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REVIEW

Immunological aspects of atherosclerosis

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Abstract Atherosclerosis is a complex chronic inflammatory and metabolic disease that involves the collaboration of several cellular components of the immune system and results in thickening of the arterial wall. Atherosclerosis is also the primary cause of coronary artery and cerebrovascular diseases. A multitude of immune cell subsets, soluble molecules such as chemokines and cytokines, and circulating lipids play pivotal roles in atherosclerosis development. In this review, we highlight the role of the immune system in the course of atherosclerotic disease development and discuss the mechanisms involved.

Keywords Atherosclerosis · Macrophage · Dendritic cell · T lymphocyte · Immunotherapy

Abbreviations

ApoE	Apolipoprotein E
DC	Dendritic cell
FGF	Fibroblast growth factor
GM-CSF	Granulocyte macrophage colony-stimulating factor
HDL	High density lipoprotein
ICAM-1	Intercellular Adhesion molecule
IFN-γ	Interferon γ
IL	Interleukin
JAM	Junctional Adhesion Molecule
LDL	Low density lipoprotein
LFA-1	Leukocyte function-associated antigen 1
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage colony-stimulating factor
MMP	Matrix metalloproteinase
NF-κB	Nuclear Factor kappa-B
PDGF	Platelet-derived growth factor
PPAR	Peroxisome proliferator-activated receptor
ROS	Reactive oxygen species
SMC	Smooth muscle cells
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
VCAM-1	Vascular-cell adhesion molecule 1
VLA-4	Very late antigen 4
VLDL	Very low density lipoprotein

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Introduction

According to the World Health Organization, approximately 30 % of global worldwide mortality can be attributed to cardiovascular diseases (CVDs), and their incidence is expected to increase mostly in low- and middle-income countries [70, 195]. It is also expected that CVD alone will cause more deaths than infectious and perinatal diseases and nutritional disorders combined. The main cause of CVD is atherosclerosis, which is characterized by local asymmetric thickening of the vessel intima [77]. Atherosclerosis is a multifactor chronic inflammatory disease of the arterial wall that leads to symptomatic pathologies such as acute coronary syndromes, stroke, and peripheral artery occlusions [77]. In addition, physiological factors such as lipid metabolism and

hypertension and behaviors such as alcohol consumption and smoking promote the development of atherosclerosis [70, 171].

Metabolic syndromes exacerbate the atherogenic phenotype by inducing dyslipoproteinemia, which results from high serum levels of low-density lipoprotein (LDL), a weak cholesterol transporter, and low levels of high-density lipoprotein (HDL), a strong cholesterol transporter [128]. The risk for atherogenicity is expressed as an LDL/HDL ratio greater than 3, and hypertension further increases the atherosclerosis risk by 60 % [109]. The effect of these physiological factors can also be highly exacerbated by behavioral factors such as lack of exercise, excessive alcohol consumption, and smoking, which increase the risk for CVDs by 200 % [5]. Based on these findings, it is important to understand the physiological processes underlying the formation of atherosclerotic plaques to better treat CVD patients.

Animal models have been useful to understand the molecular and cellular mechanisms implicated in the development of atherosclerotic lesions. Because wild-type mice are resistant to lesion development, genetic deletion models have been developed. The two most currently used models consist of mice with a deficiency in the apolipoprotein (ApoE) or low-density lipoprotein receptor (LDLR) genes [91, 145, 216]. Because LDLR-deficient mice do not properly clear very low density lipoprotein (VLDL) and LDL from the plasma, the blood slowly accumulates cholesterol and animals develop atherosclerotic lesions under a normal diet [91], although a high-fat diet exacerbates this phenotype [101]. ApoE deficiency also leads to defective cholesterol transport and sub-endothelial accumulation of cholesterol under a normal diet. This model best recapitulates the chronology of lesion development in humans and is therefore useful for identifying candidate genes implicated in the formation of lesions as well as testing novel therapies to block lesion progression [207, 216]. Although these mouse models present similarities to human atherosclerosis, their usefulness is limited due to the absence of coronary plaques and the development of stable plaques instead of unstable ones, such as those observed in humans [64]. Interestingly, however, $ApoE^{-/-}$ mice crossed to scavenger receptor class B type I adaptor-deficient animals develop similar cardiac complications as humans [211].

High circulating levels of cholesterol represent a key molecular correlate of atherosclerosis development [183]. Hypercholesterolemia leads to LDL cholesterol retention in the sub-endothelial extracellular matrix and promotes the passive infiltration and deposition of other macromolecules, such as apolipoprotein B lipoproteins (ApoB-LPs), the latter of which can interact with proteoglycans and chondroitin sulfate in the sub-endothelial matrix [18, 19, 96, 188]. Deposits of ApoB-LPs are then modified by lipolysis, proteolysis, and oxidation [82, 187, 201, 202]. The 12/15-lipoxygenase, which is expressed by endothelial cells, smooth muscle cells (SMCs), and monocytes, mediates minimal oxidative modification of ApoB-LPs. Once oxidized, ApoB-LPs in turn activate endothelial cells and induce inflammation, promoting the formation of atherogenic plaques [16, 17, 62, 155] (Fig. 1a, b).

Several types of cells collaborate to carry out the atherosclerotic process. For example, endothelial cells, monocytes, monocyte-derived macrophages, foam cells, T cells and SMCs participate in establishing an inflammatory environment by secreting cytokines. Then, chronic inflammation eventually gives rise to acute cardiac disease symptoms. In the next section, we will discuss the role of immune cells and their prevalence in the evolution of atherosclerotic disease (Fig. 1c).

Pro-atherogenic role of leukocytes

Recruitment of monocytes/macrophages

Oxidized ApoB-LPs (oxysterols) are at the origin of leukocyte recruitment, as these molecules interact with the endothelial oxidized LDL (ox-LDL) receptor-1 [34, 140, 201] and lead to cyclic AMP and reactive oxygen species (ROS) production. This activates the NF-κB transcription factor and induces the expression of various pro-inflammatory molecules by endothelial cells, including adhesion molecules for circulating monocytes (vascular-cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1)), chemotactic molecules (monocyte chemoattractant protein-1 (MCP-1)/CCL2, macrophage colony-stimulating factor (M-CSF), GM-CSF), pro-coagulant tissue factors (thrombotic factors VII/VIIa), and SMC mitogenic factors (platelet-derived growth factor (PDGF) and FGF) [33, 46, 51, 55, 166, 179].

Monocytes are the first immune cells to infiltrate the intima and therefore stimulate the inflammatory process. Monocyte chemotactic protein 1 (MCP-1/CCL2) [71] and M-CSF [167] enhance the recruitment of monocytes and T cells to the vessel intima [117, 171], a process that begins with the binding of MCP-1 to proteoglycans on the endothelial plasma membrane [37, 63]. Rolling monocytes along the P- and E-selectin-expressing luminal side of the endothelial layer then bind to MCP-1 via CCR2 and, as a consequence, firmly adhere to the endothelium [153]. Furthermore, the binding of MCP-1 to CCR2 activates the integrins LFA-1 and MAC-1 (αMβ2) expressed by the monocyte, which results in sustained avidity of the monocyte to endothelial ICAM-1 [203]. In addition to the chemokine signal, rolling monocytes also detect integrin-activating signals via the selectin ligand P-selectin glycoprotein ligand-1 (PSGL-1). Finally, integrin α4β1 (VLA-4) binds to the endothelial receptor VCAM-1 in a

