Therapeutic strategies targeting inflammation and immunity in atherosclerosis: how to proceed?

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Abstract | Atherosclerosis is a chronic inflammatory disease of the arterial wall, characterized by the formation of plaques containing lipid, connective tissue and immune cells in the intima of large and medium-sized arteries. Over the past three decades, a substantial reduction in cardiovascular mortality has been achieved largely through LDL-cholesterol-lowering regimes and therapies targeting other traditional risk factors for cardiovascular disease, such as hypertension, smoking, diabetes mellitus and obesity. However, the overall benefits of targeting these risk factors have stagnated, and a huge global burden of cardiovascular disease remains. The indispensable role of immunological components in the establishment and chronicity of atherosclerosis has come to the forefront as a clinical target, with proof-of-principle studies demonstrating the benefit and challenges of targeting inflammation and the immune system in cardiovascular disease. In this Review, we provide an overview of the role of the immune system in atherosclerosis by discussing findings from preclinical research and clinical trials. We also identify important challenges that need to be addressed to advance the field and for successful clinical translation, including patient selection, identification of responders and non-responders to immunotherapies, implementation of patient immunophenotyping and potential surrogate end points for vascular inflammation. Finally, we provide strategic guidance for the translation of novel targets of immunotherapy into improvements in patient outcomes.

Atherosclerosis, the major cause of cardiovascular disease (CVD), is a chronic inflammatory disease triggered by the accumulation of cholesterol-containing LDL particles in the arterial wall[1]. The gold standard of treatment for atherosclerosis is the prevention of cardiovascular events by targeting modifiable risk factors and the re-establishment of arterial flow by percutaneous or surgical procedures[2,3]. However, the therapeutic benefit of these strategies on cardiovascular outcomes has stagnated and a huge global burden of CVD remains[4].

Evidence for the role of inflammation in atherosclerosis has accumulated over the past 35 years (FIG. 1). Attilio Maseri (1935–2021) was one of the first investigators to foresee the importance of inflammation as a component of the pathogenesis of acute coronary syndromes[5,6]. The arterial wall is populated by various immune cells, both in healthy individuals and in patients with disease[7,8]. The innate immune system is the first line of defence against invading pathogens and the innate immune response is usually initiated by pattern recognition receptors, including Toll-like receptors (TLRs)[9,10]. The innate immune response induces the activation of antigen-presenting cells such as macrophages and dendritic cells that mediate antigen presentation, co-stimulation and cytokine production in the immune synapse to trigger the adaptive immune response. The adaptive immune response involves B cells and T cells and is slower but more specific and long-lived than the innate immune response. Athero-inflammation involves the activation of both innate and adaptive immune responses, with both inherently linked[8,11] (FIG. 2). Immune cells in the arteries are activated owing to persistent inflammatory stimuli or a failure in the resolution of inflammation, leading to chronic inflammation, a hallmark of CVD[12]. To understand atherogenesis, we must consider the interplay between cellular immunity and lipid retention[13] and the complex cross-talk between and within immune and non-immune cells, as well as the advantages and disadvantages of the experimental models used in this research field (BOX 1).

A unique aspect that sets aside atherogenesis from other chronic inflammatory diseases is the crucial role of

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Key points

- Inflammation is an important component of the pathophysiology of cardiovascular disease; an imbalance between pro-inflammatory and anti-inflammatory processes drives chronic inflammation and the formation of atherosclerotic plaques in the vessel wall.
- Clinical trials assessing canakinumab and colchicine therapies in atherosclerotic cardiovascular disease have provided proof-of-principle of the benefits associated with therapeutic targeting of the immune system in atherosclerosis.
- The immunosuppressive adverse effects associated with the systemic use of anti-inflammatory drugs can be minimized through targeted delivery of anti-inflammatory drugs to the atherosclerotic plaque, defining the window of opportunity for treatment and identifying more specific targets for cardiovascular inflammation.
- Implementing immunophenotyping in clinical trials in patients with atherosclerotic cardiovascular disease will allow the identification of immune signatures and the selection of patients with the highest probability of deriving benefit from a specific therapy.
- Clinical stratification via novel risk factors and discovery of new surrogate markers of vascular inflammation are crucial for identifying new immunotherapeutic targets and their successful translation into the clinic.

lipid particles in the induction of atherogenesis. Modified lipoproteins, such as oxidized LDL (oxLDL), trigger the immune response through a unique property, whereby these particles can act as both antigens activating the adaptive immune response[8,14] and adjuvant molecular patterns activating the innate immune response[15,16]. In advanced atherosclerosis, complex chronic inflammatory processes result in the generation of a plaque with a thin fibrous cap and a large necrotic core, or in plaque erosion or other plaque morphologies associated with clinical vulnerability to rupture, which lead to ischaemic events[17]. The complexity of inflammation in atherosclerosis has been emphasized by single-cell studies in humans and mice showing the high heterogeneity of vascular leukocytes in atherosclerotic lesions[18–27]. This heterogeneity underscores the importance of targeting specific cell subsets to inhibit atherosclerosis progression while maintaining tissue homeostasis. Superimposing the single-cell transcriptional landscape of leukocytes from mouse and human atherosclerotic plaques will help identify the different pathways, genes or cells that can be used in animal models to study human disease. Moreover, emerging evidence now shows that atherogenesis is a multiorgan process with contributions from organs such as the bone marrow and spleen[28,29]. In particular, the presence of clonal haematopoiesis of indeterminate potential (CHIP), an age-related process in which certain somatic mutations in bone marrow progenitor cells confer a competitive advantage leading to the expansion of specific cell clones, has been proposed as a risk factor for CVD[30,31].

The first proof of the benefits of targeting inflammation in CVD in humans came from the 2017 CANTOS trial[32], which showed improved clinical outcomes in patients with a history of myocardial infarction (MI) who received treatment with antibodies against IL-1β (canakinumab) compared with those who received placebo (TABLE 1). This finding was quickly followed by evidence from two clinical trials published in 2019 and 2020 showing that the anti-inflammatory effects of colchicine therapy reduced the risk of cardiovascular events in patients with recent MI[33] or coronary artery disease (CAD)[34]. Evidence for the role of inflammation in CVD has also been described in other disease settings. Patients with chronic inflammatory diseases such as lupus or rheumatoid arthritis (RA) have an increased risk of CVD (tenfold and twofold, respectively) compared with healthy controls, and this risk significantly correlates with the magnitude of systemic inflammation[35]. Moreover, checkpoint inhibitor therapies used for several cancer types to improve tumour surveillance by the immune system are associated with an increased risk of CVD, adding to the challenges in the cardio-oncology field[36,37]. Together, these studies highlight immunotherapeutics as the next step in CVD therapy that will provide an opportunity to surpass the ceiling reached with the current management of classic risk factors for CVD to address the residual cardiovascular risk[38]. At present, the challenge lies in identifying crucial effectors of atherosclerosis-specific inflammation among the plethora of inflammatory mediators while sparing the host defence.

In this Review, we discuss the therapeutic potential of targeting the immune system in atherosclerosis. First, we provide an overview of immune cells involved in CVD. Next, we summarize the published and ongoing clinical trials targeting the immune system in atherosclerosis and identify important challenges that need to be addressed to advance the translation of novel immunotherapeutics into the clinic. Finally, we highlight the new therapeutic targets emerging from preclinical studies with the biggest potential for translational pay-off in the medium term.

Immune cells involved in atherosclerosis

In this section, we summarize the functional diversity of innate and adaptive immune cells in atherosclerosis and refer to previous reviews for further in-depth discussion. The role of platelets and other non-immune cells in inflammation have been previously reviewed[39–41].

