108,112,113]. Here, we provide an overview of the microbial and host-derived inflammatory cues that have been implicated in atherogenesis.

Role of infectious agents and the host microbiota.

Microorganisms can mediate chronic inflammation either through direct infection and activation of vascular cells or through indirect induction of a systemic immune response to infections at non-vascular locations[114]. Seroepidemiological studies and sequencing of microbial DNA from atherosclerotic lesions have demonstrated an association between clinical atherosclerosis and infectious agents, including bacteria such as *Chlamydia pneumoniae*, *Helicobacter pylori*, *Porphyromonas gingivalis* and *Streptococcus sanguis* and viruses such as influenza A virus, cytomegalovirus, hepatitis C virus, HIV-1, herpes simplex viruses, Epstein–Barr virus, enteroviruses and parvovirus[114]. Although most of these studies were correlative, some microorganisms have been confirmed to promote atherosclerosis progression in mouse models[114,115]. Infection with *C. pneumoniae* was shown to breach immune tolerance through molecular mimicry, which was most evident in the case of human and bacterial forms of heat shock protein 60 (HSP60), which have significant sequence homology[116]. In a transgenic model of HIV-1 infection under atherogenic conditions, the acceleration of atherosclerosis progression by viral products was linked to inflammasome activation in monocytes and macrophages[117]. Thus, both bacterial and viral modulators may impact atherosclerosis by activating pro-atherogenic innate and adaptive immune responses. The mechanisms through which infections have been proposed to influence lesion formation, growth and rupture have been extensively described elsewhere[114,115,118].

The ‘infection hypothesis’ for atherogenesis led to several randomized, placebo-controlled trials that investigated whether macrolide antibiotics, which target *C. pneumoniae*, have any beneficial impact on clinical outcomes in patients with coronary artery disease. The results of these trials were unanimously negative[119], showing that macrolide antibiotics are not atheroprotective. However, antibiotic therapy does not address possible contributions to atherogenesis from viruses[114,115]. Also, it seems unlikely that a single pathogen, such

as *C. pneumoniae*, would by itself affect the pathogenesis of a complex disease such as atherosclerosis. Antibiotics can also affect commensal microorganisms, which could have pro-atherogenic or anti-atherogenic effects.

In addition to infectious agents, recent studies have demonstrated a potential role of the host microbiota in influencing cardiovascular disease progression[120]. Postprandial increase in gut permeability in mice was shown to result in leakage of microbiota-derived endotoxins such as lipopolysaccharide into the circulation, which triggered a low-grade inflammatory response[121]. Furthermore, stress and dietary components can shape the composition of the gut microbiota, with some bacteria being able to mediate the production of pro-inflammatory metabolites such as trimethylamine *N*-oxide (TMAO) that have been associated with cardiovascular disease risk[120]. Trimethylamine, derived from microbial metabolism of dietary components such as choline, phosphatidylcholine and L-carnitine, can be converted to TMAO by liver flavin monooxygenases. Screening of isolates from the human gut has identified eight species of trimethylamine-producing bacteria, all of which are members of the phylum Firmicutes or proteobacteria[122]. The exact mechanism by which TMAO promotes atherosclerosis still remains speculative, possibly involving changes in cholesterol trafficking, induction of vascular inflammation[123–125] and platelet hyperactivation[126]. Multiple studies have explored the potential to use TMAO either as a prognostic biomarker[127–129] or as a target for therapy[130,131]. Some reports[132,133] in animal models have raised the question of whether TMAO has a causal role in atherogenesis. Furthermore, its potential role as a cardiovascular risk factor may be confounded by its possible involvement in kidney disease and diabetes[134]. Multi-omics association studies[135] in large cohorts and rigorous validation in animal models will be necessary to clarify the existing discrepancies.

Regular exercise and consumption of dietary fibres boost the levels of microbial metabolites such as short-chain fatty acids, particularly acetate, propionate and butyrate, which can reduce cardiovascular disease-associated risk factors, including high blood pressure, and regulate lipid homeostasis[120]. Butyrate derived from *Roseburia intestinalis* has been shown to correlate inversely with inflammation and atherosclerotic lesion size[136]. The cytokines IL-22 and IL-23 have an atheroprotective role by restraining the growth of a pro-atherogenic microbiota, which also decreases TMAO levels in the blood[137,138].

Endogenous triggers of inflammation.