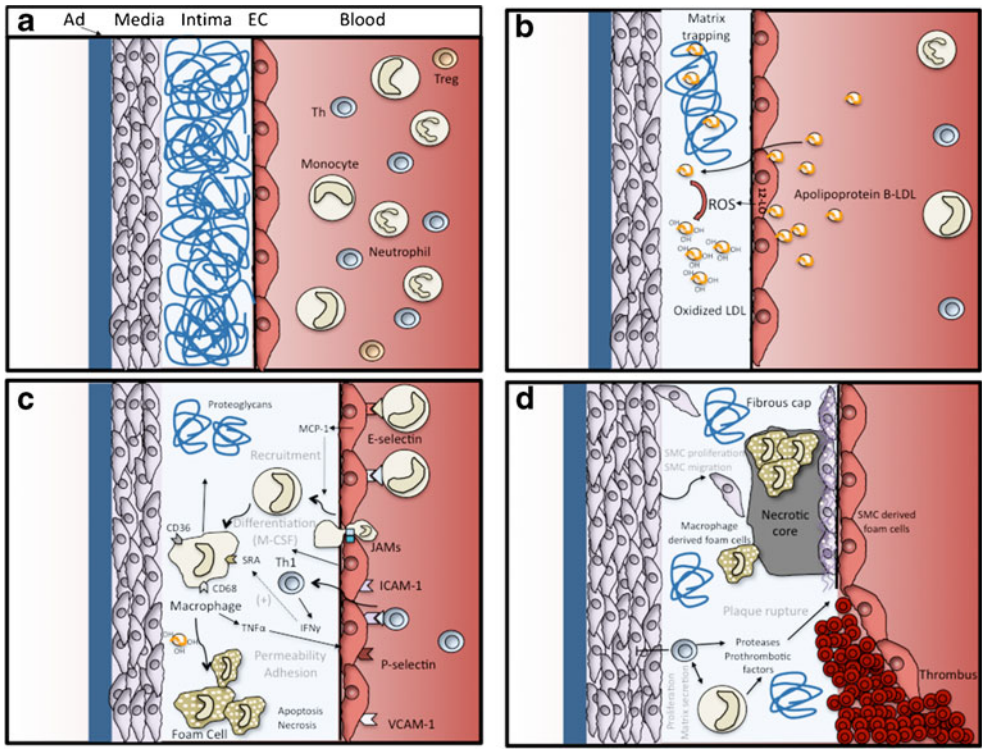

Fig. 1 Evolution of atherosclerosis lesion over time. **a** Normal vessel composed of an endothelial cell layer (*EC*), an intima layer (*Intima*), which contains the elastic lamina and extracellular matrix proteins such as collagen and proteoglycans, a tunica media (*Media*) composed of smooth muscle cells, and an the tunica adventitia (*Ad*). Under normal conditions, immune cells are mainly found in the blood compartment (*Blood*). **b** Initiation of the lesion by deposition of LDL-apolipoprotein B complexes on the endothelial cell layer and infiltration into the intima through rendering the endothelial layer permeable. The LDL–apolipoprotein B complexes are retained in the intima through their interaction with extracellular matrix components. In the intima, LDL will be oxidized through the action of ROS produced by endothelial cells. **c** Inflammatory stage of the lesion. Immune cells (monocytes, neutrophils, and T cells) infiltrate the intima by transendothelial migration using adhesion molecules (selectins, ICAM-1, VCAM-1, JAMs). Activated endothelial cells secrete M-CSF, which induces the differentiation of monocytes into macrophages that express scavenger receptors for oxidized LDL (CD68, CD36, SRA). These oxidized LDLs will accumulate in macrophages and transform them into foam cells. Foam cells will then die by necrosis or apoptosis. The accumulation of dying foam cells forms a necrotic core in the intima. **d** The advanced lesion or atheroma may degenerate and lead to rupture of the plaque depending on the thickness of the fibrous cap and the degree of inflammation. T cells stimulate the secretion by macrophages of proteases, which degrade the fibrous cap and or they inhibit SMC secretion of matrix proteins by secretion IFNγ. Degradation of the fibrous cap leads to rupture of the plaque and to the release of tissue factor into the blood, which leads to thrombus formation

low-affinity conformation [60, 89], and the resulting strength of monocyte adhesion to the endothelium promotes their patrolling along endothelial cells and leads to their trans-endothelial migration towards the intima [44]. Studies by Charo and Peters have demonstrated that *Ccr2*$^{-/-}$ mice on an ApoE-deficient genetic background demonstrate decreased monocyte/macrophage recruitment and develop smaller atherosclerotic lesions [160]. Furthermore, blocking antibodies against P- and E-selectin, both in vitro and in vivo, has been shown to reduce monocyte rolling on the aortic endothelium of ApoE-deficient atherosclerotic mice, increase the leukocyte migration velocity, and decrease the duration of the leukocyte–endothelial interaction [49]. Moreover, *LDR*$^{-/-}$ and *P-/E-selectin*$^{-/-}$ double-deficient mice, after 8 weeks on a high-fat diet, develop atherosclerotic lesions that are five times smaller than those observed in *LDR*$^{-/-}$ mice, and this difference remains as high as 40 % after 37 weeks of feeding with an atherogenic diet [46]. In addition, inhibiting the glycosylation of PSGL-1 by deleting α-(1,3)-fucosyltransferase (FucT) also decreases the interaction between monocytes and selectin-coated surfaces under flow conditions, as well as the size of atherosclerotic lesions in *ApoE*$^{-/-}$ mice [84, 86]. These experiments demonstrate the involvement of chemokines and selectins in the atherogenic monocyte homing process. Furthermore, a recent study revealed the role and therapeutic potential of a human anti-VCAM-1 antibody in ApoE deficiency; this antibody cross-reacts with murine VCAM-1 and was able to improve atherosclerotic lesions in terms of the size of the plaque and the immune cell infiltration [154].

Other proteins may also play roles in the recruitment of monocytes to the early lesion, notably junctional adhesion molecules (JAMs). JAMs are adhesion molecules belonging to the immunoglobulin superfamily and are expressed at the cell–cell junctions between endothelial and epithelial cells. JAM-A and JAM-C are involved in leukocyte recruitment to

atherosclerotic plaques, and JAM-A deficiency in $ApoE^{-/-}$ mice reduces the extent of plaques and monocyte infiltration without altering the SMC content [217]. Moreover, a circulating plasmatic form of JAM-A is detected in human atherosclerosis and has been associated with more advanced disease [27]. Two studies have also shown a role for JAM-C in atherosclerosis. The first study showed in vitro that the expression and localization of JAM-C on endothelial cells and vascular smooth muscle cells (VSMCs) could be modified by oxLDL and that leukocyte adhesion to oxLDL-activated endothelium depended on JAM-C [95]. The second study used the $ApoE^{-/-}$ mouse model and found that treating these atherosclerosis-prone mice with a specific anti-JAM-C antibody reduced the hyperplasia of the neointima and diminished the number of infiltrated macrophages without altering the VSMC content [176].

Formation of foam cells

Under stimulation by M-CSF, which is produced by activated endothelial cells, monocytes differentiate into macrophages, which phagocytize oxidized LDL via scavenger receptors [164]. Through the secretion of chemokines, activated macrophages then induce the recruitment of T cells, mast cells, and SMCs into atheroma plaques [77, 78]. This inflammatory state can be maintained for several years, thereby potentiating atherogenicity and increasing the size of plaques.