Monocytes

Monocytes are present in the blood, bone marrow and spleen during homeostasis. Monocytes can be classified into two main populations: classical monocytes (Ly6C^{high} in mice and CD14^{+}CD16^{-} in humans) and non-classical monocytes (Ly6C^{low} in mice and CD14^{low}CD16^{+} in humans). In atherosclerosis, classical monocytes are recruited to atherosclerotic plaques after engagement of the chemokine receptors CCR2, CCR5 and CX3CR1 (REFS[11,42]). In the plaque, monocytes differentiate into dendritic cells and macrophages that show high functional and phenotypic heterogeneity[43]. In both mice[42,44] and humans[45], an increase in the blood monocyte pool is associated with increased severity of atherosclerosis. Preclinical studies in mice have demonstrated that splenic Ly6C^{high} monocytes contribute to both the growing atheroma and plaque instability[29,46]. However, monocyte recruitment also has an important role in atherosclerosis regression[47], and 'patrolling' Ly6C^{low} monocytes, which are derived from Ly6C^{high} monocytes, are important for endothelial cell maintenance[48]. Hypercholesterolaemia, stress, inflammation and other

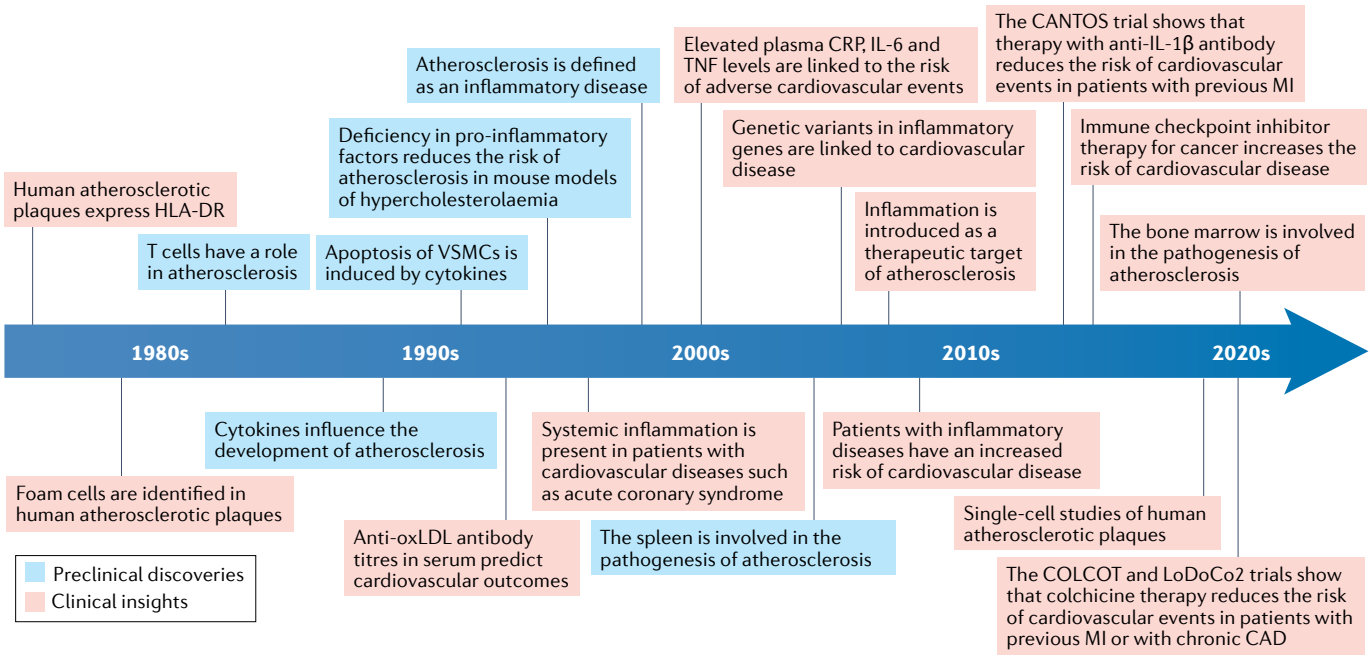

Fig. 1 | **History of research into the role of inflammation in atherosclerosis.** The timeline shows the main milestones in the past four decades of research into the role of inflammation in atherosclerosis. In the 1980s, the introduction of immunohistochemical techniques to study atherosclerotic plaques provided evidence of HLA-DR expression in human atherosclerotic plaques, followed by identification of monocytes, macrophages and T cells in the plaque[29,44,263–269]. In the 1990s, studies showed the presence of pro-inflammatory cytokines, such as tumour necrosis factor (TNF), in atherosclerotic plaques[270–274], and the association between high plasma C-reactive protein (CRP) levels and coronary artery disease (CAD)[5,275]. During this decade, the first mouse models of hypercholesterolaemia with an inflammatory gene knockout were developed[274,276,277] and titres of antibodies against oxidized LDL (oxLDL) in the serum were shown to predict cardiovascular disease outcomes[278]. In the 2000s, studies demonstrated the association between increased levels of inflammatory markers and increased risk of cardiovascular events[279,280]. An increased risk of cardiovascular disease was shown in patients with inflammatory diseases[281–283], and several studies demonstrated the association between elevated levels of CRP, IL-6 and TNF in the plasma and worse clinical outcomes in patients with cardiovascular disease[115,127,284,285]. This finding led to the introduction of inflammation as a therapeutic target in cardiovascular disease[286]. In the late 2010s, studies showed that immune checkpoint inhibitor treatment increased the risk of cardiovascular disease in patients with cancer[287,288]. In the past decade, clinical trials investigated whether targeting inflammation in cardiovascular disease is beneficial[32,34,135]. Numerous studies also demonstrated the involvement of the bone marrow in atherosclerosis[172,177,178] and performed single-cell analysis of plaque immune cells[19,25]. Preclinical discoveries are shown in blue boxes and clinical discoveries in red boxes. MI, myocardial infarction; VSMC, vascular smooth muscle cell.

risk factors for atherosclerosis can induce emergency haematopoiesis, including extramedullary haematopoiesis in the spleen[29], and contribute to disease progression by skewing haematopoietic stem cells in the bone marrow towards monopoiesis[29,44,49].

Macrophages

Two distinct resident macrophage populations are found in mouse arteries, one in the intima and the other in the adventitia[50]. Both macrophage populations originate from embryonic precursors and their survival depends on the presence of colony-stimulating factor 1. Resident adventitial macrophages are replenished by bone-marrow-derived monocytes in the period immediately after birth and are maintained by local proliferation in adulthood[51]. In atherogenesis, monocytes reconstitute the population of resident macrophages in the arterial intima during early stages of atherosclerosis[50], whereas local proliferation of lesional macrophages contributes to macrophage accumulation in advanced lesions[52]. In both health and disease, adventitial macrophages expressing lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1) prevent unfavourable arterial remodelling, largely through the regulation of collagen production in medial vascular smooth muscle cells (VSMCs)[53]. Arterial intima-resident macrophages have a pro-atherogenic function, and ablation of these macrophages prevents lesion formation[50]. A subset of LYVE1$^+$ vascular macrophages expressing the innate immune receptor C-type lectin CLEC4A2 has anti-atherogenic functions and the ablation of this macrophage population increases lesion formation[54].

Arterial macrophages have distinct functional and ontogenetic signatures and this plasticity reflects the heterogeneous environment of atherosclerotic plaques, which is increasingly being appreciated. Genetic lineage tracing and monocyte fate mapping studies have started exploring the contributions of monocytes to specific macrophage subpopulations in atherosclerosis[20,47] and have helped to understand how local progenitor cells and proliferation of resident macrophages contribute to plaque progression[50,52,55]. Three main macrophage populations with different inflammatory properties have been identified in single-cell studies of human[19] and mouse[18,27] atherosclerotic plaques, suggesting that macrophage heterogeneity in the plaques cannot be explained simply by the M1–M2 macrophage polarization paradigm[56]. Strikingly, a pro-inflammatory

macrophage population found in mice and humans expresses high levels of IL-1β[18,19], a well-recognized immune target in atherosclerosis, further highlighting the relevance of this cytokine for atherosclerosis progression. Another population of the identified macrophage subsets has a more resident-like phenotype and is enriched in transcripts of proteins involved in antigen presentation and endocytosis[18,25].