Immune cells in atherosclerotic lesions can also be activated by self-molecules that become immunogenic[86,139]. OxLDL, glycated proteins, lipids and nucleic acids in the vessel wall, crystalline cholesterol deposits, and cell death-associated molecules such as heat shock proteins are some of the best-characterized danger-associated molecular patterns implicated in promoting atherosclerosis progression[108,140]. Stimulation of TLRs, particularly TLR2 and TLR4, activates distinct transcriptional programmes in plaque macrophages that trigger the release of pro-inflammatory cytokines and chemokines[108,111]. Some endogenous TLR ligands in atherosclerotic lesions, including oxLDL, are also actively recognized by scavenger receptors[86]. SR-A (also known as MSR1) and CD36 have the highest affinity for oxLDL and have a major role in modulating atherosclerosis[141]. CD36 can trigger

macrophage differentiation into foam cells through the phosphorylation and activation of SRC and the MAPK pathway and can also promote the conversion of endocytosed oxLDL, amyloid-β and amyl peptides into crystals[142]. Crystalline cholesterol triggers NLRP3-dependent inflammasome activation[112] to produce IL-1β. Tissue damage and cell death can also induce inflammasome activation through the release of TLR ligands such as heat shock proteins and secondary inducers such as ATP and uric acid, which contribute to priming and activation of inflammasomes, respectively[113]. Although some studies have refuted the involvement of the NLRP3 inflammasome in atherogenesis[143], administration of NLRP3 inflammasome inhibitors significantly reduced lesion development in mouse models[144]. Furthermore, the CANTOS trial, which tested the efficacy of IL-1β blockade, provided the first clinical evidence that pro-inflammatory immune pathways can be viable targets for the prevention of cardiovascular events in humans[4,110].

Towards an atherosclerosis vaccine

Recent insights into the roles of innate and adaptive immune cells in atherosclerosis have prompted a flurry of research activities and clinical trials to test the viability of immunomodulatory therapies in cardiovascular diseases. Tolerogenic vaccination has emerged as a possible strategy to boost antigen-specific, anti-atherogenic humoral and cell-mediated immune responses in atherosclerosis[19].

Some immunization strategies in atherosclerosis aim to induce neutralizing antibodies that inhibit proteins associated with increased cardiovascular risk. This is the case for the secreted serine protease PCSK9, which interferes with LDL uptake in the liver by blocking LDL receptor recycling[145]. The approval of two fully human monoclonal antibodies to PCSK9 has initiated clinical trials to evaluate the safety and efficacy of human PCSK9 vaccines in lowering lipid levels through the induction of a long-term humoral response (ClinicalTrials.gov identifier NCT02508896). By contrast, tolerogenic vaccines aim to restrain atherosclerosis by mobilizing T_{reg} cells and restoring immune tolerance[146] Most of the tolerogenic vaccination efforts in mouse models have focused either on LDL or on its core lipoprotein APOB. Immunization with human oxLDL induces antigen-specific $CD4^+$ T cells in transgenic mice that express human APOB[147]. Multiple studies have established that vaccinating mice with murine APOB peptides can reduce vascular inflammation, stabilize plaques and restrain disease progression in atherosclerotic mice[146]. Such atheroprotection was mediated by T_{reg} cells and Tr1 cells that suppress inflammatory cytokine production by effector T cells[19,146].

Immunization of atherosclerotic mice with the mouse APOB peptide p18, which is identical in sequence to a peptide in human APOB, reduced aortic lesion size and induced IL-10-producing $CD4^+$ T cells and $FOXP3^+$ T reg cells[106] This work provided evidence that human APOB-derived peptides could be atheroprotective and that vaccine-based therapeutic strategies could potentially be translated to the clinic. However, p18 is only one autoreactive MHC class II-restricted epitope mapped in a single atherosclerosis antigen, APOB. Identifying the autoreactive epitopes that may be driving the adaptive immune response in human atherosclerosis is technically challenging. First, autoantigen-specific T cells are rare and therefore hard to detect. Currently, only one human APOB epitope has

been fully validated using tetramer technology[106]. Second, the use of tetramers is limited, because they require a priori knowledge of epitope specificity and HLA allelic restriction, are limited to one MHC allele at a time, are hard to validate and difficult to multiplex, and are expensive in both time and resources. To overcome this limitation, we recently introduced a restimulation-based assay that enabled simultaneous interrogation of APOB-specific effector T cell responses to 30 APOB-derived epitopes with heterogeneous HLA specificities[107]. Peptide stimulation of peripheral blood mononuclear cells elicited increased production of the pro-inflammatory cytokines IL-17, IFNγ and TNF by $CD4^+$ T cells from patients with coronary atherosclerosis but not from controls. Such restimulation assays promise to facilitate the discovery of epitope specificities, T cell phenotypes and interaction networks in human atherosclerosis. Whereas some epitopes can induce pro-inflammatory immune responses associated with adverse disease outcomes, other epitopes can elicit anti-atherogenic T_{reg} cell responses. To translate tolerogenic vaccine-based immunotherapy from mice to humans, a comprehensive list of immunomodulatory epitopes that can reliably induce T_{reg} cells and Tr1 cells must be identified for human APOB and other atherosclerosis antigens. Different tolerogenic adjuvants, administration routes and doses need to be tested in clinical trials. Strategies to stabilize vaccination-induced T_{reg} cells are currently being explored in clinical trials[148].

