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T cell subsets and functions in atherosclerosis

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Abstract

Atherosclerosis is a chronic inflammatory disease of the arterial wall and the primary underlying cause of cardiovascular diseases. Approaches including in vivo imaging, cell-lineage tracing and knockout studies in mice, as well as clinical interventional studies and advanced mRNA sequencing techniques have drawn attention to the role of T cells as critical drivers and modifiers of the pathogenesis of atherosclerosis. $CD4^+$ T cells are commonly found in atherosclerotic plaques. A large body of evidence indicates that T helper 1 (T_H1) cells have pro-atherogenic roles and regulatory T (T_{reg}) cells anti-atherogenic roles. However, T_{reg} cells can become pro-atherogenic. The roles in atherosclerosis of other T_H cell subsets such as T_H2, T_H9, T_H17, T_H22, follicular helper T cells and $CD28^-$ T cells, as well as other T cell subsets including $CD8^+$ T cells and γδT cells, are less well understood. Moreover, some T cells seem to have both pro-atherogenic and anti-atherogenic functions. In this Review, we summarize the knowledge on T cell subsets, their functions in atherosclerosis and the process of T cell homing to atherosclerotic plaques. Much of our understanding of T cell roles in atherosclerosis is based on findings from experimental models. Translating these findings into human disease is challenging, but much needed. Targeting T cells and their specific cytokines are attractive pathways for developing new preventative and therapeutic approaches including potential T cell-related therapies for atherosclerosis.

Introduction

Atherosclerosis is a chronic inflammatory disease with an autoimmune component, and is the primary underlying cause of cardiovascular diseases[1]. Studies in the past 2 decades have drawn much attention to the potential role of T cells as critical drivers and modifiers in the pathogenesis of atherosclerosis[2,3]. $CD8^+$ T cells and $CD4^+$ T cells drive immune responses to peptides presented by major histocompatibility complex (MHC) class I on all nucleated cells and MHC class II on antigen-presenting cells (APCs), respectively. These responses can occur in individuals expressing a specific MHC allele with the capacity to bind the relevant peptide epitope[4]. Binding of a specific T cell receptor (TCR) concomitant with co-

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stimulatory molecules provided by APCs activates T cells and causes their clonal proliferation[5]. In experimental models of atherosclerosis, interactions between APCs and $CD4^+$ T cells in the plaque result in the secretion of cytokines, many of which are pro-inflammatory[3,6,7]. Studies have shown that inducing the generation of regulatory T (T_{reg}) cells by targeted vaccination reduces atherosclerosis in mice[3,4,8–11].

In experimental models of atherosclerosis, many $CD4^+$ T cells in lymph nodes and atherosclerotic plaques show an antigen-experienced phenotype. Surface markers like CD44 indicate that these T cells have already been exposed to their cognate antigen and thus are poised to respond rapidly and strongly.[6]. TCR sequencing of lesional T cells revealed an oligoclonal origin, suggesting that antigen-specific T cell clones might actively expand in the atherosclerotic leasion[12,13]. These observations, which need to be confirmed by larger studies, support the notion that atherosclerosis-specific antigens drive an immune response, which is probably initiated in the lymph nodes (Fig.1). Among the candidates that might serve as T cell-activating antigens, LDL and its core protein apolipoprotein B (ApoB) show the strongest clinical correlation with atherosclerosis in humans[14]. In vitro studies in $CD4^+$ T cells from human atherosclerotic plaques show that many of these cells recognize oxidized LDL when processed and presented by APCs[7]. In particular, the TCRs in $CD4^+$ T cells bind to peptide epitopes derived from ApoB presented by MHC class II molecules on APCs[4,7,11]. Interestingly, use of an MHC tetramer of recombinant MHC-molecules loaded with an ApoB-derived peptide enabled the detection of a naturally occurring population of $CD4^+$ T cells in the blood in humans that recognizes the human peptide $ApoB_{3036–3050}$[4]. These findings strongly suggest that LDL is a relevant self-antigen that drives an autoimmune response in atherosclerotic plaques. In addition to LDL, heat shock proteins (HSPs) and peptides from pathogens such as human immunodeficiency virus (HIV) and cytomegalovirus (CMV) have been considered candidate antigens relevant in atherosclerosis[3,8,15]. These observations indicate that T cell subsets are involved in the initiation, progression, regression and ultimately rupture or erosion of atherosclerotic plaques. Much of the available evidence on the role of T cells in atherosclerosis is based on findings from experimental models. In this Review, we describe these experimental studies as well as the most relevant studies in human disease. We also discuss current knowledge on T cell homing to atherosclerotic plaques and highlight potential T cell-related therapies for atherosclerosis.

T cell research in atherosclerosis

In almost all published studies on T cells in atherosclerosis, the antigen specificity of the T cells is unknown. This is surprising, given that most T cell functions are strictly antigen dependent. However, determining the antigen specificity of T cells in atherosclerosis, or in any disease, is technically very difficult. Two methods can be used to find, isolate and study antigen-specific T cells: tetramers and restimulation assays.

MHC class II tetramers loaded with antigenic peptide can bind to $CD4^+$ T cells and MHC class I tetramers loaded with antigenic peptide can bind to $CD8^+$ T cells. Tetramers are recombinant proteins that must be specifically designed for the antigenic peptide in question and for the specific MHC allele(s) expressed by the study subject. Each tetramer must be validated against non-specific binding to other leukocytes. To establish specificity, the

tetramer must not bind to T cells from MHC-mismatched subjects. Tetramers must co-cluster with TCR on individual cells. The number of cells identified by tetramers is usually low, in the order of 10 cells per million. In atherosclerosis, very few studies on tetramer-isolated T cells exist[4].

Restimulation assays can be conducted with mixtures of antigenic peptides loaded onto APCs. Usually, the APCs are obtained from the same subject whose T cells are to be interrogated. Thus, their MHC alleles are naturally matched to the endogenous TCR repertoire. In restimulation assays, similarly to tetramers, whether the synthetic peptides that elicit a T cell response are the naturally presented peptides is not known.

T cell hybridomas have been generated with T cells from atherosclerotic *Ldlr*$^{-/-}$ mice[16]. A limitation of T cell hybridomas is that they grow independent of outside stimuli, which skews the T cell phenotype. Thus, almost all CD4^{+} T cell hybridomas reported for atherosclerosis are of T helper 1 (T_H1) cells, although many other T_H cells are found in atherosclerotic lesions in situ[17].

Because of these technical limitations, the antigen specificity of the various T cell subsets is unknown in almost all studies in atherosclerosis. This lack of definition of the T cell antigen specificity might be one reason for the vastly different effects of T cell subsets in atherosclerosis reported in experimental studies (Box1): the same T_H subset can be pro-atherogenic when recognizing an atherosclerosis-relevant antigen and potentially neutral or anti-atherogenic when detecting an unrelated antigen. This lack of knowledge of antigen specificity limits the conclusions that can be drawn about the roles of T cell subsets in atherosclerosis.

Immunohistochemistry studies[18,19], single-cell RNA sequencing (scRNA-seq)[17,20], and mass cytometry (CyTOF) approaches[17,21] (Box2) have shown that 25–38 % of all leukocytes in mouse aortic atherosclerotic plaques and human atherosclerotic plaques are CD3^{+} T cells. A study using an innovative methodology, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) (Box2), has shown that T cells account for the majority of immune cells in human atherosclerotic plaques obtained from endarterectomy[22]. However, flow cytometry, CyTOF, scRNA-seq and CITE-seq all require mechanical dissociation and enzymatic tissue digestion to prepare single-cell suspensions. The enzymes used for tissue digestion can alter cell surface molecules by proteolytic cleavage and the cellular processes during the digestion and isolation can alter single-cell transcriptomes. Moreover, the proportion of different cell types can be affected and skewed by the isolation procedures; because T cells are small, round and mechanically robust, these cells can survive the isolation procedure better than macrophages or dendritic cells, which are large, branched and fragile[23]. Analysis of gene expression signatures obtained with CITE-seq and and scRNA-seq indicated that human atherosclerotic plaques are enriched in T cells showing cytotoxicity, activation and exhaustion, whereas T cells in blood had gene signatures associated with cytokine inhibition, RNA synthesis and metabolic reprogramming[22]. Human and mouse studies indicate that T cells predominantly populate atherosclerotic lesions with an enrichment in the fibrous cap[18,19], but are also found in the adventitia of older lesions[17,24,25].