Lack of M-CSF prevents the differentiation of monocytes into macrophages, which impacts the pathophysiology of several diseases. Macrophages are major players in the atherosclerotic inflammatory process [52]. These cells act as part of the innate immune response and demonstrate the capacity to phagocytize pathogens and produce ROS, extracellular proteases, complement factors, and cytokines. Macrophages also activate the adaptive immune response by presenting pathogen-associated antigens to T cells to stimulate T cell activation. Evidence for the crucial role of macrophages in atherosclerosis has been provided by studies in which mice lacking M-CSF were crossed to ApoE-deficient mice (op/op mice), and these animals were protected from atherosclerosis despite high cholesterol levels in the blood [115].

Following deposition within atherosclerotic plaques, LDL undergoes numerous oxidative modifications, which are mainly mediated by ROS and give rise to highly oxidized metabolites such as oxysterols. Following passive LDL infiltration and minimal oxidation by 12/15-lipoxygenase and ROS, enzymes in the sub-endothelium, such as LPL, contribute to the retention of ApoB-LPs by bridging them to proteoglycans in the intima [96, 187, 188, 205]. The retention process of LDL is enhanced by the secretory sphingomyelinase, which is mainly secreted by macrophages and endothelial cells in atherosclerotic plaques [188]. Oxidized lipoproteins are bound and endocytosed by scavenger receptors expressed by macrophages that then become foam cells [53, 164, 209, 213]. Accordingly, mice deficient in scavenger receptors show a slight decrease in atherosclerotic lesions due to reduced numbers of foam cells within plaques [139]. Macrophages/foam cells express two types of scavenger receptors: class A and B. Class A receptors are trimeric proteins with a collagenous domain that is essential for ligand binding, whereas class B receptors (CD36 and SR-B1) are highly N-glycosylated and fatty-acylated protein, containing two transmembrane domains and a C-terminal cytoplasmic tail. These structures recognize a wide range of negatively charged macromolecules, such as ox-LDL, dead cell debris, and pathogenic microorganisms [209]. Furthermore, the absence of CD36 protects against the development of atherosclerosis, and $Cd36^{-/-}/ApoE^{-/-}$ double-knockout mice have a 74 % decreased aortic lesion area and a less pro-inflammatory phenotype compared to single $ApoE^{-/-}$ knockout mice. However, the combined deficiency of scavenger receptor A (SRA) I/II and CD36 provides no additional protection [103].

The uptake of ox-LDL by macrophages through scavenger receptors is tightly regulated by cytokines. The T helper (Th) 2-associated cytokine interleukin (IL)-4 induces the expression of CD36 and enhances ox-LDL uptake, while the Th1-associated cytokines interferon γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-6 induce SR-A downregulation, resulting in the impairment of cholesterol metabolism [76].

Scavenger receptors may also form a link between the innate and adaptive immune responses. When ox-LDL particles bind to scavenger receptors on macrophages, they stimulate phagocytosis and antigen processing for the presentation of MHC class II-restricted peptides to T cells. In addition, ox-LDL particles can stimulate macrophages by other means; for example, ox-LDL contains platelet-activating factor-like lipids, which are strongly inflammatory and can activate macrophages as well as endothelial cells [202]. Furthermore, ox-LDL can bind to cell surface signaling receptors such as Toll-like receptors, which can also activate macrophages [76].

Internalized ox-LDL is processed by enzymes such as cholesterol 27-hydroxylase, which converts ox-LDL into its soluble form, 27-OH-cholesterol [13]. The massive cytoplasmic accumulation of cholesterol in macrophages then leads to the formation of typical atherogenic foam cells. However, efflux of cholesterol via membrane transporters is possible and is considered anti-atherogenic.

Avoiding a surplus of intracellular cholesterol is important for the survival of macrophages/foam cells. Within atherosclerotic lesions, macrophages are exposed to an excess of cholesterol stemming from cell debris, lipoproteins, and ox-LDL particles. The rapid internalization of these particles leads to the aberrant accumulation of cholesterol in macrophages, and this toxicity affects their function and

survival. One strategy macrophages use to manage excess cholesterol uptake is the efflux of cholesterol by cholesterol efflux transporters, including a family of ATP-binding cassette (ABC) proteins and ApoE [177]. The expression of these cholesterol efflux receptors is regulated by the peroxisome proliferator-activated receptor (PPAR) family of nuclear transcription factors, which is also known for its involvement in the regulation of lipid metabolism and inflammation.

The three PPAR subtypes (α, γ, δ) are often co-expressed by cells [73]. PPARα and PPARγ promote the expression of cholesterol transporters for lipid uptake and efflux in macrophages residing in atherosclerotic lesions, and PPARα activation leads to expression of the scavenger receptor CD36 and the cholesterol efflux transporter ABCA1. The role of PPARα activators is to induce the uptake of highly atherogenic ox-LDL by CD36, followed by efflux and extracellular transport of cholesterol through HDL particles. The net effect of PPARα is pro-atherogenic because PPARα deficiency reduces atherosclerosis in $ApoE^{-/-}$ mice [194]. Loss of PPARγ disables cholesterol export and thereby accelerates the progression of atherosclerosis. PPARδ, on the other hand, regulates the inflammatory status of macrophages/foam cells, and PPARδ deficiency decreases atherosclerosis in $LDR^{-/-}$ mice mainly due to increased expression of inflammatory suppressor genes such as BCL-6 in macrophages [106].

Ox-LDLs (oxysterols) ingested by macrophages bind to the liver X receptor (LXR) nuclear hormone receptor and induce its activation and heterodimerization with the retinoid X receptor (RXR). The LXR/RXR heterodimer then forms a transcription factor that binds to specific promoters by recognizing the LXR response element sequence (LXRE), which activates the expression of target genes involved in cholesterol metabolism, including ABCA1, ApoE, and LXR itself. Treatment of $ApoE^{-/-}$ or $LDR^{-/-}$ mice with LXR or RXR activators was shown to reduce the size of atherosclerotic lesions, mainly by promoting the expression of the cholesterol efflux transporters ABCA1 and ABCG1 [94, 191].

As mentioned above, ABCA1 is a transmembrane cholesterol transporter that contributes to the export of cholesterol and fatty acids from the cytoplasm to the extracellular space. The binding of cholesterol by circulating apolipoproteins (ApoA through ApoI) then generates nascent HDL particles and directs cholesterol to the liver, where it can be secreted into the bile [28]. The role and the involvement of ABCA1 in cholesterol efflux and atherosclerosis have been described in vivo by performing bone marrow transfers from ABCA1-deficient mice to ApoE-deficient mice fed a high-fat diet. This specific ABCA1 inactivation in macrophages resulted in markedly increased atherosclerotic lesions and foam cell accumulation. However, double knockout of $ABCA1^{-/-}$ and either $ApoE^{-/-}$ or $LDLR^{-/-}$ did not affect the development, progression, or composition of atherosclerotic lesions, likely due to the observed reduction in plasma cholesterol [1, 2].

The accumulation of excess free cholesterol particles in the internal compartments of macrophages will lead to apoptosis, which contributes to the formation of the necrotic core in later plaques. Several studies have described a link between impairment of macrophage cholesterol metabolism and endoplasmic reticulum (ER) stress, both of which lead to foam cell death and consequently necrotic core formation [141, 150, 192]. The initiating event in this process is the accumulation of free cholesterol within the plasma membrane of the ER, where normally the cholesterol/phospholipid ratio should be low. Increased cholesterol induces stiffness of the ER membrane, which consequently leads to dysfunction of integral ER proteins, such as the sarco-/endoplasmic reticulum ATPase, a calcium pump involved in calcium recapture from the cytoplasm to the ER reservoir.