Conclusions

Atherosclerosis involves a complex interplay between metabolic imbalances and maladaptive immune responses that drive a state of chronic inflammation in the artery wall. While our understanding of the immune-mediated processes in atherosclerosis will continue to evolve, recent research has vastly expanded our knowledge of the immune cell interaction network, mainly through high-dimensional methods such as genome-wide association studies[149], mass cytometry[46,150], scRNA-seq[31,45,46,48] and combined protein and RNA sequencing[14]. Interactions between pro-atherogenic conditions and haematopoiesis (BOX 3), immune cell metabolism (BOX 4) and the microbiota are now firmly established. This new knowledge has not only improved our mechanistic understanding of the disease pathophysiology but has also fuelled new research efforts aimed at developing strategies for immunomodulatory therapies. These have included cytokine targeting, adoptive transfer of regulatory cells, passive immunization with monoclonal antibodies and tolerogenic vaccines. However, there are major obstacles to the translation of interventions in animal models to the clinic, some of them related to the limited extent to which mouse models recapitulate human atherosclerosis (BOX 1). Recent advances in multidimensional approaches promise in-depth characterization of the cellular and molecular mediators of human atherosclerosis. Bioinformatics-based integration of datasets from plaque cells and blood cells at the genomic, transcriptomic, proteomic and metabolomic levels will help to define the human immune atlas of atherosclerosis in a more holistic manner. Large-scale longitudinal studies involving multiple cohorts will be necessary to establish which imaging, cellular and molecular biomarkers truly correlate with disease progression after confounding factors have been accounted for.

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Glossary

Major adverse cardiovascular events
(MACEs). A composite end point frequently used in cardiovascular research that may include clinical complications such as myocardial infarction, stroke, heart failure, need for coronary revascularization, recurrent angina and death from cardiovascular disease.

Low-density lipoprotein
(LDL). A group of apolipoprotein B-containing macromolecular lipid carriers in the blood that transport cholesterol and triglycerides from the liver to other body tissues.

Atherothrombosis
The state of pathological complication when erosion or rupture of atherosclerotic plaques leads to thrombosis or clot formation, resulting in stroke or myocardial infarction.

Atherogenesis
The process of atherosclerotic plaque formation in the intimal layers of the artery, which is mediated by chronic inflammation and lipid deposition in the vessel walls.

Apolipoprotein B
(APOB). The amphipathic protein backbone of most lipid-transport particles in the plasma, including very-low-density lipoprotein, low-density lipoprotein and chylomicrons.

Efferocytosis
The process by which apoptotic cells are engulfed and cleared by phagocytic cells.

Classical monocytes
Ly-6C^{+} monocytes in mice and the CD14^{hi}CD16^{-} subset in humans. They are CCR2^{hi}Cx3CR1^{low} and are important mediators of tissue inflammation.

Non-classical monocytes
Ly-6C^{-} monocytes in mice and the CD14^{low}CD16^{+} subset in humans. They are CCR2^{low}Cx3CR1^{hi} and patrol the vessel walls to maintain endothelial integrity and homeostasis.

High-density lipoprotein
This is the only group of lipoproteins that do not contain apolipoprotein B. High-density lipoprotein is involved in reverse cholesterol transport, delivering excess cholesterol from tissues to the liver.

Inflammasome

A multiprotein cytosolic complex containing members of the NOD-like receptor (NLR) family (such as NLRP3) that integrates signals from several pattern recognition receptors and results in the maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18.

Trained immunity
The reprogramming of innate immune cells, mostly through epigenetic modifications, that creates a 'memory' of the initial insult and generates long-lasting innate immunity to specific triggers.

Very-low-density lipoprotein
The primary transporter of endogenous triglycerides from the liver to other tissues in the body.

Peripherally induced T_{reg} cells
Regulatory T (T_{reg}) cells that arise outside the thymus from conventional T cells that acquire FOXP3 expression in response to various stimuli. Unlike thymus-derived T_{reg} cells, peripherally induced T_{reg} cells do not express neuropilin 1.

Tertiary lymphoid organs
Lymphoid structures that form in peripheral tissues in response to chronic inflammation and that have functional and morphological similarities with secondary lymphoid organs.

Molecular mimicry
The possible cross-reactive activation of autoreactive B cells or T cells that are specific for self-derived epitopes that have sequence similarity or structural homology with pathogen-derived foreign epitopes.

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Box 1 |

Limitations of mouse models of atherosclerosis

Coronary artery disease

The commonly used mouse models of atherosclerosis, *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice, do not develop coronary artery disease. Spontaneous atherothrombotic events resembling myocardial infarction or stroke do not occur in these mice. However, several mouse strains that have mutations in the high-density lipoprotein receptor SR-BI or its adaptor protein PDZK1 (REFS[153,154]) do develop occlusive coronary arterial atherothrombosis, myocardial infarction, heart failure and premature death. In some of these mice, the disease develops spontaneously when they are fed a standard diet (low fat and low cholesterol), whereas other mice develop the disease only after being fed an atherogenic diet. These mice are available from the Jackson Laboratory (B6;129S-*Scarb1*^{tm1Kri}/J mice and B6;129-*Scarb1*^{tm1Kri}*Apoe*^{tm1Unc}/J mice) but are not widely used.