CD4+ T cells

CD4$^+$ T cells are critical regulators of the adaptive immune response with the capacity to differentiate into distinct T_H cell or T_{reg} cell subtypes (Fig. 1). T_H cells and T_{reg} cells can either activate or dampen the response of other immune cells, exert direct pro-inflammatory or anti-inflammatory effects on tissue-resident cells, help B cells produce high-affinity IgG antibodies, or have cytolytic activity[26]. Therefore, the function of CD4$^+$ T cells in atherosclerosis is multifaceted. Each CD4$^+$ T cell subset has specific transcriptional programmes and patterns of cytokine secretion that can accelerate or attenuate atherosclerosis. A 2019 study showed that CD4$^+$ effector memory T cells are enriched in plaques of patients with recent history of stroke compared with those with asymptomatic atherosclerosis[22]. In mice, genetic or antibody-mediated depletion of CD4$^+$ T cells protects from atherosclerotic lesion development[27,28]. Depletion of CD4$^+$ T_{reg} cells accelerates atherosclerosis[29]. After antigen presentation by APCs, lesional CD4$^+$ T cells differentiate into functionally distinct T_H subtypes (T_H1, T_H2, T_H9, T_H17, T_H22, follicular helper T (T_{FH}) and CD28$^-$ T cells) and T_{reg} subtypes (FOXP3$^+$ T_{reg} cells and type 1 regulatory T (Tr1) cells)[3,26]. Single cell data from human atherosclerotic plaques and peripheral blood mononuclear cells (PBMCs) showed that the majority of CD4$^+$ T cells in the plaque are T_H1 and T_H2 cells, and that T_H1 cells are enriched in atherosclerotic lesions compared with PBMCs[22].

Antigen presentation can initiate and modulate CD4$^+$ T cell responses in atherosclerosis. T cell immune responses are initiated by antigen-loaded APCs migrating to the lymph nodes[30]. Several types of cells function as APCs, including macrophages in the atherosclerotic plaque, B cells in the adventitia and some dendritic cell subsets such as conventional, plasmacytoid[31] or IRF8-dependent[32] dendritic cells, inducing antigen-experienced effector memory T cells[6]. Depending on co-stimulatory signals and cytokines provided by these APCs, the CD4$^+$ T cell immune response can be polarized towards an immunogenic response or a tolerogenic response. For example, CD40 on APCs interacts with CD40 ligand on T cells, contributing to T_H1 polarization[33]. Disrupting this signal reduces atherosclerosis in mice and cynomolgus monkeys[34,35]. CD80 and CD86 on APCs interact with the co-stimulatory receptor CD28 on T cells; deficiency of CD80 and CD86 in mice with atherosclerosis suppresses T_H1 responses[36]. CD80 and CD86 on APCs also interact with cytotoxic T-lymphocyte antigen 4 (CTLA4), which is expressed on T_{reg} cells and activated T cells. CTLA4 overexpression in *Apoe*$^{-/-}$ mice decreases atherosclerosis development compared with control mice[37]. Programmed cell death 1 ligand 1 (PDL1) on APCs interacts with programmed cell death 1 (PD1) and CD80 on T cells. PD1 might have a major role in limiting early T cell activation and T cell exhaustion in atherosclerosis, as has been shown in chronic inflammation and cancer[38]. Interestingly, T cells from human atherosclerotic plaques express high levels of PD1[22], and activation of the PD1–PDL1 pathway limits pro-atherogenic T cell responses in mice[39]. Interaction of OX40 ligand on APCs with OX40 on T cells increases T_H2 cell responses; blockade of this pathway in *Ldlr*$^{-/-}$ mice with atherosclerosis led to plaque regression[40]. Interaction of CD137 ligand on APCs with CD137 on T cells increases T cell proliferation and survival, which can promote atherosclerosis development and progression as shown in mice with hyperlipidaemia[41].

Activation of transforming growth factor-β (TGFβ) receptor II signalling in dendritic cells inactivates pro-inflammatory T cell responses in *Apoe*$^{-/-}$ mice, thereby dampening inflammation in atherosclerotic plaques[42]. In mice with atherosclerosis, MyD88 activation in dendritic cells is required for the induction of T_{reg} cells[43], whereas C-C motif chemokine 17 (CCL17) secreted by dendritic cells suppresses T_{reg} cell responses[44]. Interferon-α (IFNα), and presumably other type I interferons, secreted be dendritic cells in atherosclerotic plaques activates T_H1 cell responses in mice[45].

T_H cells

T_H1 cells.—TH1 cells express the defining transcription factor T-bet, chemokine receptors like CXCR3 and CCR5, and secrete interferon-γ. Several experimental studies have shown that T_H1 cells promote atherosclerosis and are the most prominent T_H cell subset in the plaque[46,47]. In atherosclerotic *Apoe*$^{-/-}$ mice, T_H1 cells express the atherosclerotic plaque-homing receptor C-C motif chemokine receptor 5 (CCR5) in lymph nodes[48]. CyTOF and CITE-seq data from human atherosclerotic plaques show that CCR5 expression is upregulated in plaque-derived T cells[22]. In addition, T_H1 cells are enriched in the plaques from patients with recent stroke compared with patients with asymptomatic atherosclerosis[22]. T_H1 cells from mouse atherosclerotic lesions secrete IFNγ and express the T-box transcription factor TBX21 (also known as T-bet)[48,49], Moreover, many $CD4^+$ T cells in the atherosclerotic plaque express other T_H1-associated pro-inflammatory cytokines in addition to IFNγ, such as IL-2, IL-3, tumour necrosis factor (TNF) and lymphotoxin, which can all activate macrophages, T cells, and other plaque cells and thereby accelerate the inflammatory response[3]. Deficiency of IFNγ, its receptor, or T-bet protects mice from atherosclerosis[46,50,51]. Consistently, IFNγ administration in *Apoe*$^{-/-}$ mice increases atherosclerosis compared with untreated mice[52]. IFNγ could directly reduce plaque stability by inhibiting vascular smooth muscle cell (VSMC) proliferation[53], affecting macrophage polarization[54] and modulating cardiovascular risk factors[55]. Some studies have shown that IFNγ induced VSMC proliferation[56,57], which can stabilize the plaque. In one study, IFNγ-deficient bone-marrow cells transplanted into *Ldlr*$^{-/-}$ mice led to larger diet-induced atherosclerotic lesions compared with control mice[58]. However, this finding is confounded by the use of a cholic acid-containing diet[58] (Box 1), which has known pro-inflammatory effects[59]. Other T_H1 cytokines besides IFNγ might be important for the development of atherosclerosis, but no specific studies of other T_H1 cytokines in T_H1 cells are available.

T_H2 cells.—Th2 cells are involved in defense against parasites, in asthma and other allergic diseases. The main T_H2 cytokine is IL-4. IL-4 binds to IL-4 receptor in T cells and activates signal transducer and activator of transcription 6, which leads to the expression of the transcription factor GATA3, the master regulator of T_H2 differentiation. In mouse atherosclerotic plaques, a substantial proportion of T cells express transcripts for T_H2-associated cytokines such as IL-4, IL-5, IL-10 and IL-13[17]. However, whether T_H2 cells are pro-atherogenic or atheroprotective remains unclear. Individuals with a high number of T_H2 cells in the PBMC fraction have lower subclinical atherosclerosis burden, as measured by common carotid intimal media thickness, than individuals with low T_H2 cell counts[60]. Moreover, IL-4 release from activated mononuclear leukocytes negatively correlate with clinical atherosclerosis[61].

However, the role of IL-4 in atherosclerosis is unclear. IL-4 was shown to antagonize T_H1 responses and diminish atherosclerotic lesion formation in *Apoe*$^{-/-}$ mice[62], whereas other studies have shown that depletion of IL-4 is atheroprotective in *Ldlr*$^{-/-}$ mice fed a high-fat diet[63] and administration of exogenous IL-4 did not decrease atherosclerosis in *Apoe*$^{-/-}$ mice with angiotensin II-induced atherosclerosis[64]. Immunization of *Apoe*$^{-/-}$ mice fed a high-fat diet with an ApoB-peptide increased IL-4 expression in T cells, which resulted in no difference in atherosclerotic lesions compared with control mice[65].

Unlike IL-4, most evidence suggests that other T_H2-associated cytokines, such as IL-5 and IL-13, are atheroprotective. In humans, plasma IL-5 levels inversely correlate with carotid intima media thickness[66,67], but elevated IL-5 levels in plasma are associated with the presence of unstable angina and myocardial infarction[68]. The immunization of *Ldlr*$^{-/-}$ mice with modified LDL is atheroprotective, inducing a T_H2-skewed immune response that was characterized by antigen-specific production of IL-5 and IL-13 with small amounts of IL-4 and IFNγ compared with non-immunized mice[69]. IL-13 administration in *Ldlr*$^{-/-}$ mice fed a high-fat diet modulates established atherosclerotic lesions by increasing lesional collagen content and reducing vascular cell adhesion molecule 1 (VCAM1) expression, resulting in decreased macrophage infiltration in plaques compared with mice treated with phosphate-buffered saline (PBS)[70]. IL-33 predominantly induces the production of T_H2 cytokines, and IL-33 treatment reduced atherosclerosis development in high-fat diet-fed *Apoe*$^{-/-}$ mice and increased the levels of IL-4, IL-5 and IL-13 and decreased levels of IFNγ in serum and lymph nodes compared with PBS-treated mice[71]. By contrast, high-cholesterol diet-fed *Apoe*$^{-/-}$ mice with deficiency in IL-33 or its receptor ST2 had no differences in atherosclerosis development compared with control mice[72]. Of note, IL-4, IL-5 and IL-13 are also produced by subtype 2 innate lymphoid cells (ILC2)[73]. Genetic ablation of ILC2 in *Ldlr*$^{-/-}$ mice fed a high-fat diet accelerated atherosclerosis development, which was corrected by reconstitution with wild type ILC2 but not by reconstitution with IL-5-deficient or IL-13-deficient ILC2[74]. The role of T cell-derived IL-5 and IL-13 has not been studied yet. Mice with specific deficiency of T cells-are needed to distinguish the effects of cytokines derived from T_H2 cells and ILC2 on atherosclerosis.