Although the majority of foam cells in atherosclerotic lesions derive from macrophages, SMCs can also give rise to foam cells. This finding comes from histological observations of simultaneous staining for both smooth muscle markers and lipid droplets within intimal SMCs. Moreover, the ability of SMCs to generate foam cells was demonstrated by the fact that they express a wide variety of lipid and cholesterol uptake receptors, including scavenger receptors and cholesterol efflux transporters [50, 100, 113, 129, 172, 189, 206]. In vitro, exposure of rat and rabbit SMCs to inflammatory cytokines increases their expression of LDL and VLDL receptors as well as the binding of LDL [171, 172, 189, 199].

Dendritic cells

The precise role and location of antigen-presenting dendritic cells (DCs) in atherosclerotic lesions is not well understood, although several hypotheses have been proposed. Under hypercholesterolemia, DCs encounter and scavenge atherosclerotic-related antigens in peripheral organs such as the skin or spleen or directly within the initial lesion site itself. These antigen-loaded DCs mature and emigrate to lymphoid organs, where they present antigen epitopes to naïve T cells to activate these cells and generate atherogenic T cells. These antigen-specific T cells then migrate to the lesions, where they can be reactivated and drive the atherogenic inflammatory process. In addition, DCs may also activate T cells within the lesion site itself, although this mechanism of activation is less efficient and will instead function by limiting the formation of T regulatory cells (Tregs) due to the inflammatory environment [127]. Both plasmacytoid DCs (pDCs) and conventional DCs (cDCs) are found within atherosclerotic lesions [147]. pDCs promote the development of atherosclerotic lesions, as depletion of pDCs in $ApoE^{-/-}$ mice using an anti-mPDCA-1 antibody was shown to be anti-

atherogenic; this treatment reduced the size of the plaques and the number of macrophages but increased the collagen content. Moreover, IFN-γ-producing pDCs, when activated by antimicrobial peptide complexes, promote the development of atherosclerotic lesions in *ApoE*$^{-/-}$ mice [47], and oxLDL has been shown to transform pDCs into IL-12-producing inflammatory cells [158] and induce the upregulation of scavenger receptors. Therefore, the uptake of oxLDL by DCs and the secretion of IL-12 initiate the formation of lesions [146, 157]. cDCs also contribute to the progression of lesions, as shown by the expression of CCL17 in a subset of cDCs in the aortae of *ApoE*$^{-/-}$ mice during disease formation. These DCs also recruit T cells to the lesions, which drives lesion progression [204]. In conclusion, DCs can induce atherosclerotic plaque formation directly or indirectly by inducing T cell activation.

T helper cells and their secretion products

Although T cells are present in atherosclerotic lesions in lower numbers than monocytes and macrophages, they play an important role in triggering and controlling inflammation via adaptive immune responses to modified self-antigens such as ox-LDL particles, heat shock proteins, β2 glycoprotein I, and likely apoptotic/necrotic debris [61, 97, 184]. Initial studies showed that plaque formation under a Western diet was not different in immunodeficient *ApoE*$^{-/-}$ mice compared to immunocompetent *ApoE*$^{-/-}$ animals [38]. However, under moderate hypercholesterolemia, plaque formation is decreased in immunodeficient *ApoE*$^{-/-}$ mice. The relevance of antigen presentation to atherosclerosis progression comes from adoptive transfer experiments, in which the injection of ox-LDL-reactive T cells into *ApoE*$^{-/-}$*/scid/scid* mice fed a high-fat diet accelerated lesion formation compared to the transfer of T cells with no reactivity to plaque-derived antigens [219]. The presence of T cells and the expression of MHC class II by phagocytes within lesions sustain the pro-atherogenic role of antigen presentation. Furthermore, multiple studies in atherosclerotic mice have shown that various Th cell subsets have specialized roles in atherosclerosis. For example, Th1 cells exert a pro-inflammatory and pro-atherogenic effect by secreting the cytokines IFN-γ, IL-2, TNF-α, and TNF-β, which activate endothelial cells, inhibit the synthesis of extracellular matrix by SMCs, and promote Th1 immune responses. In contrast, Th2 cells are anti-inflammatory and anti-atherogenic. These cells secrete IL-4, IL-5, and IL-10, which inhibit the expression of inflammatory genes but stimulate the growth and proliferation of B cells, as well as inhibit Th1 immune responses but promote the proliferation and activation of Tregs [58]. Furthermore, Th1 cells are more abundant in atherosclerotic lesions than Th2 cells.

IFN-γ is one of the most important cytokines for the development of atherosclerotic lesions. This cytokine acts on multiple cell types and induces proliferative as well as secretory functions; furthermore, IFN-γ signaling affects approximately 25 % of the transcriptome of macrophages [48]. IFN-γ is mainly secreted by natural killer cells and activated Th1 cells, but recent evidence indicates that IFN-γ may also be produced by monocytes/macrophages, DCs, and B cells. IFN-γ induces its pro-atherogenic effects through multiple mechanisms and cell targets; for example, it enhances leukocyte recruitment to the lesion site through the activation of endothelial cells and SMCs and the expression of adhesion molecules such as P- and E-selectin, ICAM-1, and VCAM-1 [31]. *ApoE*$^{-/-}$ or *LDR*$^{-/-}$ mice deficient in IFN-γ or its receptor present reduced inflammatory cell content in atherosclerotic lesions [24]. In addition, IFN-γ induces the expression of several other genes, including the MHC class II complex in macrophages, thereby activating the antigen presentation process. IFN-γ also induces the expression of α5β1 integrin by vascular SMCs, which enables these cells to bind to the extracellular matrix molecule fibronectin. Furthermore, this interaction enhances the proliferation and migration of SMCs and blocks collagen secretion. IFN-γ is also responsible for stimulating the secretion of chemokines involved in the recruitment of immune cells; for example, the chemokine MCP-1/CCL2 can polarize the immune response towards a Th1 (pro-inflammatory) response [79].

Another cytokine involved in the development of atherosclerosis is IL-12, which is produced by DCs and monocytes/macrophages and plays an essential role in Th1 cell polarization. IL-12 induces activation of the STAT4 transcription factor and the T-box transcription factor T-bet, resulting in upregulation of IFN-γ expression in Th1 cells. IL-12 deficiency in *ApoE*$^{-/-}$ mice fed a high-fat diet significantly reduces plaque development [41], and IL-12 administration enhances IFN-γ production as well as atherosclerosis development [107].

Th2 cells secrete IL-4, IL-5, and IL-10, which promote athero-protective effects by stimulating the production of antibodies against specific atherosclerotic-associated antigens by B cells. During the development of atherosclerosis, Th2 immune responses also antagonize Th1 responses [85]. IL-4 drives Th2 cell differentiation by inducing the phosphorylation (and activation) of STAT6, a transcription factor that drives the expression of GATA3 and promotes the upregulation of IL-4 and IL-5 and the down-regulation of IFN-γ gene expression. IL-4 is typically considered an anti-inflammatory cytokine that promotes B cell proliferation and antibody production [85]. However, there is some evidence that IL-4 may also play a pro-atherogenic role through induction of VCAM-1 and MCP-1/CCL2 expression by endothelial cells [88]. Thus, the effects of IL-4 on atherosclerosis remain controversial and may differ depending on the stage and/or site of the lesion as well as the

experimental model. The relative resistance to atherosclerosis of BALB/c mice fed with high-fat diet has also been attributed to Th2 responses, although BALB/c mice deficient in STAT6 lack Th2 responses and are prone to atherosclerosis, likely due to a prominent Th1 response [85]. However, in more permissive models using $LDR^{-/-}$ mice transplanted with bone marrow cells from mice deficient in IL-4, atherosclerotic lesions were unexpectedly reduced [98]. This anti-atherosclerotic effect may have been due to the lack of the pro-atherogenic effect of IL-4 on endothelial cells. Similarly, $IL\text{-}4^{-/-}/ApoE^{-/-}$ mice have been shown to develop reduced numbers of lesions compared to $ApoE^{-/-}$ single KO mice [41].