Lipoproteins

Blood lipoprotein profiles in mice are characterized by high levels of high-density lipoprotein, low levels of low-density lipoprotein, and some very-low-density lipoprotein and intermediate-density lipoprotein[155]. By contrast, humans have high levels of low-density lipoprotein. Total cholesterol levels in *Apoe*$^{-/-}$ mice fed a Western-style (high-fat, high-cholesterol) diet are extremely high (~1,000 mg dl^{-1}), levels that are not observed in humans except in cases of genetic familial hypercholesterolaemia. Also, the genetic background of the mice matters. Most atherosclerosis studies are conducted in a single mouse strain (C57BL/6), which cannot capture the genetic diversity seen in humans. In *Apoe*$^{-/-}$ mice on the C57BL/6 background, atherosclerotic lesions are larger in female mice than in male mice, whereas premenopausal female humans are protected from atherosclerosis[156].

Genetics

In C57BL/6 knockout mice, contamination with 129/Sv DNA near the targeted gene is possible if embryonic stem cells from the 129/Sv mouse strain were used to generate the knockout mice. This 129/Sv genetic contamination can affect the susceptibility to atherosclerosis[157]. Genetic diversity is known to modulate the response to antigens and atherosclerosis-relevant stimuli within a spectrum of responses ranging from pro-inflammatory to anti-inflammatory[158]. For example, a study of more than 100 inbred mouse strains from the hybrid mouse diversity panel found very large differences in atherosclerosis susceptibility and sex distribution[159].

Evolution of the immune system

Some cytokines, chemokines and immune receptors (for example, CXCL8 and CXCR1) are not conserved between mice and humans because the immune systems of both species are under intense evolutionary pressure. Mice represent a simplified model system for antigen presentation and recognition; whereas C57BL/6 mice have just one MHC class II protein with one allele (I-Ab), humans express several alleles of more than 10,000 MHC class II variants.

Diet and gut microbiota

The levels of cholesterol in different diets (chow, Western, high-cholesterol or cholate-containing diets) used for experimental studies of atherosclerosis can directly affect the phenotype and proliferation of T cells[78,160,161]. Furthermore, mice are housed in specific-pathogen-free facilities, which influences immune cell activation, differentiation and formation of their antigenic repertoire in response to microbial ligands[162]. The gut microbiota of mice differs between animal facilities and even between cages in the same facility[162]. The gut microbiota can have large effects on atherosclerosis development and progression[163,164].

Box 2 |

Role of innate lymphoid cells and unconventional T cells in atherosclerosis

Innate lymphoid cells (ILCs) include the cytotoxic natural killer (NK) cells and the non-cytotoxic group 1, group 2 and group 3 ILCs (ILC1s, ILC2s and ILC3s). Limited numbers of NK cells have been detected in mouse aortas before and after the onset of atherosclerosis[8] and in human lesions[165]. Antibody-based depletion of NK cells and adoptive transfer studies suggest that perforin and granzyme B produced by NK cells promote lesion growth in atherosclerotic mice[166]. However, a direct effect of NK cells on cholesterol-induced atherosclerosis was not confirmed when more precise NK cell loss-of-function genetic models were used[167]. ILC1s, ILC2s and ILC3s share transcription factor and cytokine expression profiles with T helper 1 (T_H1) cells, T_H2 cells and T_H17 cells, respectively. As a result of this overlap, mechanistic studies exploring the relative contributions of ILCs and T_H cells in atherosclerosis are scarce[73]. Recently, genetic depletion of ILC2s and transfer of specific ILC2 subsets have shown their direct role in mediating atheroprotection[89].

Mass cytometry and single-cell RNA-sequencing studies have recently shown that $CD8^+$ T cells accumulate in atherosclerotic mouse aortas[8] and are significantly enriched in human plaques[14]. Interferon-γ, perforin and granzyme B production by $CD8^+$ T cells promote vascular inflammation and lesion growth through apoptotic cell lysis[168,169] and induction of monopoiesis[170]. Although some $CD8^+$ T cells recognize apolipoprotein B-derived epitopes[171], the antigen specificities of most $CD8^+$ T cells in atherosclerotic plaques remain unknown[172]. Minor populations of invariant NK T cells have also been described in atherosclerosis[173]. These cells can be activated by self-antigens or microbial glycolipid antigens presented by CD1d molecules on antigen-presenting cells or by cytokines such as IL-12 and IL-18, which are secreted by Toll-like receptor-stimulated antigen-presenting cells. T_H cell-associated cytokines and cytotoxic molecules produced by activated NK T cells promote inflammation and necrotic core formation[174]. γδ T cells also respond to innate immune stimulants and produce inflammatory cytokines. Although γδ T cells are found in mouse atherosclerotic lesions[175], genetic deficiency of γδ T cells had no effect on the development of diet-induced atherosclerosis in mice[176].