Foam cells are a hallmark of atherosclerosis. These cells are derived from macrophages, dendritic cells and VSMCs[57]. Foam cells drive necrotic core formation through uptake of intraplaque lipids, which leads to increased endoplasmic reticulum stress and cell death[57]. A single-cell study of mouse atherosclerotic lesions showed that plaque *Trem2*^{high} macrophages, a subset that has also been identified in adipose tissue, express genes associated with lipid handling and have a profile consistent with a foamy macrophage phenotype[27]. *TREM2*^{high} macrophages in human and mouse atherosclerotic lesions do not express genes encoding inflammatory factors, suggesting that these subsets have a homeostatic lipid-handling role in the plaques[18,22,25,58]. The profile of this macrophage subset is consistent with evidence showing that intracellular accumulation of desmosterol, a precursor in cholesterol biosynthesis, maintains macrophage homeostasis through the activation of transcription of liver X receptor target genes and the suppression of inflammation[18,22,25,58]. This discovery draws important parallels between the pathophysiology of CVD and obesity, highlighting a common blueprint between the two most prevalent metabolic diseases at present[59,60]. At the same time, these findings call into question the concept

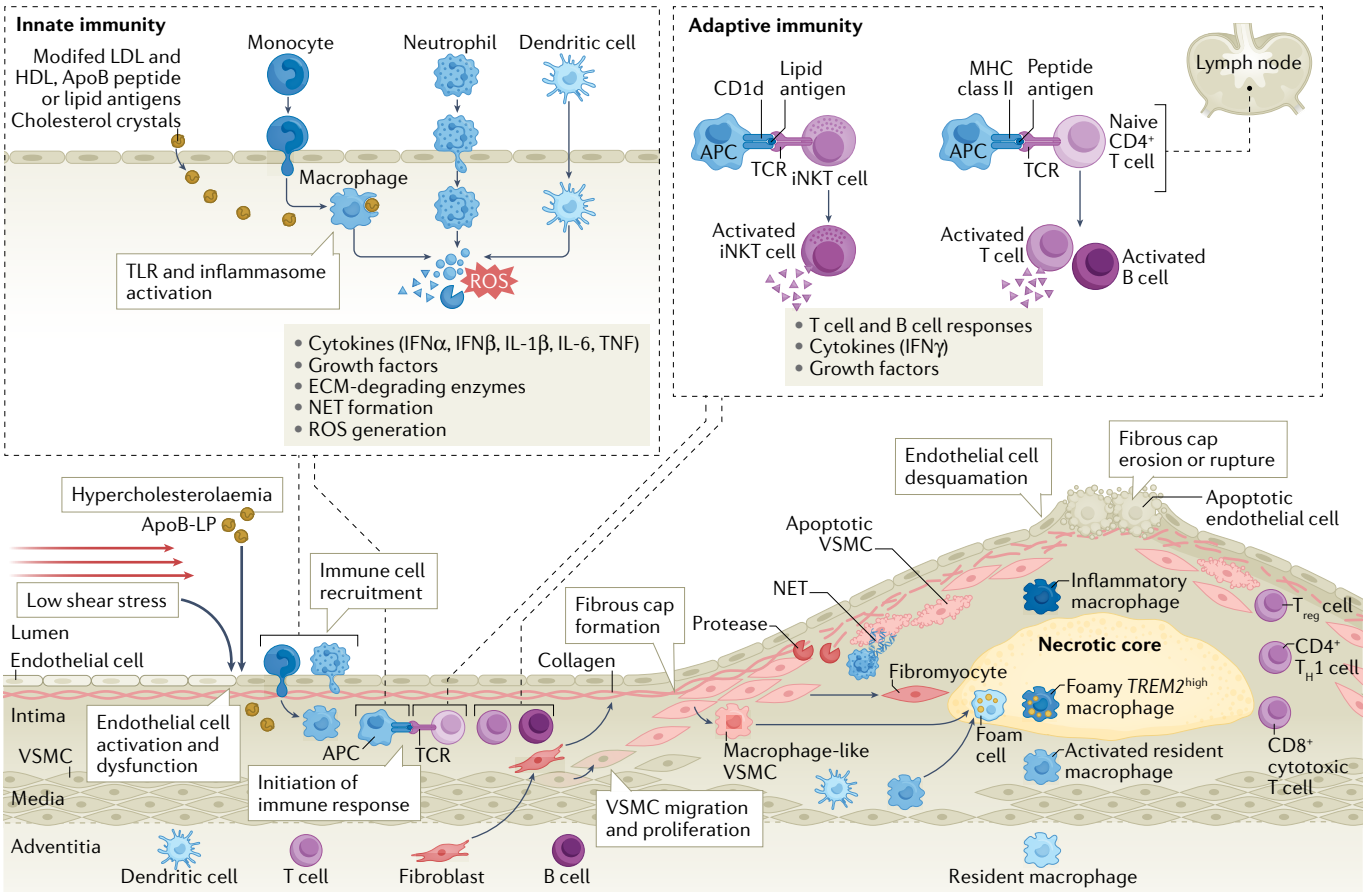

Fig. 2 | **Inflammation in atherosclerosis.** In medium and large arteries, haemodynamic forces create areas of low shear stress that are often predictors of atherosclerotic plaque location. As the atherosclerotic plaque begins to form, circulating apolipoprotein B (ApoB)-containing lipoproteins (ApoB-LP) and ApoB peptides enter the subendothelial space, where they can be modified and recognized by innate immune cells as danger signals. These danger signals activate Toll-like receptor (TLR) signalling and the inflammasome in innate immune cells, eliciting responses that drive inflammation, including production and secretion of cytokines, release of neutrophil extracellular traps (NETs), upregulation of co-stimulatory molecules and promotion of monocyte recruitment to the plaque[2–8,9]. Macrophages derived from monocyte differentiation, local proliferation or from transdifferentiation of vascular smooth muscle cells (VSMCs) take up lipoproteins present in the plaque and become lipid-laden foam cells that lay the foundation for the formation of the plaque necrotic core. At the immune synapse, antigen-presenting cells (APCs), including macrophages, dendritic cells and B cells, present lipid antigens to invariant natural killer T (iNKT) cells and peptide antigens to T cells, the latter engaging adaptive T cell and B cell responses. Antigen presentation occurs in the plaque and in secondary lymph organs, such as the lymph node[8]. Together, all these processes contribute to endothelial dysfunction, leading to further aggravation of inflammation through continued monocyte recruitment, increased uptake of lipoproteins adding to the plaque lipid burden, VSMC activation and proliferation, and fibroblast migration contributing to fibrous cap formation. ECM, extracellular matrix; IFN, interferon; MHC, major histocompatibility complex; ROS, reactive oxygen species; TCR, T cell receptor; T_H1, T helper 1; TNF, tumour necrosis factor; T_{reg} cell, regulatory T cell.

of lipid-driven inflammation. Further studies are warranted to reconcile inflammatory and lipid drivers of the disease. Another aspect of plaque macrophage biology to consider is the role of these cells in plaque rupture and thrombosis through the production of matrix metalloproteinases and tissue factor[61], and the coordination of intraplaque efferocytosis, a crucial mechanism for resolving inflammation in atherosclerosis[62] (BOX 2).