T_H9 cells.—T_H9 cells, a subset initially associated with the T_H2 phenotype, are the primary source of IL-9. The production of IL-9 in T_H9 cells is stimulated by TGFβ and IL-4, and inhibited by IFNγ[75]. It is not known whether Th9 cells are pro- or anti-atherogenic. IL-9 plasma levels are increased in patients with coronary atherosclerosis compared with healthy individuals, and IL-9 levels were higher in human atherosclerotic plaques than in non-atherosclerotic vessel samples[76]. Moreover, plasma IL-9 levels are increased in patients with acute coronary syndrome compared with healthy individuals, but no differences were observed in the number of T_H9 cells in blood[77]. In vitro, IL-9 administration increased IL-17 secretion from PBMCs of patients with unstable angina and from healthy individuals[77]. In *Apoe*$^{-/-}$ mice fed a Western diet, IL-9 administration exerted pro-atherogenic effects, at least partially by inducing VCAM1 expression in aortic endothelial cells, which mediates inflammatory cell infiltration into atherosclerotic lesions[78].

T_H17 cells.—T_H17 cells are characterized by expression of the T_H17-defining transcription factor nuclear receptor RORγt (RORC), are activated by IL-23 and IL-17 is their signature cytokine[79]. T_H17 cells have distinct plasticity in different inflammatory settings[80–84]. In immune, endothelial and stromal cells, IL-17 induces the secretion of the pro-inflammatory cytokines IL-6, granulocyte-colony stimulating factor) and granulocyte-macrophage colony-stimulating factor, and chemokines[79], all of which can be pro-atherogenic. By contrast, IL-6 and TGFβ induce a subtype of T_H17 cells that produce IL-10 concomitantly with IL-17[85,86], and IL-10 can be atheroprotective[87]. Moreover, IL-17 is also produced by γδT cells and ILC3 cells[88]. Because of this complexity, experimental studies in *Apoe*$^{-/-}$ mice on the role of IL-17 have yielded discrepant results: some studies suggest that IL-17A is pro-athrogenic[89–91], others atheroprotective[92] and other studies suggest that IL-17 has no effect on atherosclerosis[93]. *Il17a*$^{-/-}$*Apoe*$^{-/-}$ mice and *Il17ra*$^{-/-}$*Apoe*$^{-/-}$ mice fed a Western diet had smaller atherosclerotic plaques in the aortic arch and aortic roots, but similar plaque burden in the thoracoabdominal aorta, compared with *Apoe*$^{-/-}$ mice[94]. By contrast, in vivo administration of IL-17A reduced plaque burden in aortic roots in *Ldlr*$^{-/-}$ mice compared with control mice[95]. Moreover, some studies in mouse models of atherosclerosis indicate that IL-17 can promote plaque stability by increasing the production of type I collagen by VSMCs[95–97].

Some clinical studies showed that plasma IL-17 levels and the number of peripheral T_H17 cells are increased in patients with unstable angina or acute myocardial infarction compared with patients with stable angina and healthy individuals[98,99]. However, in larger clinical studies, plasma IL-17 levels were similar in individuals with or without coronary artery disease[100], and low serum IL-17 levels were associated with a higher risk of cardiovascular events in patients with acute myocardial infarction[101]. In human atherosclerosis, IL-17 is produced concomitantly with IFNγ by T cells infiltrating into coronary artery plaques, and IL-17 and IFNγ synergistically increase pro-inflammatory responses by VSMCs[100]. By contrast, consistent with some experimental studies, IL-17 expression in human carotid atherosclerotic plaques was associated with a stable plaque phenotype with a lower macrophage content and a higher VSMC content[95,97].

T_H22 cells.—T_H22 cells are unique in that they express IL-22 but not IL-17 and IFNγ, and express the master transcription factor aryl hydrocarbon receptor (AHR). The cytokines responsible for inducing T_H22 cell differentiation are unknown[102]. It is also unknown whether Th22 cells are pro- or anti-atherogenic. Increased circulating levels of T_H22 cells have been reported in patients with acute coronary syndrome compared with healthy controls[77,103]. IL-22 levels are higher in plasma from patients with symptomatic atherosclerotic disease compared to patients with asymptomatic atherosclerosis[77,103,104]. In *Apoe*$^{-/-}$ mice, IL-22 is involved in activation of vascular repair by stimulating medial VSMC differentiation into a synthetic phenotype, which might contribute to atherosclerotic plaque growth by enabling VSMC migration into the intima[105]. Moreover, IL-22 reduced atherosclerosis by repressing pro-atherogenic gut microbiota, shown by a study in IL-22-deficient atheroprone mice[106].

T_{FH} cells.—T_{FH} cells, which express the defining transcription factor B-cell lymphoma 6 (BCL6), are found in B cell follicles and maintain and form germinal centres together with B cells. T_{FH} cells are required for antibody isotype switching in germinal centre B cells[107]. TFH cells are most likely pro-atherogenic. The atherogenic environment increases the autoimmune responses of $CXCR3^+$ T_{FH} cells in atherosclerosis-prone mice[108]. However, in *Apoe*$^{-/-}$ mice fed a high-cholesterol diet, marginal zone B cells inhibit the response of T_{FH} cells, thereby limiting atherosclerosis development and progression[109]. Moreover, the pro-atherogenic T_{FH} cell activity in germinal centres can be regulated by a subset of $CD8^+$ T cells with regulatory function, which might be defective in *Apoe*$^{-/-}$ mice[110]. The pathway involving the co-stimulatory molecule inducible T-cell co-stimulator (ICOS) and its ligand ICOSL is crucial for the differentiation and maintenance of T_{FH} cells: blocking ICOS–ICOSL signalling in *Apoe*$^{-/-}$ mice reduces atherosclerosis burden, with reduced numbers of T_{FH} cells in secondary lymphoid organs[111]. Ageing increases the proportion of T_{FH} cells in *Apoe*$^{-/-}$ mice, but not in wild-type mice, whereas ageing did not modify the overall percentage of $CD4^+$ T cells in *Apoe*$^{-/-}$ mice[110]. Interestingly, T_{FH} cells can derive from T_{reg} cells. These 'switched' T_{FH} cells are pro-atherogenic and their depletion reduces atherosclerosis in *Apoe*$^{-/-}$ mice[111]. In patients with coronary artery disease, plasma levels of IL-21, a major T_{FH} cytokine, negatively correlated with FOXP3 expression in T_{reg} cells[111].

$CD28^{null}$ T cells.—$CD4^+CD28^-$T cells are characterized by the lack of CD28 expression, which is the main co-stimulatory receptor of naive $CD4^+$ T cells. $CD28^-$ T cells are pro-inflammatory and cytotoxic. Interestingly, $CD28^-$ T cells are only found in humans and non-human primates, not in mice[112]. Patients with acute coronary syndrome have a higher number of $CD28^-$ T cells in blood than healthy individuals[113–115], and $CD28^-$ T cells from these patients are resistant to apoptosis[116]. Moreover, human unstable atherosclerotic plaques can be invaded by clonally expanded T cells, including a large monoclonal population of $CD28^-$ T cells[115]. In addition, some $CD28^-$ T cell subsets are reactive to HSP60 and HSP70[117], which is a possible atherosclerosis antigen[118]. Among patients with end-stage renal disease, those with atherosclerosis had higher expansion of the $CD28^-$ T cell subset than those without atherosclerosis[119].

T_{reg} cells

$FOXP3^+$ T_{reg} cells.—In humans, the classical T_{reg} cell subset is characterized by expression of the transcription factor forkhead box protein P3 (FOXP3), the IL-2 receptor subunit-α (IL-2RA; also known as CD25, which is part of the trimeric high-affinity IL-2 receptor) and CTLA4, and by lack of CD127 expression. Of note, FOXP3 is a reliable T_{reg} cell marker in mice, but is not a reliable marker for human T_{reg} cells because FOXP3 is transiently expressed by other $CD4^+$ T cells during activation[120]. In mice, T_{reg} cells protect against atherosclerosis[29,121]. Similarly, clinical data suggest a strong inverse relationship between T_{reg} cells and atherosclerosis: T_{reg} cell numbers and IL-10, a cytokine secreted by Tregs are lower in patients with myocardial infarction than in patients with stable angina or individuals without coronary artery disease[122,123], and a low T_{reg} cell to $CD4^+$ T cell ratio in blood predicted a higher rate of cardiovascular events in a large cohort study[124]. However, in a large, prospective, case-control study excluding patients with diabetes mellitus, individuals with a low T_{reg} cell to total T cell ratio in blood had a lower risk of myocardial infarction,

but the association was not significant when additional cardiovascular disease risk factors were included in the model[125]. Another study showed no correlation between T_{reg} cell numbers in blood and carotid and coronary atherosclerosis[126]. This discrepancy might be due to differences in the study designs[127].