Box 3 |

Role of haematopoiesis in atherosclerosis

The development of atherosclerosis depends on the haematopoietic supply of immune cells, particularly classical monocytes[25]. In turn, hypercholesterolaemia, stress and inflammation associated with atherosclerosis induce the proliferation and myeloid-biased differentiation of haematopoietic stem and progenitor cells (HSPCs) in the bone marrow, the release of immune cells into the circulation and the diversion of haematopoiesis to extramedullary sites[177]. Mechanistically, cholesterol enrichment in membrane lipid rafts triggers the myeloproliferation of HSPCs through increased sensitivity to IL-3 and granulocyte–macrophage colony-stimulating factor (GM-CSF) and increased RAS–ERK signalling[178]. Proteoglycan-bound apolipoprotein E was shown to regulate cholesterol efflux from HSPCs and inhibit their growth factor-induced hyperproliferative response in a cell-intrinsic manner[179]. Hypercholesterolaemia can also promote a loss of cellular quiescence in HSPCs and skewing towards pro-inflammatory myeloid cell types, a cell-intrinsic effect that was maintained even after return to normocholesterolaemic conditions[180]. During atherosclerosis, myeloid-biased haematopoiesis in the GM-CSF-rich and IL-3-rich environment of the splenic red pulp significantly contributes to the systemic abundance of inflammatory monocytes and neutrophils[181]. Granulocyte colony-stimulating factor-dependent mobilization of HSPCs to the red pulp can be triggered by a high-fat diet and impaired cholesterol efflux, which elicits IL-23 production by splenic dendritic cells and macrophages[182]. Activation of the sympathetic nervous system in acute cardiovascular events, such as myocardial infarction, can also release HSPCs from the bone marrow and drive extramedullary myelopoiesis[183]. Noradrenaline-dependent sympathetic nervous system signalling promotes proliferation of HSPCs and augments plaque inflammation under conditions of chronic stress[184]. Disease-promoting lifestyle choices such as inadequate sleep[185] and insufficient exercise[186] can impact atherosclerosis progression by influencing haematopoiesis[187]. Finally, ageing increases the likelihood of clonal haematopoiesis of indeterminate potential, whereby somatic mutations in *DNMT3A*, *TET2* and *ASXL1* lead to the formation of a genetically distinct subpopulation of haematopoietic cells, which is strongly associated with increased risk of cardiovascular events[188].

Box 4 |

Modulation of atherogenesis by immune cell metabolic pathways

Hypoxia and lipid imbalances can influence immune cell phenotypes through metabolic rewiring[189]. Pro-inflammatory macrophages and T cells favour catabolic processes such as aerobic glycolysis, fatty acid synthesis, the pentose phosphate pathway and glutaminolysis. Indeed, increased glucose uptake by plaque macrophages forms the basis of ^{18}F-fluorodeoxyglucose positron emission tomography of vascular inflammation[190]. By contrast, anti-inflammatory macrophages preferentially use anabolic processes such as mitochondrial fatty acid β-oxidation, oxidative phosphorylation and tryptophan metabolism. Molecular disruptions that shift the balance from aerobic glycolysis to mitochondrial oxidative respiration can lead to anti-inflammatory polarization of immune cells and suppress plaque inflammation[191]. Leptin, an adipocyte-secreted hormone associated with obesity, causes pro-inflammatory reprogramming of T cells through upregulation of glucose metabolism[192]. Leptin deficiency inhibits atherosclerosis through improved regulatory T (T_{reg}) cell function[193] A molecular connection between glucose metabolism and monocytic inflammation was also found in human atherosclerosis[194]. Metabolomic profiling of 159 human carotid plaques identified inflammatory metabolic signatures such as increased glycolysis, increased amino acid utilization and impaired fatty acid β-oxidation in those plaques that were prone to rupture[195].

Intracellular cholesterol metabolism can also regulate immune cell activation in atherosclerosis. In mouse models, cholesterol feeding not only had immediate effects such as inflammasome activation but also triggered epigenetic reprogramming in bone marrow myeloid precursors that allowed for the maintenance of metabolic adaptations even after diet reversal[196]. Indeed, mechanistic studies involving pharmacological inhibitors have shown that mevalonate, an intermediate in the cholesterol biosynthesis pathway, can imprint a memory of inflammatory insults in myeloid cells[197], a phenomenon known as trained immunity[198]. So far, the trained immunity hypothesis of atherosclerosis is lacking a specific molecular mechanism that, when suppressed, would have an impact on the disease process. Other reports have suggested an anti-inflammatory effect of cholesterol uptake, whereby inflammatory gene expression in macrophages is suppressed, either through sterol-dependent signalling[32] or through impairment of the pentose phosphate pathway[199]. Similar effects were observed for T cells, in which excess cholesterol was shown to favour T_{reg} cell differentiation[160,200] By contrast, cholesterol enrichment in lipid rafts may facilitate assembly of the immune synapse and boost T cell receptor signalling in effector T cells, thereby rendering them more resistant to T_{reg} cell-mediated suppression[160]. Cholesterol-induced dysregulation of lipid uptake and efflux pathways in dendritic cells can modulate inflammation and atherogenesis through regulation of T cell activation and polarization[201].