Dendritic cells

Dendritic cells are another crucial cell type driving atherosclerotic plaque inflammation that bridges the innate and adaptive immune responses. Dendritic cells can be classified into three main subsets: plasmacytoid dendritic cells, type 1 conventional dendritic cells (cDC1s) and type 2 conventional dendritic cells (cDC2s). Plasmacytoid dendritic cells are generally located in blood and lymphoid tissues. After encountering pathogens, these cells produce large amounts of type I interferon (IFN). By contrast, conventional dendritic cells are found in lymphoid and non-lymphoid sites. cDC1s are involved in cross-presentation of antigens and drive cytotoxic immune responses, whereas cDC2s are involved in T cell priming[63].

In humans, plaque dendritic cell numbers positively correlate with plaque vulnerability[64]. Dendritic cells have been found to have both pro-atherogenic and anti-atherogenic functions in mouse models, as reviewed previously[8]. Dendritic cells elicit an adaptive immune response that encompasses both T cells and B cells[8]. During atherosclerosis regression in mice, dendritic cells can leave the lesions and migrate to the lymphatic tissue in a process mediated by the chemokine ligands CCL19 and CCL21 and their receptor CCR7 on the surface of dendritic cells[65]. Dendritic cells expressing CCL17 have a pro-atherogenic role in mice[66].

Box 1 | Can we learn from mouse models?

Laboratory mice have provided invaluable insights into the mammalian immune system, diseases and drug development. These models are economical, easy to breed and straightforward to manipulate genetically and for these reasons, they are here to stay. The generation of *Apoe*$^{-/-}$[290] and *Ldlr*$^{-/-}$[291] mouse models has led to scientific advances in the field of lipoprotein metabolism as well as in research on inflammation in atherosclerosis, and validated the discovery of PCSK9 as a novel therapy[292,293]. However, the translation of beneficial responses to therapeutics from mice to humans has not always been successful[294–297].

The cardiovascular system of mice and humans differs in the levels of shear stress in the vasculature[298], the degree of fibrosis[299] and the content of T cells (higher in humans[25]). Furthermore, atherosclerotic lesions in mice form predominantly at the aortic root, a pattern observed in patients with familial hypercholesterolaemia but not relevant to the general population[299]. Plaque rupture frequency in mice is very low and when it happens spontaneously (usually in the brachiocephalic artery), the rupture is not at the same site as in humans (carotid artery)[300]. Moreover, only a few genes linked to atherosclerosis in mice have shown a genetic association with human atherosclerosis, raising questions as to the use of mouse models of atherosclerosis[301]. As a result, to study the spectrum of human cardiovascular disease thoroughly we have to use several experimental models.

The immune system of mice and humans is also dissimilar; mice have higher lymphocyte levels (70–90%) and fewer neutrophils (10–25%) in the blood. Toll-like receptor (TLR) expression, antibody subsets, levels of defensins and nitric oxide production are also different in mice and humans, as reviewed previously[294]. In addition, many cytokines and chemokines in humans have no known orthologues in mice and vice versa[295]. These differences are partly attributable to variations in protein expression and signalling. Genomic comparisons revealed substantial transcriptional overlap between mice and humans but raised noteworthy differences[302]. Moreover, the immune response varies between mouse strains as a result of genetic variations and polymorphisms arising from genetic drift and/or intentional breeding[303].

Pig models have similar cardiovascular anatomical features to and higher genetic homology with humans. For instance, as in humans, pigs have ten TLRs (TLR1–10) and duplication of the *IL1B* gene, and pig TLRs have significant homology with their human counterparts[304]. However, distinguishing dendritic cells from macrophages and B cells in pigs is difficult owing to common markers in these cell types, and variations in the morphology and function of neutrophils have been reported between pig breeds[305]. Lymph node histology is also different between pigs and humans; in pigs, the medullary tissue is located in the periphery and the cortical part in the central area[305]. Finally, there is a severe lack of reagents for pig models compared with their availability for humans and mouse models. Altogether, the study of the immune system and its role in cardiovascular diseases in pig models presents challenges.

Organoid systems and lab-on-a-chip technology are being devised to fill the gap in translation between mouse models and humans[306]. In the meantime, we strive to improve mouse models. To reduce variability, laboratory mice are kept in specific pathogen-free conditions, leading to a low density of mature T cells, scarceness of neutrophils and low lipopolysaccharide responsiveness compared with mice in the wild, which more closely resembles the human immune system[294,307,308]. Therefore, part of the problem is not inherent in the use of mouse models per se but how we use them. Perhaps better models will emerge by dialling back our efforts towards pathogen-free environments. Moreover, humanized mice are a powerful tool to improve research into human cardiac disease[309].

In summary, mouse models are still the foundation of basic research and offer too many advantages to be discarded. Albeit useful, no organoid or lab-on-a-chip system can fully reproduce the advantage of a structured immune system. Therefore, several questions must be considered when choosing a model: What aspect of human cardiovascular disease is addressed with the model? Does the model recapitulate the human immune response in the disease condition or a particular stage of disease? Is the species or strain appropriate to model the question? Are there reagents available to study the immune system? How could the genetic background influence the study outcome? Considering these questions, we need to keep learning from a variety of biological systems, using each one to address the appropriate question to which it can provide the answer.

Table 1 | **Immunotherapies proven to be effective in phase III clinical trials in cardiovascular disease**

Trial (year)	Agent	Drug target	Trial design	Patient cohort	Primary end point	Main outcomes	Ref.
CANTOS (2017)	Canakinumab	Inhibition of the IL-1β pathway	Randomized, double-blind, placebo-controlled	10,061 patients with previous MI and elevated plasma CRP levels	Non-fatal MI, non-fatal stroke or death from cardiovascular causes	The 150-mg dose of canakinumab reduced cardiovascular events compared with placebo, independent of lipid level reductions	32
COLCOT (2019)	Colchicine	Broad cellular effects, including inhibition of tubulin polymerization, alteration of leukocyte responsiveness, and inhibition of inflammasome assembly and IL-1 release	Randomized, double-blind, placebo-controlled	4,745 patients with MI within 30 days before enrolment	Death from cardiovascular causes, resuscitated cardiac arrest, MI, stroke, or hospitalization for angina leading to coronary revascularization	Colchicine decreased the risk of the composite end point compared with placebo	33
LoDoCo2 (2020)	Colchicine	Broad cellular effects, including inhibition of tubulin polymerization, alteration of leukocyte responsiveness, and inhibition of inflammasome assembly and IL-1 release	Randomized, double-blind, placebo-controlled	5,522 patients with chronic coronary artery disease	Death from cardiovascular causes, spontaneous MI, ischaemic stroke or ischaemia-driven coronary revascularization	Colchicine decreased the risk of the composite end point compared with placebo	34

CRP, C-reactive protein; MI, myocardial infarction.

CD103$^+$ cDC1s can promote atheroprotective regulatory T (T_{reg}) cell responses[67]. Loss of myeloid differentiation factor 88 (MyD88) signalling in CD11c$^+$ dendritic cells leads to loss of T_{reg} cells and increased atherogenesis in mice[68]. By contrast, plasmacytoid dendritic cells have been reported to have both pro-atherogenic and anti-atherogenic roles in mice, possibly owing to subtle cellular heterogeneity in this subset[69,70].