Like in other T cells, the activity of T_{reg} cells increases when their TCR binds its cognate antigenic peptides presented by MHC class II molecules. However, like for other T cell subsets, in studies on atherosclerosis the antigen specificity of T_{reg} cells is usually unknown. Upon stimulation, T_{reg} cells can produce high levels of IL-10 and TGFβ. IL-10 is an anti-inflammatory cytokine, whose deficiency increases atherosclerosis in atheroprone mice[87]; of note, in this early study the source of IL-10 was not identified. TGFβ has plaque-stabilizing effects in *Apoe*$^{-/-}$ mice[128]. T_{reg} cells exert their atheroprotective properties by secreting IL-10 and TGFβ, and by suppressing the proliferation of pro-inflammatory effector T cells[129]. The atheroprotective effects of treatment with IL-2 complexes[130] and anti-CD3 treatment[131,132] in atheroprone mice have been attributed to an increase of T_{reg} cell numbers relative to other T cells.

A splice variant of FOXP3 controls some T_{reg} cell effector functions and is associated with human atherosclerotic plaque stability[133]. A study in *Apoe*$^{-/-}$ mice with MHC class II deficiency showed that lack of antigen presentation on MHC class II molecules aggravates atherosclerosis via decreased T_{reg} cell numbers[134]. In patients with subclinical atherosclerosis, T_{reg} cell numbers in blood correlate positively with plasma LDL levels[135]. Likewise, in mice, hypercholesterolaemia initially favours the differentiation of T_{reg} cells followed by increased TCR downstream signalling events[136], an effect that might be a response to elevated inflammation[137], intracellular lipid accumulation in T_{reg} cells[138] or an antigen-specific response. These findings suggest that a subpopulation of T_{reg} cells responds to antigens associated with increased plasma LDL levels or to components of LDL particles. Such LDL-reactive or ApoB-reactive T_{reg} cells have TCRs specifically responding to atherosclerosis antigens. One class of atherosclerosis-related antigens are ApoB peptides. The existence of ApoB peptide-reactive T cells was shown in humans and mice with the use of human and mouse MHC class II tetramers loaded with a sequence-identical human and mouse ApoB peptide[4].

Pathological conversion of T_{reg} cells.—The plasticity of FOXP3^{+} T_{reg} cells has been investigated in experimental atherosclerosis[139]. Circulating and atherosclerotic plaque T_{reg} cell numbers in established mouse atherosclerosis decline in later stages of the disease, whereas total CD4^{+} effector T cells and splenic T_{reg} cells increase with increased atherosclerotic lesion size[137]. T_{reg} cells in late atherosclerosis in mice simultaneously express FOXP3 and T-bet, lose their capacity to regulate and to protect from atherosclerosis, while retaining some phenotypic similarity with T_{reg} cells[48,49,137]. These findings suggest that the immunosuppressive phenotype of T_{reg} cells disappears as atherosclerosis progresses.

In autoimmune conditions, such as experimental autoimmune encephalitis or arthritis, instability of FOXP3 expression triggers the formation of antigen-specific, but dysfunctional, partially non-protective former T_{reg} cells (known as exT_{reg} cells)[140–142]. A study in *Apoe*$^{-/-}$ mice fed a Western diet showed that T_{reg} cells lose FOXP3 expression and

their immunosuppressive function during atherosclerosis progression, leading to the conversion of a fraction of these cells into pro-atherogenic T_{FH} cells expressing BCL6[111]. Transgenic $CD4^+$ T cells expressing a TCR reacting to human LDL differentiated into T_{FH} cells after transfer into transgenic *Ldlr*$^{-/-}$ mice expressing human ApoB100[143]. In humans, 67% of $ApoB_{3036–3050}$-reactive $CD4^+$ T cells from individuals without cardiovascular disease expressed FOXP3, indicative of ApoB-reactive T_{reg} cells[4], but not the T_H17-defining transcription factor RORγt or the T_H1-associated transcription factor T-bet, commonly found in ex-Tregs. By contrast, in patients with subclinical atherosclerosis, the percentage of T cells expressing only $FOXP3^+$ declined to about 30%, and a substantial proportion of the remaining $FOXP3^+$ T cells acquired simultaneous expression of the Th17-defining transcription factor RORγt or the TH1-asscoiated transcription factor T-bet[4].

The instability of FOXP3 expression might be caused by increased methylation of the *FOXP3* locus, which is observed in patients with severe coronary artery disease[144]. T_{reg} cell instability might also be induced by increased intracellular cholesterol levels or by decreased IL-2 signalling[111,140]. In addition, the function of FOXP3 might be regulated by alternative splicing favouring a FOXP3 isoform that induces pathogenic transcriptional programmes[133]. These data suggest that the initial protective immune response by T_{reg} cells can shift to a pathogenic response as atherosclerosis progresses[139]. Although some possibilities have been suggested, the mechanisms by which this switching occurs remain largely unknown. Inhibiting the enzyme MALT1, which is part of the CARMA1–BCL10–MALT1 (CBM) signalosome complex, promotes T_{reg} cell conversion towards IFNγ expression and T_H1 cell polarization[145,146]. This combination is reminiscent of the $FOXP3^+$T-bet^+ T cell type observed in atherosclerosis in mice[48,49]. Conversely, blocking or knocking out cyclin-dependent kinase 8 (CDK8) or CDK19 stabilizes T_{reg} cells even under conditions of inflammation[147].

Tr1 cells.—Tr1 cells lack FOXP3 expression and are characterized by the expression of lymphocyte activation gene 3 protein and CD49b, which is the α_2 subunit of the $\alpha_2\beta_1$ collagen receptor. Tr1 cells prominently secrete IL-10[148]. An experimental study in *Apoe*$^{-/-}$ mice showed that Tr1 cells are atheroprotective via IL-10 secretion[149]. A study in humans has shown that the frequency of Tr1 cells secreting IL-10 is significantly lower in the blood of patients with coronary artery disease than in healthy individuals[150].

Other T cell subsets

$CD8^+$ T cells

CD8 T cells recognize antigenic peptides presented by MHC-I. They can mature to cytotoxic T cells that can kill virus-infected and other abnormal (like cancer) cells using various cytotoxic pathways. Patients with coronary artery disease have increased levels of cytotoxin-producing $CD8^+$ T cells in blood compared with healthy individuals[151,152], and $CD8^+$ T cells are abundant in atherosclerotic plaques in humans and mice[153–155]. In advanced human atherosclerotic lesions, $CD8^+$ T cells outnumber $CD4^+$ T cells[22,153,156], and are predominantly found in fibrous cap areas[157]. TCR sequencing of T cells from human atherosclerotic plaques identified a subpopulation of $CD8^+$ T cells whose frequencies

correlated with TCR clonality, suggesting clonal expansion in the plaque[22]. However, similar to $CD4^+$ T cells, in most studies on atherosclerosis the antigen specificity of plaque $CD8^+$ T cells is unknown.

In experimental studies, both pro-atherogenic and atheroprotective roles of $CD8^+$ T cells have been reported. The cytotoxic activity of $CD8^+$ T cells towards lesion-stabilizing cells such as VSMCs and the inflammatory cytokines produced by of $CD8^+$ T cells might exacerbate the inflammatory responses in atherosclerotic plaques and drive the progression and instability of the lesions (Fig. 2a). Conversely, $CD8^+$ T cell cytotoxic activity towards APCs might limit atherosclerosis (Fig. 2a). MHC class I-dependent cytotoxic $CD8^+$ T cells have been suggested to contribute to plaque inflammation and the build-up of the necrotic core. One study identified ApoB-reactive $CD8^+$ T cells in *Apoe*$^{-/-}$ mice[158]. $CD8^+$ T cell depletion with antibodies reduced atherosclerosis in atheroprone mice[159–161], suggesting that $CD8^+$ T cells are pro-atherogenic. These pathogenic $CD8^+$ T cells have higher IFNγ and granzyme B production than CD8 T cells from non-atherosclerotic mice[161]. A study in *Ldlr*$^{-/-}$ mice fed a high-fat diet showed that $CD8^+$ T cells promote atherosclerosis by regulating monopoiesis and the levels of peripheral monocytes via IFNγ production[159]. However, another study showed that IFNγ produced by $CD8^+$ T cells does not have a role in atherosclerosis in *Apoe*$^{-/-}$ mice fed a high-fat diet[160]. In this mouse model, studies of adoptive transfer of $CD8^+$ T cells deficient in either perforin, granzyme B, TNF or IFNγ into lymphopaenic *Apoe*$^{-/-}$ mice suggested that $CD8^+$ T cells promote atherosclerotic plaque development by perforin-mediated and granzyme B-mediated apoptosis of macrophages, VSMCs and endothelial cells, followed by increased inflammation promoted by TNF secretion[160]. Of note, in these adoptive transfer studies, most $CD8^+$ T cells are probably not specific for atherosclerosis antigens.