Atherogenesis can also be modulated by amino acid metabolism. Indoleamine 2,3-dioxygenase 1 (IDO1), a key rate-limiting enzyme in tryptophan degradation, mediates the formation of immunomodulatory metabolites such as kynurenine and anthranilic acid[202]. Inhibitor-mediated antagonism[203] or global genetic abrogation[204] of IDO1 activity has been shown to accelerate vascular inflammation and lesion formation in

mouse models of atherosclerosis. However, whereas anthranilic acid-induced IL-10 production, particularly from B cells, reduced plaque inflammation[204], kynurenine, which correlates with poor prognosis in patients with cardiovascular disease, was shown to promote atherosclerosis in hypercholesterolaemic mice through inhibition of IL-10 production[205].

Box 5 |

Antigen presentation in atherosclerosis

Atherosclerosis is associated with loss of immune tolerance to self-antigens. $CD4^+$ T cells are the central mediators of this process because they help B cells and antibodies to mature and $CD8^+$ T cells to become fully cytotoxic. Activation of $CD4^+$ T cells requires the recognition of antigens presented by antigen-presenting cells (APCs), such as dendritic cells (DCs), on MHC class II molecules. Atherosclerotic mice that lack the invariant chain, a molecule involved in the formation and transport of peptide–MHC class II complexes, have defects in antigen-specific activation of adaptive immune responses and develop significantly smaller diet-induced aortic lesions than control mice[206]. Co-stimulatory molecules on the APCs and cytokine-mediated crosstalk instruct T cells regarding the nature of the response to be elicited[74]. Thus, MHC class II-dependent interactions between T cells and APCs are highly context dependent and can either promote pro-atherogenic inflammatory responses of effector T cells[57,64] or mediate the generation of immunosuppressive regulatory T cells[207]. DCs are the most potent activators of naive T cells in lymphoid organs and instruct the differentiation of T helper (T_H) cell subsets. Enrichment of cholesterol in membrane microdomains was shown to boost the antigen-presenting functions of DCs through enhanced MHC class II clustering[208]. Naive T cells probably encounter antigens in secondary lymphoid organs and in tertiary lymphoid organs found in the adventitia of large arteries in aged atherosclerotic mice[104].

Other APCs, including macrophages and B cells, also express MHC class II molecules and support recall (reactivation) T cell responses in lesions and germinal centres, respectively. Macrophages far outnumber DCs in atherosclerotic arteries. Abrogation of IL-27 receptor signalling resulted in the upregulation of MHC class II expression and pro-inflammatory cytokine secretion by myeloid cells, which engaged in longer and more frequent interactions with T cells in the aortic walls[209]. Unlike for myeloid cells, the role of B cell-mediated T cell activation is still controversial. Expression of MHC class II molecules on B cells was shown either to promote atherosclerosis[210] or to have no effect on lesion progression[211]. ‘Innate response activator’ B cells producing granulocyte–macrophage colony-stimulating factor, which accumulate in secondary lymphoid organs after the onset of atherosclerosis, indirectly modulate T_H1 cell skewing through the expansion of IL-12-secreting $CD11b^+$ DC populations[212].