Another product of T helper cells is IL-17A, a chronic, inflammatory cytokine produced by Th17 cells as well as several other subclasses of leukocytes. Although previous work demonstrated a pro-atherogenic effect of IL-17A, a recent genetic study found that IL-17A deficiency in all cell types in $ApoE^{-/-}$ mice accelerated atherosclerosis during an initial phase of high-fat feeding [39], which was likely due to increased IFNγ production. Furthermore, treatment of $ApoE^{-/-}$ animals with IL-17A was shown to reduce atherosclerosis progression. However, additional work is needed to definitively determine whether IL-17A could be a promising target for treating atherosclerosis.

Mechanisms leading to advanced lesions

Lesion progression is associated with a high inflammatory state. Although advanced atherosclerotic lesions (atheroma) that can lead to ischemia via arterial obstruction, myocardial infarction, and stroke are more strongly associated with plaque rupture and consequent thrombosis. The majority of myocardial infarctions result from atheroma with less than 50 % stenosis of the artery [110]. Thrombosis occurs after the rupture of plaques often found at lesion edges, where foam cells are abundant. The cellular composition of atherosclerotic plaques directly affects the stability of plaques, and determining their cellular composition is necessary for an accurate prognosis of a patient's risk to develop atherosclerosis. Macrophages play a key role in the thrombotic process by secreting extracellular proteinases, which degrade the extracellular matrix components of the fibrous cap. Under the shear stress associated with blood flow, the fibrous cap detaches, which exposes intimal tissue components to the blood, activates platelets, and initiates thrombosis (Fig. 1d).

Stable plaques show reduced numbers of inflammatory cells but increased numbers of proliferating and ECM-producing SMCs. On the other hand, unstable or vulnerable plaques generally show a thin fibrous cap and a massive accumulation of inflammatory and foam cells. Unstable plaques present a massive inflammatory necrotic core and accumulate non-proliferative and non-secreting SMCs [66, 186]. As a result, lesion stability and vulnerability are highly dependent on SMC survival and the content of their secretions within the plaque [32].

High levels of inflammation within plaques are the result of collaboration between the innate and the adaptive immune systems, which is mediated by the interactions between macrophages, macrophage-derived foam cells, and T cells. T cell–macrophage interactions and consequent cytokine secretion play an important role in the evolution of plaques from the stable to the unstable state. IFN-γ also plays a pivotal role in stabilizing plaques by inhibiting the expression of matrix metalloproteinase (MMP)-9, a protease that degrades extracellular matrix, and by upregulating superoxide dismutase, a protein involved in reducing ROS. However, as mentioned previously, IFN-γ also destabilizes plaques by increasing the production and secretion of chemokines and downregulating ApoE expression. Altogether, the prevailing effect of IFN-γ is increased size and vulnerability of the lesion [108]. Indeed, IFN-γ receptor deficiency in ApoE-deficient mice reduces the size of the atherosclerotic lesions, the lipid content, and the cellularity but increases the collagen content [72].

Other elements of the immune system that have been studied in plaques include the co-stimulatory molecule CD40, which is expressed by antigen-presenting cells, and its ligand CD40L, which is expressed on T cells. Both CD40 and CD40L are expressed within atheroma plaques, and they co-localize on atheroma-associated endothelial cells, SMCs, and macrophages. Moreover, chronically activated $CD4^+$ T cells express CD40L within human atherosclerotic lesions as well as the atheromatous tissues of hypercholesterolemic mice [120]. Accordingly, interruption of CD40 signaling, either by injecting anti-CD40L antibodies into LDR-deficient mice or using CD40/LDR double-deficient mouse strains, has been shown to alter the composition of the atheroma by reducing the content of pro-atherogenic molecules and increasing the content of SMCs and fibrillar collagen [118, 119, 175], and these effects were recently confirmed using CD40 RNAi lentiviral constructs in mice. Furthermore, CD40 knockdown not only reduced MMP-9 expression, chemokine production, and the lipid content of existing plaques but also increased the collagen content, which led to plaque stabilization [200].

Anti-atherogenic role of leukocytes

Although the immune system predominantly promotes the development of atherosclerosis, the immune system consists of a balance of pro- and anti-inflammatory signals, and some actors therefore counterbalance the pro-atherosclerotic effects of macrophages and T cells.

Anti-inflammatory cytokines and their atheroprotective mechanisms

Several studies have shown a protective role for IL-10 in the development of atherosclerotic lesions. In particular, this IL-10 anti-atherogenic effect has been demonstrated in vivo using atherosclerotic animal models. Mice deficient in IL-10 and fed an atherogenic cholate-containing diet showed formation of early atherosclerotic lesions that were characterized by an accumulation of immune cells, T cell activation, and increased production of pro-inflammatory cytokines [162]. However, this effect was reversed by the local or systemic overexpression of adenoviral IL-10 gene constructs. In addition, $ApoE^{-/-}$ mice crossed to IL10-deficient mice presented greater numbers of less stable lesions, which again suggested a role for IL-10 in atheroprotection [26]. It is interesting to note that in a model of atherosclerotic plaque regression induced by administration of conjugated linoleic acid, IL-10 expression was highly upregulated, and this may be the mechanism by which linoleic acids mediate plaque regression [130]. Several subsequent studies have tried to dissect the anti-atherosclerotic effect of IL-10; one of them found that IL-10 prevented the formation of oxLDL aggregates in macrophages and thereby reduced foam cell formation, and another study showed that IL-10 regulates oxLDL-induced apoptosis in macrophages and endothelial cells [210, 212].

The Th2 cytokine IL-10 is produced by Th2 cells, B cells, monocytes, and macrophages. In addition to the mechanisms described above, the atheroprotective effect of IL-10 is also mediated by its ability to inhibit the expression and secretion of pro-inflammatory cytokines, including IL-1β, TNFα, and IL-8. IL-10 also inhibits the production of the chemokine MCP-1, resulting in reduced immune cell recruitment to atherosclerotic lesions. Furthermore, IL-10 can block MMP production by macrophages and SMCs; MMP acts to degrade extracellular matrix, which leads to unstable plaques with a thin fibrous cap and increases the risk of plaque rupture and thrombosis. Thus, IL-10 may also have a protective effect against plaque rupture and thrombus formation [74], as well as an effect on the secretion of tissue factors and the production of thrombin by peripheral blood mononuclear cells and macrophages. Another atheroprotective effect of IL-10 is apoptosis limitation within atherosclerotic lesions by limiting ROS production. ROS are responsible for several cellular modifications, including endothelial damage, oxidation of lipids, and recruitment of inflammatory cells to the lesion site. The preventative effect of IL-10 against ROS production is mediated by its inhibition of the iNOS enzyme, which is the most important ROS producer within atherosclerotic lesions. This results in the reduction of LDL oxidation and atherosclerotic-related antigens [124], and IL-10 also reduces ER stress-induced apoptosis in macrophages by stimulating the production of survival molecules and the production of cholesterol efflux molecules such as the ABCA1/ABCG1 transporters [173]. In conclusion, IL-10 is an extremely versatile molecule, and future investigations will be necessary to establish its potential use as a target for atheroprotective strategies.