Other experimental studies suggest that $CD8^+$ T cells can also have an atheroprotective role. $CD8^+$ T cells with regulatory functions in atherosclerosis have been assessed in immunization studies. $CD8^+$ T cells mediate the atheroprotective effects of immunization with an ApoB-related peptide (p210) in *Apoe*$^{-/-}$ mice[162]. Adoptive transfer of $CD8^+$ T cells from p210-immunized mice reduced atherosclerosis in *Apoe*$^{-/-}$ mice compared with transfer of $CD8^+$ T cells from control mice[162]. Immunization with p210 decreased number of dendritic cells at the site of immunization and in the atherosclerotic plaque and reduced immunoreactivity of plaque macrophages compared with mice injected with PBS[162]. Another study of vaccination with p210 in *Apoe*$^{-/-}$ mice showed that $CD8^+$ T cells from immunized mice had higher lytic activity against macrophages and inhibited the polarization of $CD4^+$ T cells into T_H17 cells compared with $CD8^+$ T cells from control mice[163]. Depletion of $CD8^+$ T cells in advanced atherosclerosis in *Ldlr*$^{-/-}$ mice fed a Western diet for 10 weeks resulted in less stable lesions with significantly reduced collagen content in the aortic valve area, increased macrophage content and increased necrotic core area compared with controls[164]. Furthermore, a specific subset of $CD8^+$ regulatory T cells reduces atherosclerosis in *Apoe*$^{-/-}$ mice by blocking ICOS–ICOSL signalling between T_{FH} cells and germinal centre B cells, leading to reduced levels of potentially atherogenic IgGs[110].

Natural killer T cells

Natural killer T (NKT) cells are divided into two subgroups: invariant NKT (iNKT) cells (or type I), which have few variant TCRs, and type II NKT cells, which have more variable TCRs. So far, only iNKT cells have been studied in atherosclerosis. iNKT cells can be activated by the interaction of the TCR with antigen-presenting CD1d molecules containing antigenic glycolipids present on APCs[165] (Fig. 2b). Some glycolipids are of microbial origin and some are self-glycolipids[166]. Activation of iNKT cells results in the rapid release of T_H1, T_H2 and T_H17 cytokines, depending on the expression of the same transcription factors that define the corresponding T_H cell subtypes: T-bet, GATA3 and RORγt, respectively[167]. Like $CD8^+$ T cells, iNKT cells can also express the cytotoxic proteins perforin and granzyme B[167]. Most studies using *Apoe*$^{-/-}$ mice[168–172] and *Ldlr*$^{-/-}$ mice[168,169,171,173–175] fed a Western or Chow diet suggest that iNKT cells are pro-atherogenic. iNKT cells are thought to promote atherosclerosis by secreting cytokines, which can activate other immune cells present in the atherosclerotic lesion.

γδT cells

Unlike T cells expressing αβ T cells, γδT cells do not recognize specific antigens[176]. Only a few mouse studies on γδT cells in atherosclerosis have been published[177–179] since the presence of γδT cells in human atherosclerotic lesions was reported in 1993[180]. γδT cells are among the T cell subsets described in mouse atherosclerotic lesions[179] (Fig. 2c). Interestingly, the intracellular cholesterol content in γδT cells regulates their activation, proliferation and effector functions[177]. In addition, γδT cells are an abundant source of IL-17 in mice[88], and could modulate atherosclerosis via IL-17 production. However, *Apoe*$^{-/-}$ mice with genetic deficiency of γδT cells had similar early atherosclerosis development as *Apoe*$^{-/-}$ mice with γδT cells[178]. To date, the exact role of this T cell subset in atherosclerosis is unclear.

T cell homing to atherosclerotic lesions

Upon activation, inflammatory cells including T cells increase the expression of various chemokine receptors. Many of their corresponding chemokines are overexpressed in atherosclerotic lesions[181], suggesting that chemokine–chemokine receptor interactions are important for the recruitment of T cells to atherosclerotic lesions. Naive $CD4^+$ T cells are recruited to atherosclerotic lesions via L-selectin[182] or via the P-selectin glycoprotein ligand 1 (PSGL1)[183]. T cell recruitment to the plaque can occur via C-C motif chemokine receptors (CCRs) and their ligands (CCLs), and C-X-C motif chemokine receptors (CXCRs) and their ligands (CXCLs). The main chemokine–chemokine receptor pairs that have been studied in the context of atherosclerosis are CCL5 binding to CCR1 or CCR5, CXCL10 binding to CXCR3, CXCL16 binding to CXCR6, and CCL19 and 21 binding to CCR7[184]. CCR2 has been reported to be involved in T_{reg} cell recruitment[185], but this role has not been shown in atherosclerosis.

CCR1/CCR5–CCL5 axis

CCR1 and CCR5 are expressed on various cells including T cells in atherosclerotic plaques[48,186,187]. CCR1 deficiency enlarged atherosclerotic plaque size with increased T cell

content in *Apoe*$^{-/-}$ mice[186], increased plaque inflammation and IFNγ production from spleen T cells[187]. Consistently, transplantation of *Ccr1*$^{-/-}$ bone-marrow cells into *Ldlr*$^{-/-}$ mice increased atherosclerotic plaque size with increased T cell infiltration into the lesions compared with transplantation of *Ccr1*$^{+/+}$ bone-marrow cells[187]. These data suggest that CCR1 has an atheroprotective role.

By contrast, CCR5 has a pro-atherogenic role. In *Apoe*$^{-/-}$ mice, CCR5 deficiency in *Apoe*$^{-/-}$ mice reduced diet-induced atherosclerosis and induced a more stable plaque phenotype with reduced T cell infiltration[186]. CCL5 blocking in *Ldlr*$^{-/-}$ mice reduced the progression of established atherosclerosis, with decreased CD4^{+} T cell infiltration into the lesions[188]. Interestingly, CCR5 is involved in the homing of T_H1-like exT_{reg} cells to atherosclerotic lesions in mice[48]. CCR5 is involved in promoting unstable plaque formation in *Ldlr*$^{-/-}$ mice partially via increasing TNF production from T cells[189]. However, CCR5 deficiency does not have an effect on early atherosclerosis in *Apoe*$^{-/-}$ mice[190], and the role of CCR5 might be T cell-independent in advanced atherosclerosis[191].

In addition, the structural similarity of chemokines allows them to form unique heterodimers to shape the overall signalling response of their receptors. Heterodimers of CCL5 with CXCL4, CCL2 or CCL17 act synergistically to increase T cell and monocyte arrest on human aortic endothelial cells in vitro[192]. Peptide inhibitors against CCL5–CXCL4 reduce atherosclerosis in mice[193]. Dendritic cell-derived CCL17, which is present in advanced human and mouse atherosclerosis[44], limits T_{reg} cell expansion and homing to atherosclerotic lesions in *Apoe*$^{-/-}$ mice[44]. In these mice, CCL17 deficiency reduced atherosclerosis, and a CCL17 blocking antibody expanded T_{reg} cell numbers and reduced atherosclerosis progression[44].

CXCR3–CXCL10 axis

T_H1 cells and CD8^{+} T cells in mouse and human atherosclerotic plaques express high levels of CXCR3[194,195], which accompanies T_H1 cell differentiation[196]. A single-cell gene expression analysis of human samples showed that some clusters of blood CD4^{+} T cells or CD8^{+} T cells express higher CXCR3 levels in patients with symptomatic atherosclerosis than in patients with asymptomatic atherosclerosis, suggesting that these circulating cells are activated and might be prone to migrating to the atherosclerotic site[22]. Administration of a CXCR3 antagonist significantly inhibited atherosclerotic lesion formation in *Ldlr*$^{-/-}$ mice[197], in which the draining lymph nodes of the aorta were significantly smaller, contained fewer activated T cells and more T_{reg} cells than those from vehicle-treated mice[197]. Moreover, administration of an antagonist of both CCR5 and CXCR3 significantly reduced atherosclerosis in *Ldlr*$^{-/-}$ mice, with significant reduction of T cell accumulation and IFNγ expression in plaques compare with control mice[198]. Consistently, *Cxcr3*$^{-/-}$ *Apoe*$^{-/-}$ mice had smaller atherosclerotic lesions than *Cxcr3*$^{+/+}$ *Apoe*$^{-/-}$ mice, which correlated with a decrease in the total number of T cells, an increased number of T_{reg} cells and an upregulation of the production of anti-inflammatory molecules including IL-10 within atherosclerotic lesions[194].

Plasma CXCL10 levels are elevated in patients with coronary atherosclerosis compared with individuals without coronary atherosclerosis[199]. In *Apoe*$^{-/-}$ mice, *Cxcl10* deletion

significantly decreased atherosclerotic plaque size, with an increased number and activity of T_{reg} cells despite a decreased overall accumulation of $CD4^+$ T cells in the lesions[200]. Moreover, CXCL10 neutralization in mice prevented IFNγ-mediated T cell responses, resulting in restoration of endothelial healing[201].