Neutrophils

Neutrophils are involved in all stages of atherosclerosis[71]. In mice, neutrophil depletion reduces atherosclerosis, whereas increased levels of circulating neutrophils exacerbate plaque formation, suggesting a role of this cell type in lesion development[72]. Neutrophils promote vascular inflammation through the secretion of reactive oxygen species, which leads to increased permeability of the endothelial cell barrier[73]. Neutrophils attract monocytes via secretion of chemotactic molecules and can activate macrophages via extrusion of their nuclear material as neutrophil extracellular traps (NETs)[74]. NETs contain histone H4, which binds to VSMCs and induces cell lysis, resulting in plaque destabilization[75]. In addition, NETs induce plaque erosion and platelet aggregation, leading to thrombosis[76]. Overall, neutrophils have a pro-atherogenic role. However, during thrombotic events, neutrophils have reparative functions through the promotion of endothelial repair and angiogenesis[77].

T cells

T cells are important for atherosclerosis initiation and progression, as reviewed previously[78,79]. A mass cytometry study revealed that T cells outnumber macrophages in human carotid artery plaques[25], in contrast to plaques in mice, in which the overall proportion of T cells is lower[24]. T cells in human atherosclerotic plaques show more activation-related and exhaustion-related gene expression than peripheral blood T cells. High expression of the inhibitory molecule PD1 as a consequence of chronic antigen stimulation can result in inefficient T cell effector function and dysregulation of the immune response within the plaque[19,25]. Once activated, T cells directly mediate effector functions in the arterial wall or help B cells produce antibodies. CD4$^+$ T cells are the most abundant T cells in mouse atherosclerotic plaques, and are polarized predominantly towards a pro-inflammatory phenotype (T helper 1 (T_H1) cells)[79]. CD4$^+$ T cells have been shown to both protect against and promote atherogenesis depending on the subset involved. T_H1 cells have been consistently shown to have pro-atherogenic roles, whereas T_{reg} cells are thought to have atheroprotective roles via IL-10 and TGFβ secretion[78]. The role of T_H2 cells and T_H17 cells in atherosclerosis is controversial[78]. Phenotyping of CD4$^+$ T cells in a mouse model of atherosclerosis with the use of single-cell RNA sequencing revealed a CD4$^+$ T cell population[80] that shared transcriptional similarities with apolipoprotein B (ApoB)-reactive CD4$^+$ T cells[81]. During atherosclerosis progression, ApoB-reactive CD4$^+$ T cells undergo a transition from a T_{reg} cell to a pro-inflammatory phenotype, which might contribute to further disease progression[81].

CD8$^+$ T cells in atherosclerotic lesions have also been found to have dual functions, with pro-atherogenic effects mediated by IFNγ production and macrophage activation, and atheroprotective effects via B cell modulation[78]. CD8$^+$ T cells in mice have been identified as drivers of plaque inflammation and apoptosis, promoting unstable plaque phenotypes and plaque erosion[82,83]. CD8$^+$ T cells outnumber CD4$^+$ T cells in

advanced human atherosclerotic plaques[25,82], and an increase in $CD8^+$ T cell numbers in blood is associated with the presence of CAD[84,85].

Invariant natural killer T (iNKT) cells are a distinct subset of T cells that express unique invariant T cell receptors and natural killer cell surface molecules, such as CD161 (also known as NK1.1 in mice) and killer cell immunoglobulin-like receptors (analogous to the Ly49 family in mice)[86]. Given the central role of lipids in atherosclerosis, iNKT cells are a relevant cell type because they respond to lipid antigens presented by CD1d on antigen-presenting cells. In mice, iNKT cells are considered to be pro-atherogenic owing to their production of pro-inflammatory cytokines such as IFNγ[86]. In humans, rupture-prone plaques have higher numbers of iNKT cells than stable plaques[87] but the exact mechanism underlying this observation is unknown.

B cells

B cell subpopulations make different contributions to atherogenesis[88]. B cells are central to humoral immunity and mediate the production of antibodies against oxidation-specific epitopes to help dampen inflammation. B cells are classified into two lineages: B1 cells, which are mainly produced in the fetal liver, and B2 cells, which originate in the bone marrow. B1 cells are further subdivided into B1a and B1b subsets. B2 cells can differentiate into transitional (T1 and T2 marginal zone progenitor) B cells, marginal zone B cells, follicular B cells and antibody-secreting plasma cells[88].

Box 2 | **Rebalancing the immune system in cardiovascular disease**

The balance between pro-inflammatory and anti-inflammatory immune processes is important for tissue homeostasis and to control inflammation. A failure to resolve acute inflammation results in the development of chronic inflammation, as seen in atherosclerosis. Crucial mechanisms in the resolution of inflammation in atherosclerosis involve efferocytosis and a rebalance of the levels of pro-inflammatory lipid mediators towards specialized pro-resolving mediators (SPMs). Non-specific targeting of inflammation in cardiovascular disease might affect immune subsets with homeostatic functions and induce the inhibition of endogenous plaque-resolving immune processes, such as efferocytosis.

Targeting efferocytosis

Defective efferocytosis and lack of immunomodulation promote an inflammatory environment in the atherosclerotic plaque, the formation of the necrotic core and plaque destabilization owing to secondary necrosis of apoptotic cells[62]. Efferocytosis is mediated through phagocytic receptors, such as tyrosine–protein kinase MER (MERTK) or LDL-receptor-related protein 1 (LRP1), and apoptotic cell ligands[310–312]. In atherosclerosis, impaired efferocytosis can be attributed to the downregulation or cleavage of efferocytosis receptors[310,312] and dysregulated expression of 'eat me' signals[313,314]. Atherosclerotic mice with increased MERTK expression have higher levels of efferocytosis and less necrotic core formation than control *Ldlr*$^{-/-}$ mice[310,311]. Loss of LRP1 in macrophages or haematopoietic cells in atheroprone mice leads to increased lesion area and necrotic core size[315], highlighting the potential of therapies aimed at increasing efferocytosis. One avenue for increasing efferocytosis is masking the 'don't eat me' signal CD47 on apoptotic cells. Blocking CD47 with a neutralizing antibody improved efferocytosis and ameliorated atherosclerosis in *Apoe*$^{-/-}$ mice[314]. Drugs targeting CD47 (Hu5F9-G4 and TTI-621) are currently being tested in clinical studies as cancer therapies[316,317]. However, the use of anti-CD47 in a clinical setting might have various adverse effects because of the role of CD47 in the regulation of other cellular processes[318], such as anaemia owing to high CD47 expression on haematopoietic stem cells and erythrocytes[319]. In addition, total loss of CD47 or its ligand thrombospondin, which is associated with the regulation of inflammatory responses rather than efferocytosis, increased the size of the necrotic core in mice[320]. Therefore, the pharmacological properties of the antibody and target accessibility should be considered before advancing this therapy into the clinical arena. Of note, concomitant inhibition of CD47 and tumour necrosis factor (TNF) using anti-CD47 antibody therapy and commercially available anti-TNF antibodies, such as infliximab or etanercept, offers a synergistic benefit in the clearance of apoptotic cells in mice[314]. The observation that anti-TNF therapy reduces the risk of future cardiovascular events in patients with rheumatoid arthritis[321] provides a strong rationale for combining anti-inflammatory and pro-efferocytic therapies for the treatment of advanced atherosclerosis.

Specialized pro-resolving mediators

Mediators involved in the resolution of inflammation in atherosclerosis include IL-10, annexin A1 and SPMs, such as resolving D1, 15-epi-lipoxin A4 and resolvin E1 (REF.[322]). Chronic inflammation in mouse and human atherosclerotic plaques is characterized by an imbalance between SPMs and pro-inflammatory mediators, such as leukotrienes[323]. In addition, a low resolvin D1 to leukotriene ratio in saliva has been proposed as a biomarker of the presence of non-resolving inflammation[324].