Similar to IL-10 and because of its multiple targets, transforming growth factor-β (TGF-β) also inhibits atherosclerosis development in multiple ways. First, it stimulates the secretion of collagen, which stabilizes plaques against rupture. In vivo, anti-TGF-β antibodies exacerbate the development of atherosclerotic lesions by decreasing the collagen content and increasing the number of inflammatory cells [126]. Moreover, the level of TGF-β in the sera of patients has been shown to inversely correlate with the advancement of atherogenic lesions [69]. Furthermore, TGF-β can regulate T cell activity [68, 170], and larger, more inflamed atherosclerotic plaques were observed in $ApoE^{-/-}$ or $LDR^{-/-}$ animals expressing a dominant-negative TGF-β receptor under control of a T cell-specific promoter. Thus, we conclude that TGF-β serves as another potential target for treatments against atherosclerosis.

Regulatory T cells

Although several cell types can produce IL-10 and TGF-β, these cytokines are mainly produced by Tregs. In recent years, several studies have reported a major role for Tregs in the anti-atherogenic process [4, 125]. Early evidence suggesting that Tregs have a protective role in atherosclerosis came from a study published in 2006; in this study, the transplantation of natural Tregs from healthy mice to Treg-deficient mice led to a reduction in atherosclerotic lesions, whereas depletion of Tregs increased these lesions [3]. Other studies have confirmed the protective effect of Tregs in atherosclerosis, showing, for example, that a low number of Tregs in patients is associated with severe outcomes, such as acute coronary syndrome. Downregulation of the number of Tregs seems to be associated with epigenetic modulations, and a high level of methylation of the FOXP3 gene in response to oxLDL was shown to be responsible for this downregulation [92]. As Tregs have a protective role, they have been proposed as a therapeutic tool for atheroma prevention. Along this line, a recent study used immunization with apolipoprotein B100-derived peptides and found that this treatment induced increased numbers of Tregs, reduced lesion development in young $ApoE^{-/-}$ mice and even allowed for regression of previously established lesions in older $ApoE^{-/-}$ mice [81].

Additional roles for dendritic cells

The DC population is heterogeneous in terms of cell phenotype and function. As previously described, most DCs

are pro-atherogenic, although one subset of DCs has been identified as anti-atherogenic, and these monocyte-derived DCs ($CD11c^{high}MHCII^{high}CD11b^{-}CD103^{+}$) are able to induce Tregs [30]. In addition, immature DCs ($CD86^{-}CD80^{-}CD40^{-}MHCII^{-}$) are tolerogenic because they produce TGF-β and IL-10, which enables them to mediate anti-atherosclerotic responses. In the $ApoE^{-/-}$ transgenic model in which TGF-βR signaling in $CD11c^{+}$ DCs is disrupted, the DCs present a more inflammatory phenotype, which leads to more advanced lesions [112].

Antibodies

As mentioned previously, humoral immunity has been implicated in atherosclerosis, and splenectomy increases atherosclerosis both in $ApoE^{-/-}$ mice and humans [25]. Moreover, the transfer of splenocytes from atherosclerotic mice to $ApoE^{-/-}$ animals protects against the development of disease. Clearly, modified LDLs are immunogenic, and antibodies against ox-LDL are found in human atherosclerosis patients and animal models [152]. Antibodies against the heat shock protein HSP60 are also found in experimental models of atherosclerosis [208]. Most of the antibodies specific for ox-LDL or HSP60 also react with microbial components, such as *Streptococcus pneumoniae*, and this cross-reactivity may be explained by the molecular mimicry theory. Furthermore, the presence of these antibodies is protective against the development of atherosclerotic plaques, and immunization of $LDR^{-/-}$ mice with the pneumococcal vaccine prevents the development of atherosclerotic plaques [12].

As the immune system is one of the major factors in atherosclerosis disease development and progression, several components of the immune system may be targeted in therapeutic approaches. Thus, immune targeting will be discussed in the last section of this review.

Therapeutic immune targeting in atherosclerosis

Theoretically, the prevention of atherogenesis would require life-long preventive therapies. Thus, new anti-atherogenic drugs should meet the criteria of being safe and inexpensive and should have significant benefits beyond those of currently available therapies. The improvement of the circulating lipid profile (mainly lowering LDL cholesterol and increasing HDL cholesterol) using statins or other drugs is the recommended pharmacologic approach to stabilize atherosclerotic plaques [168]. In fact, not only statins but also antiplatelet and antihypertensive medications play protective roles in both primary and secondary prevention of acute cardiovascular events in high-risk patients [9, 57, 80, 144, 193]. A recent systematic review and meta-analysis of clinical trials concluded that different antiplatelet agents might be useful for preventing acute cardiovascular disorders. In particular, acetyl salicylic acid (at the dose of 150 mg/day) was strongly recommended for both primary and secondary prevention of acute major events; however, because this study was mainly based on cost-effectiveness estimations, this approach should be validated in subsequent studies [80].

In comparison, randomized clinical trials have shown angiotensin-converting enzyme inhibitors and angiotensin II type I receptor blockers to reduce acute cardiovascular events and associated atherosclerotic inflammation [215]. The beneficial effects of these medications (all commercial drugs approved by regulatory authorities) were proposed to be improved by combined therapies [214]; however, complete abrogation or regression of atherogenesis has not been observed. Interestingly, during the last decade, novel therapies selectively targeting immune and vascular mediators (such as cytokines and chemokines) have been developed and tested in animal models of CVD for their ability to further reduce atherosclerosis and its acute ischemic complications. In the following subsections, we will present the evidence from both basic research and clinical studies on the most promising treatments that selectively target atherosclerotic inflammation and plaque destabilization.

"Pleiotropic" immunomodulatory activities of statins

Statins (inhibitors of 3-hydroxyl-3-methylglutaryl coenzyme A reductase) are the most commonly used lipid-lowering drugs worldwide [133]. Despite some safety issues (mainly myopathy and hepatotoxicity), the majority of these drugs (e.g., rosuvastatin, atorvastatin, simvastatin, and pitavastatin) are well tolerated and effective at improving cardiovascular outcomes in clinical trials [10]. The pharmacologic improvement in lipid profile has been indicated as the main mechanism underlying statin-mediated anti-atherosclerotic effects [133]. This approach is particularly relevant in the clinical management of high-risk patients, as it allows for the development of novel treatment strategies to inhibit lipids in patients intolerant to statins [65, 185]. In this regard, promising antibody-mediated approaches capable of reducing LDL cholesterol (in combination with statins or alone) were recently investigated in large clinical trials [65, 185]. Furthermore, during the last decade, additional roles for statins in atherosclerosis treatment were identified. For example, statins were extensively investigated as immunomodulatory drugs, and this additional anti-atherosclerotic potential was defined as a "pleiotropic" activity independent of their ability to ameliorate the lipid profile [104, 105] (Fig. 2). In particular, statins were found to directly reduce the production of inflammatory molecules (such as C-reactive protein) by human hepatocytes in vitro [8]. In addition, statin use is associated with beneficial coronary angiogenesis and a reduction in blood pressure in humans [20, 131, 148]. Finally, statins were shown to improve the serum levels of pro-