CXCR6–CXCL16 axis

CXCR6 is expressed on several NKT cells and other T cell subsets, and regulates T_H1 cell homing[202]. *Cxcr6*$^{-/-}$*Apoe*$^{-/-}$ mice show reduced atherosclerotic plaque formation and decreased IFNγ expression in the aorta[203]. CXCR6 regulates the recruitment of atherogenic IL-17A-producing cells (T_H17 cells and γδT cells) into aortic atherosclerotic lesions in *Apoe*$^{-/-}$ mice[204]. Endothelial CXCL16 expression is probably involved in the recruitment of $CXCR6^+$ cells to the arterial wall. CXCL16 is upregulated in experimental atherosclerosis[205] and in patients with acute coronary syndrome compared with healthy controls[206]. However, *Cxcl16* deletion in *Ldlr*$^{-/-}$ mice increased atherosclerosis without changes in T cell recruitment but with increased macrophage recruitment to the plaques compared with *Cxcl16*$^{+/+}$*Ldlr*$^{-/-}$ mice[207]. CXCL16 is also a scavenger receptor[208], and this function might be more important in atherosclerosis than its chemokine function.

CCR7

CCR7 regulates T cell, B cell and mature dendritic cell homing to lymph nodes and Peyer's patches. CCR7 is expressed on naive T cells and central memory T cells[209]. Both CCR7 and its ligands, CCL19 and CCL21, have been detected in mouse and human atherosclerotic lesions[210]. CyTOF of immune cells from human atherosclerotic plaques and blood showed CCR7-expressing central memory $CD4^+$ T cells[22]. In addition, some clusters of $CD8^+$ effector memory T cells are enriched in atherosclerotic plaques compared with blood[22]. These observations suggest a possible pathological role of CCR7 in atherosclerosis. However, the role of CCR7 in atherosclerosis is still controversial because the findings from different experimental studies are contradictory[211–213]. One limitation of these studies is that they used mice with global knockout of *Ccr7*, and CCR7 is also expressed on activated dendritic cells and B cells. A second limitation is that the antigen specificity of the identified CCR7-expressing T cells is unknown.

Potential T cell-related therapies

Currently no clinically applicable therapies for atherosclerosis related to T cells are available; however, some therapeutic candidates are emerging. Current data point to IL-1β, IL-17 and TNF as effective targets to reduce cardiovascular disease progression, at least in some settings[214,215]. Whether the autoimmune component of atherosclerosis can be addressed by anti-inflammatory therapies targeting cytokines and inflammatory pathways is currently unknown; however, vaccination and immunomodulation might provide a future antigen-specific therapy that is expected to avoid weakening the host defence[8]. Tolerogenic vaccines against atherosclerosis are focused on inducing the generation of atheroprotective T_{reg} cells against various atherosclerosis antigens. Other vaccines induce neutralizing antibodies against proprotein convertase subtilisin/kexin type 9 (PCSK9) or cholesterol ester transfer protein (CETP)[8]. The concept of T_{reg} cell-inducing vaccines for atherosclerosis is

based on the idea that induction of antigen-specific T_{reg} cells would suppress atherosclerosis by suppressing effector T cell expansion, especially of T_H1 cells. T_{reg} cells would also reduce inflammatory cytokine secretion. Preventative vaccination with ApoB-peptide, LDL or HSP has been shown to reduce atherosclerotic lesions in experimental models[4,11,216]. Currently no evidence indicates whether any of these vaccines can be therapeutic, that is, whether vaccination would work after the onset of atherosclerosis. One concern would be that the induced T_{reg} cells could switch their phenotype towards a pathogenic phenotype, as detailed above. A different strategy to reduce atherosclerosis by T_{reg} cell expansion is used in clinical trials on low-dose IL-2 therapy[217]. The CCR5 antagonist maraviroc is currently used as a treatment in patients with HIV infection, given that some HIV strains use CCR5 to enter $CD4^+$ T cells. Treatment of atherosclerosis-prone mice with this CCR5 antagonist reduced atherosclerotic lesions[218]. People living with HIV infection have an increased incidence of cardiovascular disease. One study showed that in people with HIV infection, treatment with the CCR5 antagonist reduced atherosclerotic progression[219].

Conclusion

Emerging evidence indicates that atherosclerosis is a chronic inflammatory disease with an autoimmune component. LDL and ApoB peptides seem to be the most relevant self-antigens that can both drive an autoimmune response in the atherosclerotic plaque and be atheroprotective in a vaccination setting. T_H cells have been well studied in the context of atherosclerosis, and are considered to have important roles in atherosclerosis. The pro-atherosclerotic role of T_H1 cells and the anti-atherogenic role of T_{reg} cells have been clearly shown in mice. The role of the other T_H subtypes and $CD8^+$ T cells is less clear and discrepant between different studies (Box 1). Advances in single-cell analysis technologies such as scRNAseq and CyTOF (Box 2) are providing much deeper insights into T cell subsets in atherosclerotic lesions, lymph nodes and blood (Fig. 3). Techniques for sampling local molecules released from coronary plaques[220,221] or debris from ruptured plaques[222] are available (Fig.3a). However, all these approaches do not (yet) address the problem of identifying antigen specificity of the T cells. Newly identified MHC class II-restricted epitopes in ApoB have been used to build tetramers to detect antigen-specific T cells in atherosclerosis development in humans and mice[4] (Fig.3b). Prophylactic tolerogenic vaccines that are based on ApoB and other atherosclerosis antigens are effective in animal models, but whether and how these approaches can safely be translated to the clinical setting is currently unknown.

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Glossary terms

Major histocompatibility complex

Family of molecules that present antigen peptides to T cells. MHC class I molecules present peptides to $CD8^+$ T cells and MHC class II molecules to $CD4^+$ T cells. In humans, MHC

molecules are very diverse (thousands of known alleles), but some alleles are more common (~10–20%) in some populations.

T cell receptor
The TCR is a heterodimer that consists of either αβ or γδ chains, and expression of each heterodimer is mutually exclusive on the same cell. TCR signalling proceeds through transmembrane and intracellular CD3 subunits associated with the TCR. TCRs are highly polymorphic through recombination of V and J (and in some cases D) segments and through template-free nucleotide addition.

Co-stimulatory molecules
Co-stimulatory molecules are cell surface receptors that are ligated when the TCR engages the MHC-peptide complex. The nature of the co-stimulatory molecules determined the outcome of antigen presentation[248].

TCR sequencing
Technique that uses specific primers to identify the V, D and J segments used in each α, β, γ and δ subunit. The assembly of short reads and specialized reconstruction software such as TRACER[249] allow the reconstruction of the entire TCR sequence, often of paired α and β or γ and δ chains.

MHC tetramers
Reagents used to analyse antigen-specific T cells; constructed from four recombinant truncated MHC class I or MHC class II molecules with the antigenic peptide naturally (non-covalently) or covalently bound and tetramerized by streptavidin; the streptavidin can be labelled by fluorochromes for detection with flow cytometry (Fig. 3b).

$CD4^+$ effector memory T cells
A type of antigen-experienced CD4 T cells that has effector functions but also forms memory (recall response after restimulation).

T cell exhaustion
Progressive loss of T-cell functions under chronic antigen stimulation, for example in cancer or persistent virus infetions. Exhaustion can result in the deletion of the responding cells.

Innate lymphoid cells
Cells of the innate immune system with similarity to T helper cells in the expression of key transcription factors and effector molecules, but that lack antigen-specific receptors characteristic of the adaptive immune system

Necrotic core
The necrotic core of atherosclerotic lesions is composed of cholesterol crystals, debris of apoptotic cells such as smooth muscle cells, foam cells and macrophages, and calcium particles. Large necrotic cores are associated with vulnerable plaques.

Central memory T cells
A type of antigen-experienced CD4 T cells that has no effector functions but forms memory and is able to recirculate to secondary lymphoid organs.

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Key points

- Atherosclerosis is a chronic inflammatory disease, and accumulating evidence supports the critical role of T cells as drivers and modifiers in of this condition.
- T cells drive immune responses to peptide epitopes related to atherosclerosis, such as peptides derived from apolipoprotein B.
- $CD4^+$ T helper 1 (T_H1) cells and natural killer T cells have pro-atherogenic roles, whereas regulatory T (T_{reg}) cells have anti-atherogenic functions.
- The role of other T_H cell subsets, such as T_H2, T_H9, T_H17, T_H22 and follicular helper T (T_{FH}) cells, and of $CD8^+$ T cells and γδT cells remains controversial.
- During atherosclerosis progression, T_{reg} cells can convert into pro-inflammatory T cell subsets, such as T_H1, T_H17 or T_{FH} cells.
- T cell recruitment to the atherosclerotic plaque occurs via chemokines and chemokine receptors such as CCR5, CXCR3 and CXCR6.

Box 1 |

Possible causes of discrepancies in the findings of experimental studies on atherosclerosis

Several factors can account for the discrepancies in the effects of T cell subsets reported in experimental studies on atherosclerosis.

Mouse strains

One possible cause for conflicting findings is the vastly different susceptibility to atherosclerosis of inbred mouse strains[223]. The role of T cells in atherosclerosis has been mostly explored in *Apoe*$^{-/-}$ mice or *Ldlr*$^{-/-}$ mice on a C57BL/6 background. However, 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. 129/Sv contamination can decrease the susceptibility to atherosclerosis.