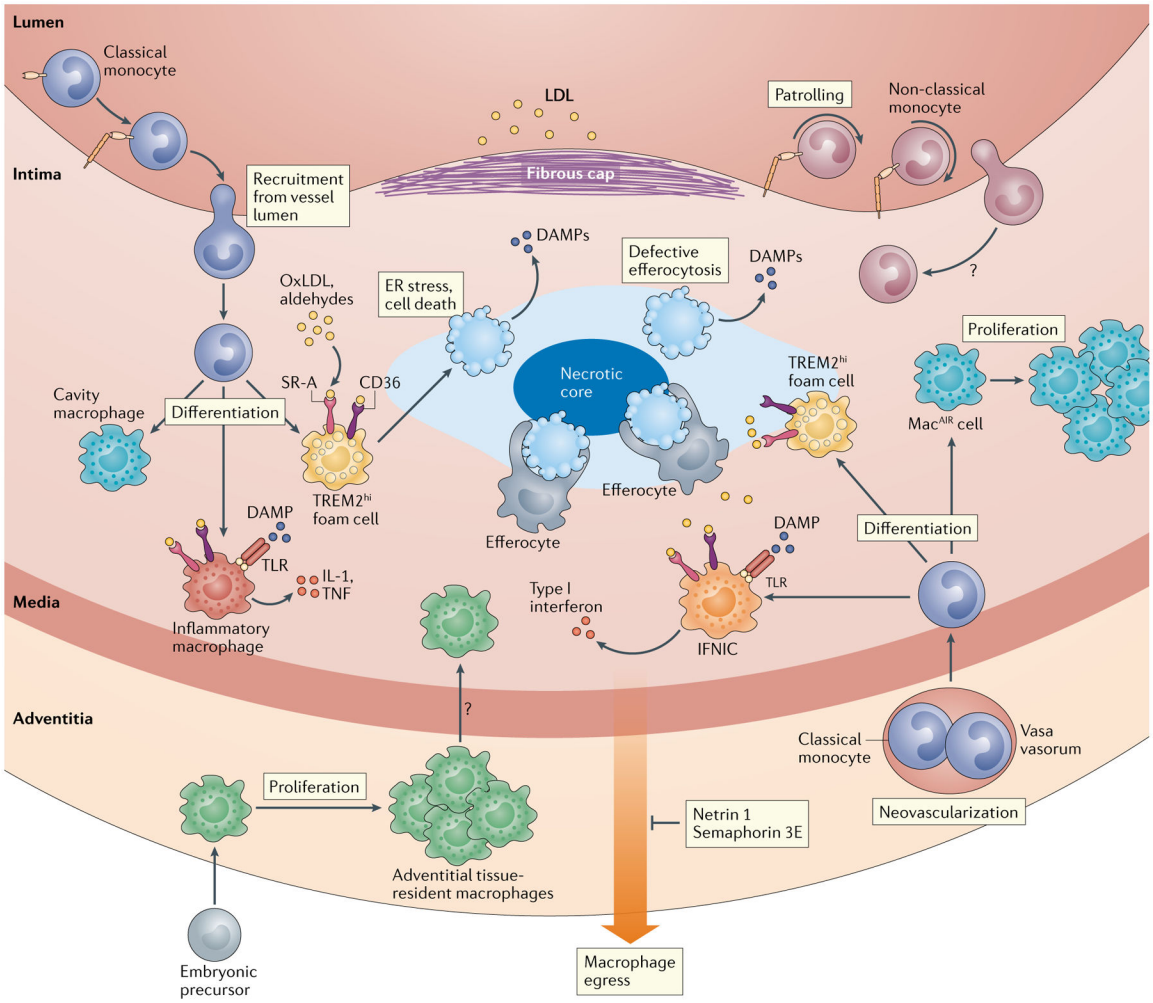

Fig. 1 |. Vascular macrophage populations in mouse atherosclerotic lesions.

The adventitia of the healthy artery wall contains tissue-resident vascular macrophages. The adventitial macrophage subset is derived from embryonic precursors and is maintained by local proliferation. These tissue-resident macrophages help to regulate arterial stiffness, but their role in plaque progression is unknown. The aortic intima-resident macrophage (Mac^{AIR}) subset originates from monocytes that were seeded postnatally and is sustained by local proliferation during the initial phases of atherosclerosis[49]. As disease progresses, Mac^{AIR} cells are replenished from classical monocytes recruited from the artery lumen and, in aged mice, from areas of neovascularization (vasa vasorum)[151,152]. The Mac^{AIR} subset and cavity macrophages[8] are likely to be the same cells. During atherosclerosis, macrophages in the neointima phagocytose apoptotic cells by efferocytosis. Monocyte-derived macrophages can become inflammatory macrophages that produce IL-1, tumour necrosis factor (TNF) and other cytokines and chemokines, which attract more classical monocytes into the intima. Decreased egress and increased retention of macrophages and monocytes in response to neuroimmune guidance cues such as netrin 1 and semaphorin 3E have been proposed to amplify the inflammatory response[21]. Low-density lipoprotein (LDL) from the blood in the vessel lumen can enter the intimal layer, where it can become

oxidized (oxLDL) or modified with aldehydes. Modified LDL is taken up by foam cells through scavenger receptors such as CD36 and SR-A. Single-cell studies have defined foam cells as the TREM2^{hi} macrophage subset. Endoplasmic reticulum (ER) stress of efferocytes can lead to cell death, which leads to the release of danger-associated molecular patterns (DAMPs) that are recognized by Toll-like receptors (TLRs) on inflammatory macrophages. If efferocytosis is insufficient, dead macrophages accumulate in the necrotic core. Type I interferon-inducible cells (IFNICs) are likely to produce type I interferons. IFNICs are replenished by differentiation from circulating classical monocytes. Non-classical monocytes patrol the endothelium of the blood vessel and can enter the plaque, but their further fate remains unknown. TREM2, triggering receptor expressed on myeloid cells 2.