In the atherosclerotic plaque, lipid and peptide SPMs signal through *N*-formyl peptide receptor 2 (FPR2) and chemokine-like receptor 1 (CMKLR1), which are both G protein-coupled receptors. Systemic administration of SPMs, including resolvin D1, 15-epi-lipoxin A4, Ac2-26 (a synthetic analogue of annexin A1) and resolvin E1, reduced atherosclerosis in mouse and rabbit models of advanced atherosclerosis[249,323,325–328]. These studies highlight that restoring the balance of pro-inflammatory and pro-resolving mediators to induce the resolution of inflammation is an exciting therapeutic avenue, especially given that atherosclerosis in a clinical setting is usually treated once plaques and non-resolving inflammation have been established. Resolvin E1 analogues have been tested in a phase II trial for the treatment of ocular inflammation but did not improve outcomes compared with placebo[329]. However, before moving into a clinical setting in cardiovascular disease, the effect of activation of immunosuppressive mechanisms should be evaluated. Most of the above-mentioned mediators have systemic roles in maintaining tissue homeostasis. However, specific delivery of Ac2-26 using monocyte–macrophage-targeting nanoparticles increased plaque stability in mice[249]. Therefore, selective targeting of specific pro-resolving or pro-inflammatory cell types in atherosclerosis will most probably mediate the most beneficial outcomes in the clinic.

In atherosclerosis, B cells are not always found in the plaque and are more commonly localized in the adventitia or in node-like structures, referred to as tertiary lymphoid organs, that form in the adventitia as a result of chronic inflammation[79]. B1 cells have been described as atheroprotective in mice owing to the production of IgM antibodies that block the uptake of oxLDL by macrophages in lesions[16,89]. By contrast, B2 cells have been shown overall to be pro-atherogenic, through antibody responses formed via germinal centre B cell reactions that further drive adaptive immunity[88]. In mice fed a high-cholesterol diet, subsets of B2 cells with atheroprotective functions arise in secondary lymphoid organs, such as the lymph node (T2 marginal zone progenitor B cells)[90] and the spleen (marginal zone B cells)[91]. These subsets act either through PDL1-mediated suppression of T follicular helper cells[91] or via IL-10, although the role of IL-10 varies in different mouse models (IL-10 was shown to have a role in *Apoe*$^{-/-}$ mice[90] but not in *Ldlr*$^{-/-}$ chimeric mice[92]) and is dependent on the microbiome[93] and the radioresistance of B cell subsets[94].

Clinical trials of immunotherapies in CVD

Over the past 5 years, promising results from clinical trials targeting inflammation in CVD have been reported. In this section, we summarize the positive phase III trials, promising phase II studies, ongoing trials and trials with neutral results, and the lessons learnt from these studies (FIG. 3).

Phase III clinical trials showing cardiovascular benefits

Two immunotherapeutics have been successful in improving the cardiovascular outcomes of patients with CVD: canakinumab[32] and colchicine[33,34,95] (TABLE 1).

Canakinumab. The CANTOS trial[32] was a double-blind, randomized, controlled trial investigating the effects of canakinumab, a monoclonal antibody against the pro-inflammatory cytokine IL-1β, in patients with recent MI. In total, 10,061 patients with a history of MI who were receiving optimal management for cardiovascular risk factors and had high-sensitivity C-reactive protein (hsCRP) levels of >2 mg/l were randomly assigned to receive canakinumab or placebo. Canakinumab was administered subcutaneously at doses of 50 mg, 150 mg or 300 mg every 3 months. Patients were followed up for a median of 3.7 years. The 150-mg canakinumab dose led to a significantly lower rate of recurrent cardiovascular events than placebo, independently of lipid-level lowering (HR 0.85, 95% CI 0.74–0.98; $P = 0.021$)[32]. No effect was observed on total mortality, owing to a small but significant increased risk of infection with canakinumab. Notably, among patients receiving canakinumab, those with a reduction in on-treatment hsCRP levels to <2 mg/l benefited the most from the treatment, and the effect of canakinumab at reducing hsCRP levels was dose-dependent[96]. A subanalysis extended the scope of the effects of canakinumab beyond IL-1β by showing that the modulation of plasma IL-6 levels is associated with the beneficial effects of canakinumab in reducing the risk of cardiovascular events[97]. Moreover, canakinumab reduced cancer mortality[98]. The CANTOS trial demonstrated for the first time the proof-of-principle that therapeutic targeting of the immune system can be beneficial for cardiovascular outcomes in patients.

Colchicine. Colchicine, which is widely used for the treatment of gout and pericarditis, decreases inflammation by inhibiting cytoskeletal microtubule formation[99,100]. Colchicine has broad cellular effects, including reduction of monocyte and neutrophil motility and inhibition of inflammasome assembly in vitro[101]. The LoDoCo2 trial[34] included 5,522 patients with stable chronic CAD. After 1 month of open-label use of colchicine (0.5 mg once daily), patients were randomly assigned to receive colchicine or placebo and followed up for a median of 28.6 months. Patients receiving colchicine had a 31% reduction in the incidence of the primary composite end point of cardiovascular death, MI, ischaemic stroke and ischaemia-driven coronary revascularization compared with patients receiving placebo (HR 0.69, 95% CI 0.57–0.83; $P < 0.001$). Unfortunately, data on the effects of colchicine on inflammatory markers are not available. The results of this trial are consistent with those of two phase II trials investigating colchicine, LoDoCo[95] (in patients with stable chronic CAD) and COLCOT[33] (in patients with MI), and provide further support for the potential benefits of anti-inflammatory therapy in patients with acute coronary disease. Taken together, these trials demonstrated that anti-inflammatory therapies are efficacious in reducing cardiovascular events in patients with stable CVD. Although CANTOS and LoDoCo2 have not yet changed the treatment strategy in cardiovascular risk management in clinical practice, these trials are a crucial milestone for the clinical translation of immunomodulatory therapeutics in CVD. Both treatments target innate immunity, offering proof in humans of the importance of the innate response of the immune system in triggering inflammation in atherosclerosis.

Promising phase II clinical trials

Several cytokine blockers have shown promising results in phase II trials (TABLE 2). Cytokine blockers are the first line of biologics for the treatment of chronic inflammatory diseases, including RA, inflammatory bowel disease and psoriasis[102–104]. Therefore, an arsenal of potential therapeutics for CVD is available, some of which will soon be available as generic drugs (such as tumour necrosis factor (TNF) blockers).

IL-1 blockade. IL-1 is a pro-inflammatory cytokine that drives inflammation in atherosclerosis[105]. Both isoforms of IL-1, IL-1α and IL-1β, are involved in atherosclerosis. Studies in mice have shown that IL-1α has a role in the remodelling of arteries during early atherogenesis, whereas IL-1β mainly drives vascular inflammation in later stages of atherosclerosis[106]. However, IL-1β had a protective role in advanced atherosclerosis in mice through the promotion and maintenance of a fibrous cap rich in VSMCs and collagen[107]. Additionally, IL-1α forms a link between the immune system and coagulation through the activation of IL-1α by thrombin,

underscoring the importance of this isoform in the pathogenesis of adverse cardiovascular events[108]. In humans, the levels of IL-1β in the coronary arteries are higher in patients with CAD than in patients with non-ischaemic cardiomyopathy[109], and this cytokine is considered to be therapeutically tractable. Several options are available for IL-1 blockade, including canakinumab (selective IL-1β targeting), anakinra (an IL-1 receptor antagonist, which thereby targets IL-1α and IL-1β) and xilonix (a monoclonal antibody specifically targeting IL-1α). In two separate studies, therapy with anakinra significantly reduced hsCRP levels in the acute setting in patients with ACS compared with placebo[110,111]. Therapy with xilonix plus standard of care showed a non-significant trend towards a reduction in restenosis and the incidence of major adverse cardiovascular events