T cell depletion strategy

In many experimental studies on atherosclerosis, monoclonal antibodies (mAbs) are used to deplete T cell subsets. These mAbs are usually antibodies against mouse antigens generated in rats or hamsters. Therefore, the antibody itself is a foreign antigen that elicits a vigorous immune response in the mice, resulting in the production of neutralizing antibodies[224]. If a mAb is injected more than once, subsequent doses might become ineffective[224]. Therefore, careful monitoring of $CD4^+$ T cell or $CD8^+$ T cell levels over the course of the depletion experiment is required.

Power of the studies

Another common problem is that most of the available experimental studies on the role of T cells in atherosclerosis were underpowered, which can yield spurious results[225]. This can be avoided by appropriate power calculations during the experimental design phase.

Gene knockout strategy

Data from mice with systemic gene knockout are difficult to interpret, because most genes are expressed not only in T cells but also in other cell types. As a case in point, effects on atherosclerosis that were originally attributed to T cells in studies in mice with systemic deficiency in a specific receptor, transcription factor or cytokine were later found to be caused by innate lymphoid cells[74]. Conditional knockout mice are generated most commonly with the Cre–*loxP* system, in which *Cre* is expressed under the control of a cell-specific or tissue-specific gene promoter, in the case of T cells, for example, under the *Cd4*–Cre, *Cd8*–Cre and other subset-specific drivers, so that the Cre-mediated *loxP* recombination and subsequent gene loss occurs only in the targeted cells. However, the specificity of each Cre driver must be established for each targeted T cell type. When an inducible Cre system is used, such as the Cre recombinase fused to a mutant oestrogen ligand-binding domain, in which Cre is active only after administration of tamoxifen, the degree of gene knockout in the targeted cell should be monitored before and after tamoxifen administration.

Diet

The levels of dietary cholesterol in different types of diet (Chow, Western, high-cholesterol or cholate-containing diets) used in experimental studies on atherosclerosis can directly affect the phenotype and proliferation rate of T cells[137,226,227]. Hundreds of different diets are used in experimental atherosclerosis studies.

Gut microbiota

The gut microbiota of mice differs between different animal facilities and even between cages in the same facility. The gut microbiota can have large effects on T cell functions[2,228,229] and on atherosclerosis development and progression[2,230,231].

Box 2 |

Technological advances for analyzing leukocytes in atherosclerosis

Assessment of the immune cell composition of tissues was traditionally performed by flow cytometry and immunohistochemistry. The development of high-dimensional, parametric single-cell analyses, including mass cytometry (CyTOF)[232,233] and massively parallel single-cell RNA sequencing (scRNA-seq)[234–239], have led to deeper insights into immune cell subsets.

Similar to flow cytometry, in CyTOF, single-cell suspensions are stained with antibody panels to detect cellular antigens. In contrast to flow cytometry, antibodies for CyTOF are conjugated with rare metal earth (lanthanide) isotopes, which enable the detection of up to 40 antigens. Flow cytometry requires substantial compensation to correct for the spectral overlap of fluorophores. In CyTOF, the mass spectra of different lanthanides do not overlap. CyTOF can simultaneously detect extracellular, intracellular and phosphorylated antigens[240]. CyTOF has been used to analyze immune cell composition in atherosclerosis[17,21].

scRNA-seq allows the detection of single-cell transcriptomes. The commercialization of gel-bead-based approaches revolutionized the assessment of the immune landscape of complex tissues by allowing the analysis of thousands of single-cell transcriptomes in parallel[241]. This approach sharpens the focus on the heterogeneity of immune cell populations. On the basis of the transcriptome data, the developmental ontogeny of individual cell clusters can be assessed with trajectory algorithms[242,243], the functionality of immune cell subsets can be predicted, and the likely cellular interaction partners within a tissue can be identified based on the expression of receptor-ligand pairs[244]. scRNA-seq enables a better understanding of aortic wall cells and particularly leukocytes[17,20,25,245–247]. Moreover, gene expression and cell surface phenotypes can be assessed in the same single cell with the use of antibodies conjugated with barcoded oligonucleotides, such as with the cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) or the Abseq methods[22].

A summary of analytic methods is shown in the Table below. Of note, all these methods require generating single-cell suspensions, which can introduce various artifacts.

Characteristics	**Microscopy**	**Cytometry**		**scRNA-seq with CITE-seq or Abseq**	
	Immunofluorescence	**flow cytometry**	**CyTOF**	**Antibodies**	**Genes**
Number of markers	~3	~16	~40	~50	~2,000
Number of cells	10–1,000	Millions	~100,000	~10,000	~10,000
Multiplexing	No	No	Yes	Yes	Yes
Intracellular antigens	Yes	Yes	Yes	No	N/A
Cell morphology	Yes	Some (forward	No	No	No

Characteristics	Microscopy	Cytometry		scRNA-seq with CITE-seq or Abseq	
	Immunofluorescence	flow cytometry	CyTOF	Antibodies	Genes
		and side scatter			
Surface markers and transcriptome in the same cell	No	No	No	Yes	Yes
Cost per cell	High	Low	Medium	High	High
Spectral overlap	Yes	Yes	No	No	No
Enzymatic digestion	No	Yes	Yes	Yes	Yes
Non-proportional cell death of different cell types	No	Yes	Yes	Yes	Yes

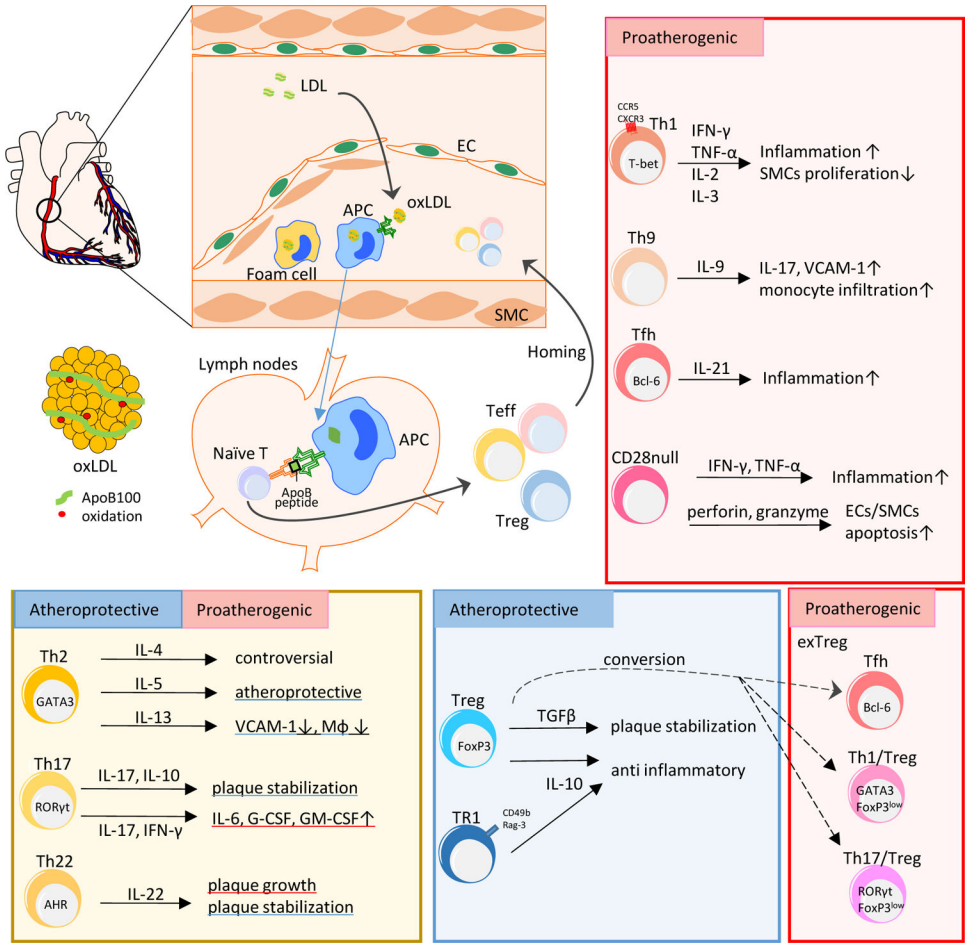

Fig. 1 |. Role of T helper cells and regulatory T cells in the pathogenesis of atherosclerosis.
a | Naive $CD4^+$ T helper (T_H) cells are primed in secondary lymphoid organs. T_H cells acquire the complete phenotype of effector T (T_{eff}) cells or regulatory T (T_{reg}) cells after encountering antigenic peptides from apolipoprotein B (ApoB) presented by antigen-presenting cells (APCs). APCs take up and process oxidized LDL (oxLDL), migrate to the draining lymph node and present peptides from ApoB on major histocompatibility complex (MHC) class II molecules. Naive T cells recognize this complex through their specific T cell receptors (TCRs). Co-stimulatory molecules induce T cells to express transcription factors that favour the differentiation into distinct T_H phenotypes. Homing receptors promote T cell migration to atherosclerotic lesions, where they secrete effector cytokines. **b-c** | $CD4^+$ T cells can act in a pro-atherogenic or atheroprotective manner. Atherosclerotic lesions contain T_H1, T_H2, T_H9, T_H17, T_H22, T_{reg}, type 1 regulatory T (Tr1) cells and follicular helper T (T_{FH}) cells. T_{reg} cells can convert to 'exT_{reg} cells (loss of CD25 and FoxP3)' (dashed arrows), acquiring properties of other T_H phenotypes such as T_H1, T_H17 and T_{FH}. Instability of forkhead box protein P3 (FOXP3) expression triggers the formation of antigen-specific, but dysfunctional, partially non-protective exT_{reg} cells. AHR, aryl hydrocarbon receptor; BCL6, B-cell lymphoma 6; CCR5, C-C chemokine receptor type 5; CXCR3, C-X-C chemokine receptor type 3; EC, endothelial cell; G-CSF, granulocyte colony-stimulating factor; GATA3, GATA-binding factor 3; GM-CSF, granulocyte-macrophage colony-