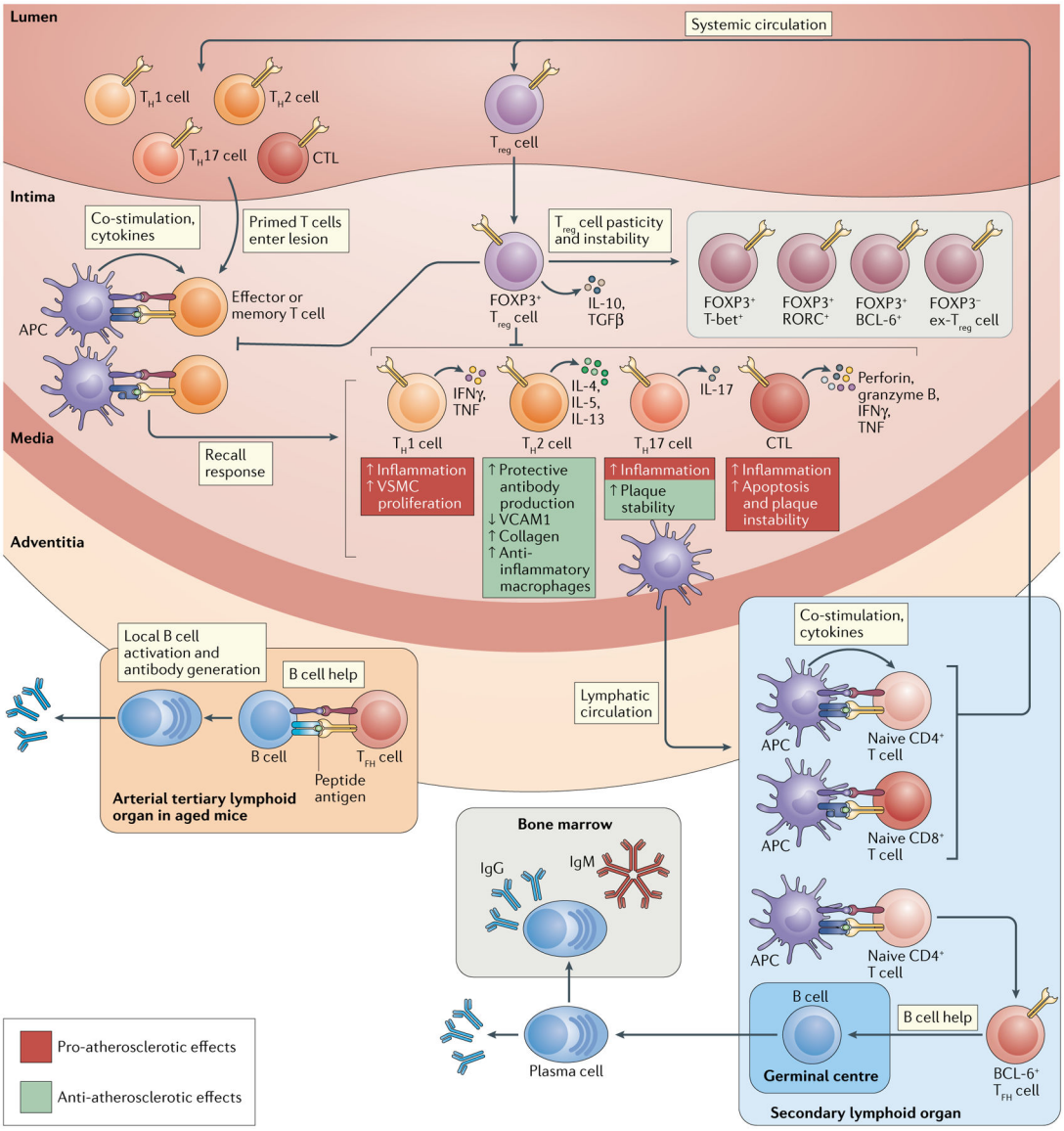

Fig. 2 |. Adaptive immune cells in atherosclerosis.

T cell subsets encounter atherosclerosis-associated antigens in secondary lymphoid organs, where antigen-presenting cells (APCs) arriving through the lymphatics present cognate peptides on MHC class I molecules to naive $CD8^+$ T cells and on MHC class II molecules to naive $CD4^+$ T cells. The nature of the co-stimulatory molecules and cytokines determines T cell polarization. BCL-6^+ T follicular helper (T_{FH}) cells stay in the secondary lymphoid organs and provide help to B cells in germinal centres. Other $CD4^+$ T cells — T helper 1 (T_H1) cells, T_H2 cells, T_H17 cells and regulatory T (T_{reg}) cells — as well as $CD8^+$ cytotoxic T lymphocytes (CTLs), enter the systemic circulation and home back to the plaque, where APCs initiate a recall response and trigger cytokine secretion (by $CD4^+$ T_H cells) or killing (by $CD8^+$ CTLs). The main cytokines produced are listed for each cell type, together with their pro-atherosclerotic effects (red) and anti-atherosclerotic effects (green). T_{reg} cells regulate T cell activation and responses through the production of IL-10

and transforming growth factor-β (TGFβ) and through contact-dependent mechanisms. $FOXP3^+$ T_{reg} cells are plastic (acquiring other transcription factors in addition to FOXP3) or unstable (losing FOXP3 expression and becoming ex-T_{reg} cells). B cell-derived plasma cells enter the bone marrow and produce IgM or, if class-switched, IgG antibodies to atherosclerosis antigens. In the adventitia of arteries with advanced atherosclerosis, arterial tertiary lymphoid organs form, which contain germinal centres, T_{FH} cells and B cells that mature to antibody-secreting plasma cells. IFNγ, interferon-γ; TNF, tumour necrosis factor; VCAM1, vascular cell adhesion molecule 1; VSMC, vascular smooth muscle cell.