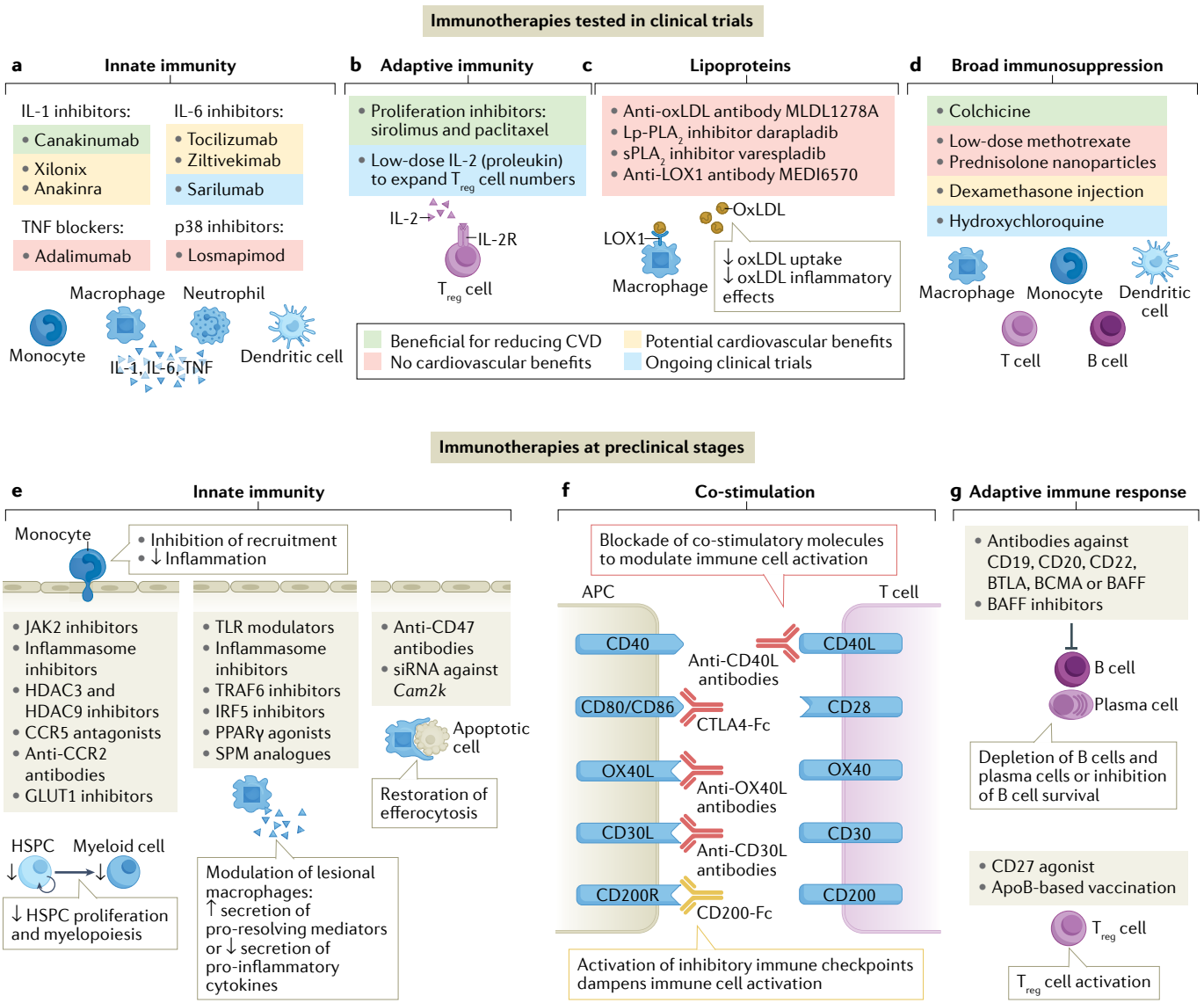

Fig. 3 | **Targeting the immune system in atherosclerosis. a–d** | Immunotherapies for the treatment of atherosclerosis that showed benefit (green), no benefit (red) or potential benefit (yellow) in reducing inflammation or cardiovascular events in clinical trials or currently being tested in ongoing clinical trials (blue) are shown. Therapeutics targeting innate immunity include IL-1 inhibitors, IL-6 inhibitors, tumour necrosis factor (TNF) blockers and p38 inhibitors (panel **a**). Therapeutics targeting adaptive immunity include local proliferation inhibitors in drug-eluting stents and low-dose IL-2 targeting regulatory T (T_{reg}) cells (panel **b**). Therapeutics targeting lipoproteins to reduce inflammation include antibodies against oxidized LDL (oxLDL), lipoprotein-associated phospholipase A2 (Lp-PLA_2), secretory phospholipase A2 (sPLA_2) and lectin-like oxidized LDL receptor 1 (LOX1) (panel **c**). Therapeutics with broad immunosuppressive effects include colchicine, low-dose methotrexate, glucocorticoids and hydroxychloroquine (panel **d**). See TABLES 1,2 and 3 and Supplementary Table 1 for further details. **e–g** | Overview of therapeutics in preclinical development targeting innate immunity (panel **e**), co-stimulation pathways (panel **f**) and B cell and T cell regulation (panel **g**). APC, antigen-presenting cell; ApoB, apolipoprotein B; BAFF, B cell activating factor; BCMA, B cell maturation antigen; BTLA, B and T lymphocyte attenuator; CCR, C-C chemokine receptor; CD30L, CD30 ligand; CD40L, CD40 ligand; CTLA4, cytotoxic T lymphocyte antigen 4; CVD, cardiovascular disease; GLUT1, glucose transporter 1; HDAC, histone deacetylase; HSPC, haematopoietic stem and progenitor cell; IRF5, interferon regulatory factor 5; OX40L, OX40 ligand; PPARγ, peroxisome proliferator-activated receptor-γ; siRNA, small interfering RNA; SPM, specialized pro-resolving mediators; TLR, Toll-like receptor; TRAF6, tumour necrosis factor receptor-associated factor 6.

Table 2 | **Potentially effective immunotherapies in phase II clinical trials in cardiovascular disease**

Study (year)	Agent	Drug target	Study design	Patient cohort	Primary end point	Main outcomes	Ref.
El Sayed et al. (2016)	Xilonix	Monoclonal antibody specifically targeting IL-1α	Randomized, placebo-controlled	43 patients undergoing percutaneous SFA revascularization	Clinically significant target vessel restenosis, time to restenosis and incidence of major adverse cardiovascular events	At 12 months of follow-up, no difference between Xilonix and placebo; at 3 months, trend towards decreased restenosis (0% versus 10%) and cardiovascular events (9% versus 24%) in the Xilonix versus placebo groups	112
MRC-ILA heart study (2015)	Anakinra	IL-1 receptor antagonist	Randomized, double-blind, placebo-controlled	182 patients with NSTE–ACS presenting <48 h from onset of chest pain	hsCRP AUC over the first 7 days after treatment initiation	Decrease in hsCRP levels after 14 days of treatment with anakinra; similar risk of MACE at 30 days and 3 months but significant increase in MACE at 1 year in the anakinra group compared with the placebo group	111
VCU-ART3 (2020)	Anakinra	IL-1 receptor antagonist	Randomized, double-blind, placebo-controlled	99 patients with STEMI	hsCRP AUC at baseline and at 72 h and 14 days after treatment initiation	Decrease in hsCRP AUC after 14 days of treatment with anakinra; reduced incidence of new-onset heart failure, death and hospitalization for heart failure in the anakinra group compared with the placebo group	110
DANCE (2018)	Dexamethasone delivered to the adventitial tissue surrounding target lesions	Broad anti-inflammatory effect	Prospective, single-group, open- label; data compared with findings from contemporary trials	262 patients with symptomatic PAD receiving PTA ($n = 124$) or atherectomy ($n = 159$)	12-month primary patency (composite of freedom from binary restenosis and clinically driven target-lesion revascularization)	Reduced restenosis after 12 months of follow-up	243
Kleveland et al. (2016)	Tocilizumab	Monoclonal antibody against IL-6 receptor	Randomized, double-blind, placebo-controlled	117 patients with NSTEMI, included in the randomization at a median of 2 days after symptom onset	hsCRP AUC at 1–3 days of treatment initiation	Tocilizumab reduced hsCRP levels compared with placebo	119
ASSAIL-MI (2021)	Tocilizumab	Monoclonal antibody against IL-6 receptor	Randomized, double-blind, placebo-controlled	199 patients within 6 h of STEMI and undergoing PCI	Myocardial salvage index measured by MRI 3–7 days after treatment initiation	Tocilizumab increased the myocardial salvage index and reduced CRP levels compared with placebo	118
RESCUE (2021)	Ziltivekimab	Monoclonal antibody against IL-6	Randomized, double-blind, placebo-controlled	264 patients with chronic kidney disease and hsCRP >2 mg/l	hsCRP measured 12 weeks after treatment initiation	Ziltivekimab reduced hsCRP levels at all doses compared with placebo	120