stimulating factor; IFNγ, interferon-γ; LAG3, lymphocyte activation gene 3 protein; OxPL, oxidized phospholipid; PL, phospholipid; RORγt, nuclear receptor RORγt; T-bet, T-box transcription factor TBX21; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; VSMC, vascular smooth muscle cell, VCAM1; vascular cell adhesion molecule 1.

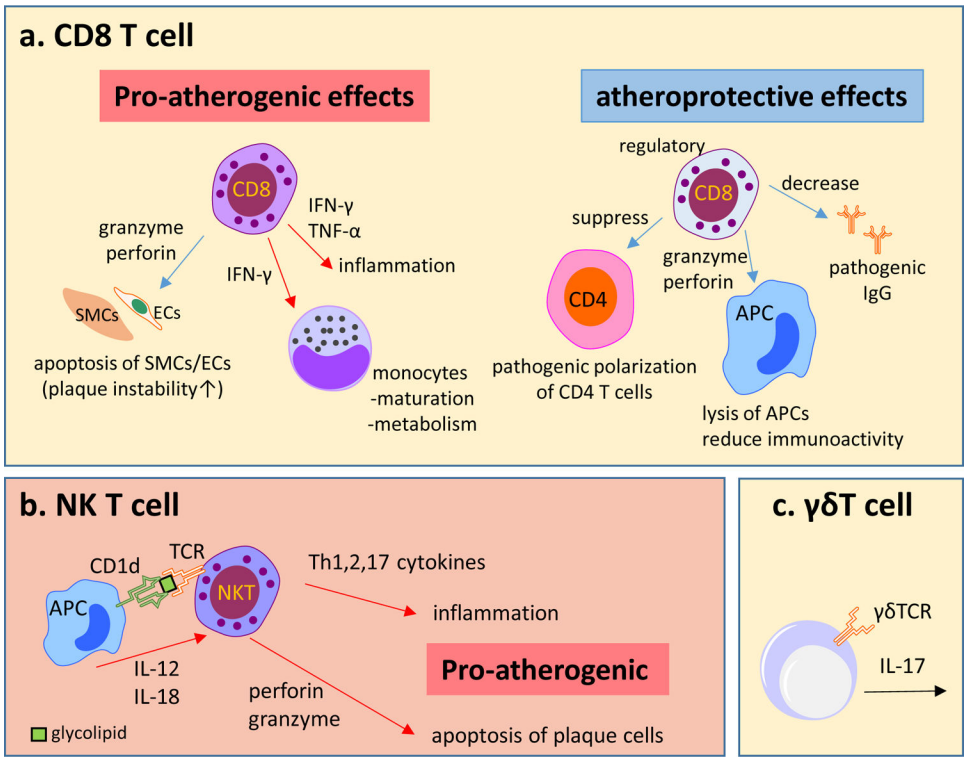

Fig. 2 |. Role of CD8$^+$ T cells, iNKT cells and γδT cells in atherosclerosis.

a | Overview of atheroprotective and pro-atherogenic functions of CD8$^+$ T cells. The cytotoxic activity of CD8$^+$ T cells towards atherosclerotic lesion-stabilizing cells, such as vascular smooth muscle cells (VSMCs) and endothelial cells (ECs), and the secretion of interferon-γ (IFNγ), tumour necrosis factor (TNF) and other pro-inflammatory cytokines exacerbate the inflammatory responses and drive the progression and destabilization of atherosclerotic lesions. Regulatory CD8$^+$ T cell subsets can have atheroprotective effects, with high cytotoxic activity towards antigen-presenting cells (APCs) and inhibition of CD4$^+$ T cell polarization into pro-atherogenic phenotypes. **b** | Invariant natural killer T (iNKT) cells can be activated by the interaction of the T cell receptor (TCR) with CD1d molecules containing antigenic glycolipids present on APCs. iNKT cells can also be activated in a CD1d-independent manner by Toll-like receptor stimulation and by the activation of APCs, which in turn secrete cytokines that activate iNKT cells, such as IL-12 and IL-18. Activation of iNKT cells results in the rapid release of T_H1, T_H2 and T_H17 associated cytokines, which activate other immune cells in the atherosclerotic lesion. iNKT cells can also promote atherosclerosis by the induction of apoptosis of plaque cells through the release of cytotoxic proteins such as perforin and granzyme B. **c** | γδT cells are among the T cell subsets described in mouse atherosclerotic lesions. The intracellular cholesterol content in γδT cells regulates their activation, proliferation and effector functions. In addition, γδT cells are an abundant source of IL-17; therefore, these cells could modulate atherosclerosis via IL-17 production. However, the exact role of γδT cells in atherosclerosis is unclear.

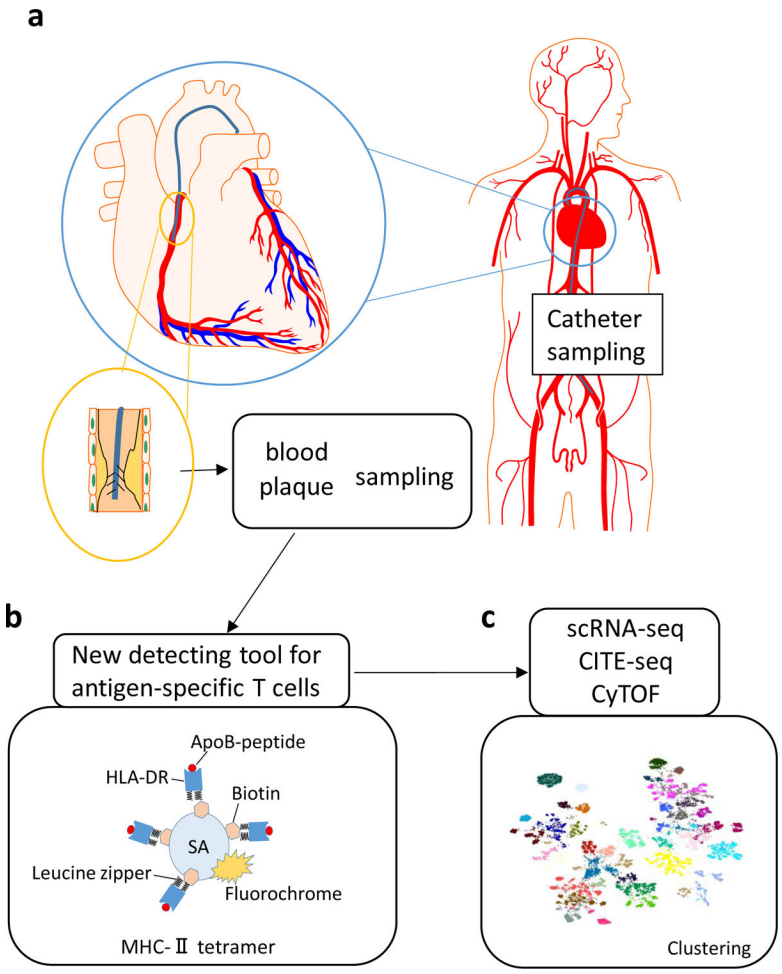

Fig. 3 |. Tools for sampling and analysis of T cells in atherosclerosis.

a | Advanced percutaneous catheter devices can be used to sample the boundary layer of blood close to the vessel wall upstream and downstream of intact and disrupted human coronary atherosclerotic plaques or to sample plaque debris generated by plaque disruption through stent placement. A distal protection device can be positioned in the coronary artery downstream from the atherosclerotic plaque. **b** | Tetramer technology to identify apolipoprotein B (ApoB)-specific T cells. The tetramer reagent consist of four specific recombinant major histocompatibility complex (MHC) class II molecules tetramerized with streptavidin (SA) and loaded with ApoB-peptide for detecting ApoB-specific T cell receptors. **c** | Single-cell RNA sequencing (scRNA-seq), cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) and mass cytometry (CyTOF) provide deep transcriptome data, cell surface phenotypes and T cell receptor sequencing data for ApoB-specific T cells in atherosclerosis.