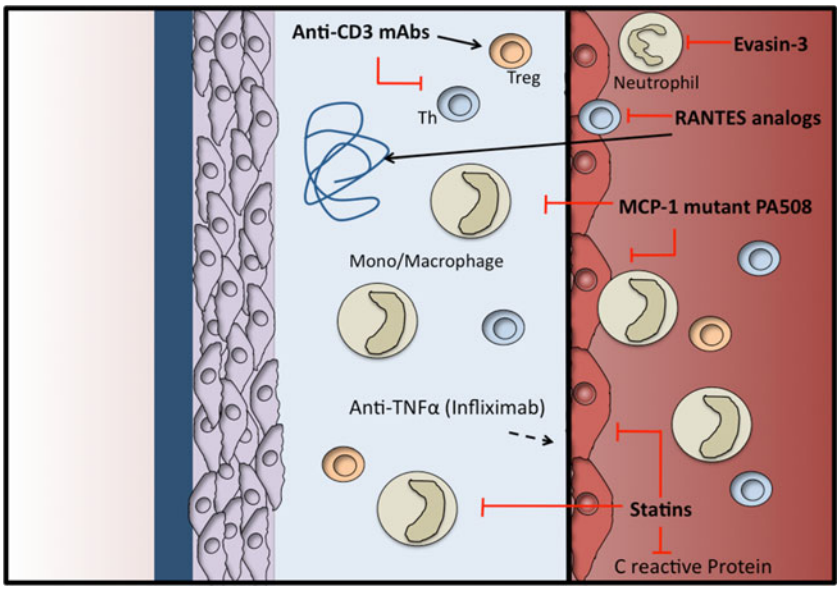

Fig. 2 Immune targeting of atherosclerosis lesion. Several actors of the immune system can be targeted to reduce atherosclerotic lesions. Statins, lipid-lowering drugs, also have anti-inflammatory properties by preventing EC activation, blocking recruitment of monocytes, and reducing the level of C reactive protein. Recruitment of immune cells to lesions may be blocked by MCP-1 or RANTES analogs. Moreover, anti-CD3 antibodies have been shown to inhibit the activity of Th cells and to increase the proportion of Treg cells in the lesion. Anti-TNFα antibodies, which were promising, have not yet shown clear benefit

atherosclerotic molecules in clinical studies [14, 15, 67, 75, 90, 99, 121, 142, 156, 161, 180, 198]. The most famous study targeting statin use and inflammation was the JUPITER trial [169], in which Ridker and coworkers performed a randomized, double-blind, placebo-controlled, multicenter, prospective trial investigating the potential benefits of rosuvastatin for the primary prevention of major cardiovascular events in 17,802 apparently healthy persons without hyperlipidemia but with elevated high-sensitivity C-reactive protein serum levels [169]. After a median follow-up of 1.9 years, this trial was stopped for an excess of benefit, as 10 mg/day rosuvastatin significantly reduced the incidence of major cardiovascular events as well as serum CRP compared to placebo [169]. Similarly, other statins (such as pravastatin and atorvastatin) were shown to reduce macrophage accumulation within carotid plaques in humans [36, 165]. These promising results on atherogenesis and plaque vulnerability were confirmed by a meta-analysis indicating that 1-month treatment with a statin may be sufficient to reduce atherogenesis and improve intraplaque features of vulnerability [123].

Additional statin-mediated pleiotropic activities were recently discovered, including their reduction of endothelial cell dysfunction [54, 149] and activation [190] independent of their cholesterol-lowering properties. Moreover, statins were shown to potently reduce leukocyte intraplaque recruitment in vitro and in vivo in atherosclerotic mice [135, 181, 196]. Both basic and clinical research on the pleiotropic activities of statins have contributed to improvements in the clinical management and risk assessment of patients with atherosclerosis, even when considering potential discrepancies between estimated and real cardiovascular risk reduction [168]. Thus, there is no doubt that statin treatment delivers significant effects for both the secondary and primary prevention of major coronary events [144], although potential differences in the safety and efficacy of statins remain to be elucidated in future prospective studies.

Selective treatments targeting chemokines

Small proteins that attract leukocytes from the circulation to inflammatory sites in different diseases are referred to as chemokines [102]. In addition, these molecules are classified into four main subgroups, including CC, CXC, XC, and CX_3C chemokines [102]. These redundant mediators (most are able to bind to several different transmembrane receptors on the leukocyte surface) are upregulated both within plaques and in the circulation of humans with advanced atherosclerosis and animal models of CVD [42, 218]. Therefore, the selective inhibition of their expression and bioactivity has been intensively investigated to reduce atherosclerotic inflammation and post-ischemic tissue injury. In 2004, the research group of F. Mach in Switzerland focused on the selective inhibition of RANTES/CCL5-driven leukocyte recruitment within atherosclerotic plaques in vivo (Fig. 2). These authors showed that chronic treatment with the CC chemokine antagonist Met-RANTES markedly abrogated atherogenesis in comparison to the vehicle control in hypercholesterolemic mice. This improvement was accompanied by a decrease in leukocyte infiltration and a concomitant increase in intraplaque collagen, a molecule that

stabilizes atherosclerotic plaques. Importantly, these beneficial effects were independent of the serum concentrations of lipids [197]. Similar results were obtained in $LDLR^{-/-}$ mice using another RANTES/CCL5 analog, [^{44}AANA^{47}]-RANTES, which interferes with the heparin binding and oligomerization of this chemokine [23]. The selective inhibition/antagonism of CCL5 was also recently reported as a promising therapeutic strategy to reduce post-infarction cardiac inflammation in mice; Braunersreuther and collaborators showed that a single intraperitoneal injection of [^{44}AANA^{47}]-RANTES during myocardial ischemia prior to reperfusion was associated with improvements in cardiac infarct size, leukocyte infiltration, oxidative stress, and apoptosis in comparison to PBS-treated animals [22]. Accordingly, Montecucco et al. confirmed the effect of CCL5 inhibition in preventing post-ischemic cardiac remodeling and improving survival in C57BL/6 mice. Using a mouse model of permanent left coronary ligature, these authors showed that the intravenous administration of a rat anti-mouse CCL5 monoclonal antibody improved cardiac function at 21 days after cardiac ischemia onset as compared to isotype IgG control [134]. Furthermore, evidence from the research group of C. Weber in Germany recently confirmed the potential for selective inhibition of CC chemokines in mouse myocardial infarction injury and neointima formation. These authors targeted MCP-1/CCL2 with the non-agonist mutant PA508, which does not activate CCR2 (the cognate CCL2 receptor) and therefore represents a very selective and potent inhibitor of CCL2-triggered pathways. The intraperitoneal injection of PA508 significantly reduced mouse neointimal plaque area, myocardial infarction size, and monocyte tissue infiltration compared to vehicle, confirming that anti-CC chemokine treatments are a promising strategy against mouse atherosclerosis and acute ischemic events [111]. In the last decade, the research group of A. Proudfoot in Switzerland developed several small molecules capable of binding CC and CXC chemokines and inhibiting their bioactivities at nanomolar concentrations. These proteins (called Evasins) were isolated from the tick salivary gland and produced as recombinant proteins to be tested in mouse inflammatory disorders, including rheumatoid arthritis and atherosclerosis [43, 56]. Evidence from our research group showed a relevant anti-atherosclerotic effect for Evasin-3 (inhibiting CXCL1 and CXCL2) in mouse models of shear stress-induced carotid atherosclerosis and acute myocardial ischemia and reperfusion injury [35, 136]. Conversely, no effect was observed in models of mouse stroke [35]. The molecular mechanisms identified were related to the abrogation of neutrophil inflammation, which has recently been implicated as a key pathophysiological process modulating atherosclerotic inflammation. More recently, the therapeutic potential of inhibiting CXCL1 with neutralizing antibodies was not confirmed in a mouse model of post-ischemic heart failure and remodeling [151], as these authors failed to observe any benefit different treatment schedules. This study therefore highlighted important limitations in the use of anti-chemokine drugs in atherosclerosis and their potential translation to human medicine. Concomitant adverse events have also been identified, including allergic reactions, low bioavailability, immunosuppression, and risk with subsequent immunization. Thus, these side effects preclude the use of these drugs in chronic treatments; moreover, few selective anti-chemokine medications have been investigated in humans (phase I), and their development may require considerable time.

TNF-α inhibition

TNF-α is a pro-inflammatory molecule expressed by cellular components of early fatty streaks and late atherosclerotic lesions of humans [137]. Furthermore, TNF-α may be secreted by several cell types within atherosclerotic plaques, including endothelial cells, SMCs, and macrophages [29, 116, 159], and circulating levels of TNF-α are increased in advanced atherosclerosis and in patients symptomatic for acute stroke [137]. In mice, TNF-α gene knockout on the $ApoE^{-/-}$ background results in significantly smaller atherosclerotic plaque areas [21]. Together, these data suggest that TNF-α may represent a promising target to reduce atherosclerosis. In particular, patients suffering from chronic inflammatory disorders (such as rheumatoid arthritis) may be particularly sensitive to anti-TNF-α therapy, as this therapy could potentially reduce both the severity of arthritis and the concomitant acceleration of atherosclerosis. Considering the increased cardiovascular mortality affecting rheumatoid arthritis patients, possibly due to common pathophysiological mediators [138], Hürlimann and coworkers performed a pilot study investigating the potential effects of treatment with the anti-TNF-α antibody infliximab on rheumatoid arthritis activity and endothelial function in 11 subjects with active disease who were taking a stable dose of methotrexate and prednisone. After 12 weeks, treatment with anti-TNF-α was associated with improvements in the rheumatoid arthritis activity score, systemic inflammatory parameters, and endothelium-dependent vasodilation (a surrogate parameter of atherosclerotic endothelial dysfunction, assessed by high-resolution ultrasound of the brachial artery) [87].

After this initial study was published in 2002, aortic stiffness was evaluated as a potential indicator of atherosclerosis in rheumatoid arthritis patients in comparison to healthy controls. Among 77 enrolled patients with rheumatoid arthritis, the effects of concomitant anti-TNF-α therapy were prospectively investigated in a subgroup of nine subjects. Despite this low number of patients, the authors showed that aortic stiffness and endothelial function were significantly improved in patients given anti-TNF-α therapy.

The authors concluded that selective anti-TNF-α treatment might represent a promising strategy against atherogenesis [122]. Di Micco and coworkers recently addressed this point in another pilot study investigating TNF-α inhibition with infliximab in patients with rheumatoid arthritis compared to patients on recommended therapy without infliximab. This study enrolled only seven patients per group, but it prospectively evaluated the carotid intima–media thickness (another surrogate parameter of atherosclerosis) before and after 12 months of treatment. Unexpectedly, the results showed that treatment with infliximab was associated with a significant worsening of atheroprogression that was not observed in controls [45]. However, these data were not confirmed at the 3-month ($n=60$) or 1-year follow-up examinations ($n=54$) in patients with inflammatory arthropathies in the study published by Angel and coworkers [6, 7]. In conclusion, each of these studies enrolled a very small number of patients and was not focused on clinical cardiovascular outcomes; as a result, we are unable to conclude whether anti-TNF-α inhibition represents a potential therapeutic strategy against atherosclerosis, and larger clinical trials are warranted.

The CD3 blocking strategy

In 2003, Chatenoud's group showed that anti-CD3 blocking antibodies were able to reconstitute self-tolerance in established autoimmune type 1 diabetes in nonobese diabetic mice [11]. In 2009, Hirata et al. tested whether anti-CD3 blocking would also demonstrate tolerogenic functions in atherosclerosis, and these authors found that oral administration of anti-CD3 antibody significantly reduced atherosclerotic lesion formation and the accumulation of macrophages and $CD4^+$ T cells in plaques compared to control treatment (Fig. 2). These authors also observed a significant increase in latency-associated peptide-positive cells and $CD25^+Foxp3^+$ cells among the $CD4^+$ T cell population in anti-CD3-treated mice, in association with increased production of anti-inflammatory TGF-β and suppressed Th1/2 immune responses [174]. Steffens and coworkers investigated the potential effects of chronic treatment with an anti-CD3 antibody in dyslipidemic mouse atheroprogression, and as expected, these authors reported that anti-CD3 antibody treatment reduced plaque development by modulating T cell subset polarization and promoting the Treg phenotype [182]. These promising results from animal models highlight T cells as a potential anti-atherosclerotic target, based on the tolerogenic effects of anti-CD3 antibodies.

T cell inhibition for the treatment of atherosclerosis and acute myocardial infarction was recently investigated using the older and less selective immunosuppressive drug cyclosporine, as this medication abrogates T helper and cytotoxic lymphocyte activation and proliferation [178]. In humans, two pilot studies showed that acute administration of a single intravenous bolus of cyclosporine at the time of reperfusion was associated with a significant reduction in myocardial infarct size during the first days after the event [163], lasting until 6 months after treatment [132], as compared to placebo. However, these important results in humans were not confirmed in several animal models of acute myocardial infarction [114], nor in vitro in atherosclerotic cells [93, 143]. Thus, the heterogeneity between animal models and the limited number of patients investigated does not permit additional speculation, and further studies are needed to clarify the anti-atherosclerotic potential of cyclosporine.

Conclusions

Atherosclerosis is a chronic inflammatory disease that involves a multitude of complex events and risk factors. Some physiological factors, such as dyslipidemia and metabolic syndrome, in addition to behavioral factors, including smoking and lack of exercise, gradually lead to the establishment of an inflammatory immune process within vulnerable areas of arteries [70].

The early key step in atherosclerosis is the deposition of ApoB-LP particles within the intima layer of arteries and their subsequent oxidation [59, 201]. Furthermore, the effect of oxidized ApoB-LPs on disease progression is highly exacerbated by sub-endothelial enzymes. Indeed, lipoprotein lipase, sphingomyelinase and phospholipase A_2 are secreted by endothelial cells, monocytes, and SMCs and mediate the active retention of oxidized ApoB-LPs by extracellular matrix components [96, 188].

Retained oxidized ApoB-LPs stimulate endothelial cell activation and the expression of pro-inflammatory markers such as P-selectin, E-selectin, ICAM-1, and VCAM-1 and chemotactic factors such as MCP-1 and M-CSF. Activated endothelial cells then recruit monocytes from the vessel lumen to the intima through the production of chemokines [33, 179]. Once inside the intima, monocytes are activated by M-CSF and differentiate into macrophages that phagocytize retained ApoB-LP particles and transform into lipid-filled cells called foam cells.

Following the recruitment of monocytes and their differentiation into macrophages, T cells are recruited into lesions and contribute to the establishment of an adaptive immune response. The adaptive immune response is based on the co-stimulatory interaction between CD40 and CD40L, which activates T cells following antigen presentation by antigen-presenting cells [120].

Foam cells and infiltrated immune cells secrete several cytokines and chemokines [83] as well as PDGF-B, which contribute to the activation and recruitment of medial SMCs [40] that invade the intima layer. The evolution of atherosclerotic

plaques into fibroproliferative or thrombotic lesions is determined according to their cellular components, whereby the balance between pro- and anti-inflammatory cytokines as well as pro- and anti-apoptotic factors tightly regulates the cellularity of atherosclerotic lesions.

To treat atherosclerosis, several strategies have been used in recent decades. Given that the main cause of atherosclerosis is an excess of lipids in the serum, one major therapeutic approach has been to diminish this excess of lipids. For this reason, the cholesterol-lowering drugs statins were developed, and these act by blocking the main cholesterol metabolic enzyme HMG-CoA reductase. Despite the beneficial effect of statins in the treatment of atherosclerosis, the need for new therapies has arisen, and preliminary encouraging results have been obtained for the treatment of atherosclerosis by targeting immune actors such as TNF-α or CD3.

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