AUC, area under the curve; CRP, C-reactive protein; hsCRP, high-sensitivity C-reactive protein; MACE, mayor adverse cardiovascular events; MI, myocardial infarction; NSTE–ACS, non-ST-segment elevation acute coronary syndrome; NSTEMI, non-ST-segment elevation myocardial infarction; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; PTA, percutaneous transluminal angioplasty; SFA, superficial femoral artery; STEMI, ST-segment elevation myocardial infarction.

compared with standard of care only in patients undergoing percutaneous femoral artery revascularization[112]. Whereas the CANTOS trial highlighted the relevance of targeting IL-1β in stable CAD, these studies illustrate the importance of IL-1 as a target in the acute setting of thrombotic events. Additional studies in larger patient groups should be performed to further assess the effect of these therapeutics on cardiovascular outcomes.

IL-6 blockade. IL-6 is a pro-inflammatory cytokine involved in the innate immune response and a downstream mediator of a cytokine cascade featuring TNF and IL-1. IL-6 is a central stimulus for the acute phase response. In particular, IL-6 stimulates the production of CRP, among other acute phase reactants, in hepatocytes[113]. IL-6 signalling contributes to atherosclerosis and plaque destabilization in mice[114]. Data from humans show that elevated IL-6 levels in the plasma are associated with an increased risk of MI, and genetic studies have provided evidence of a causal role for IL-6 receptor signalling in CVD[115–117]. Therapy with tocilizumab, a monoclonal antibody targeting the IL-6 receptor, reduced hsCRP levels in patients with ST-segment elevation MI (STEMI)[118] or non-STEMI[119] compared

with placebo. Tocilizumab therapy also significantly increased the myocardial salvage index in patients with STEMI[118]; however, the absolute difference between the tocilizumab and placebo groups was only 5.6%, meaning that this increase might be of limited clinical relevance. In a phase II trial published in 2021, IL-6 blocking with the antibody ziltivekimab reduced hsCRP levels in patients with chronic kidney disease, who are at high risk of atherosclerosis[120]. These studies demonstrate the efficacy of IL-6 blockade for inflammation reduction. Follow-up studies, including the ZEUS trial[121], will provide a more complete picture of the clinical relevance of IL-6-targeted therapies in CVD.

Blockade of other cytokines. Alternatives to IL-1 and IL-6 blockade include TNF or IL-23 blockers, given that preclinical and clinical research has demonstrated a pro-atherogenic role for these cytokines[122–124]. TNF is a pro-inflammatory cytokine and is produced by several cells involved in atherosclerosis, including macrophages and VSMCs[125]. In mice, TNF deficiency reduced atherogenesis[126]. In humans, TNF is present in atherosclerotic plaques and the levels of TNF in peripheral blood predict future coronary events in patients with MI[125,127]. In observational studies in patients with arthritis, inflammation was a strong risk factor for cardiovascular events and TNF blockade resulted in reduced atherogenesis and lower incidence of cardiovascular events compared with patients with arthritis who did not receive TNF-blocking therapy[35]. However, in clinical trials in patients with heart failure, TNF blockade had no efficacy or even worsened the clinical outcome[128,129]. Therefore, TNF blockers might not be suitable for patients with substantial deterioration of left ventricular systolic function.

IL-23 is present in human atherosclerotic plaques, and high plasma levels of IL-23 are associated with increased mortality in patients with carotid artery stenosis[123]. Studies in mice have shown that IL-23 drives T_H17 cell function, contributing to the aggravation of atherosclerosis[130–132]. Despite the pro-atherogenic role of IL-23 in mice, several meta-analyses of studies in patients with psoriasis showed either no effect or possible worsening of cardiovascular outcomes after treatment with IL-23 blockers (ustekinumab and briakinumab) compared with placebo[133,134]. These studies were primarily designed to assess the effect of the IL-23 blockers on psoriasis and, therefore, conclusions cannot be drawn about their effect on inflammation in atherosclerosis. Other alternative therapeutic targets currently being tested in trials, including hydroxychloroquine and low-dose IL-2, are discussed in BOX 3 and TABLE 3.

Challenges

Several strategies for targeting inflammation in CVD have been tested in clinical trials but have not resulted in the reduction of inflammation markers and/or cardiovascular events (Supplementary Table 1). Notable examples are methotrexate and a p38 inhibitor, which did not reduce cardiovascular events or mortality in patients with CVD[135,136]. The majority of the trials that did not show efficacy of the drug being tested included unselected patient cohorts; therefore, a potential explanation for the lack of efficacy might be the heterogeneity of the patient group. The CANTOS trial[32] was the first trial to take a step towards the use of precision medicine by specifically selecting patients with an increased residual inflammatory risk (measured as hsCRP >2 mg/l). However, the trials investigating colchicine also included unselected patient groups and did show beneficial effects on cardiovascular outcomes[33,34]. This finding illustrates that failure to demonstrate efficacy might also be mechanism-based and that inhibiting inflammation in CVD is effective provided the correct inflammatory target or drug is chosen.

The variability of disease settings in clinical trials of CVD might explain the lack of beneficial effects of p38 inhibitors. p38 is an intracellular kinase that is activated in CVD by several stressors, such as oxLDL and hypertension, and is involved in the stabilization of mRNA encoding several inflammatory mediators that are crucial in CVD[137,138]. The first study of the p38 inhibitor losmapimod in CVD included patients with stable atherosclerosis[139]. Vascular inflammation was assessed with fluorodeoxyglucose (FDG) PET–CT imaging. Losmapimod therapy did not significantly reduce the overall uptake of FDG in the index vessel compared with placebo but reduced inflammation in the most inflamed regions[139]. However, losmapimod had no effect on clinical outcomes in subsequent trials that included larger cohorts of patients with acute MI[136,140], suggesting that p38 might have a selective role in chronic stable CVD, which is consistent with the role of p38 in prolonging inflammatory responses via modulation of mRNA stability[138].

Other studies have also used FDG PET–CT imaging to assess vascular inflammation, such as the GLACIER trial[141].

Box 3 | Ongoing clinical trials targeting atherosclerosis

Hydroxychloroquine is an antimalarial and a disease-modifying antirheumatic drug used for the treatment of inflammatory rheumatic diseases, especially systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA)[330]. In lysosomes, hydroxychloroquine inhibits the degradation of cargo by increasing the pH and preventing the activity of lysosomal enzymes. This drug can inhibit nucleic acid sensors, such as cyclic GMP–AMP synthase, and prevents ligand binding to Toll-like receptor 7 (TLR7) and TLR9, thereby reducing the production of pro-inflammatory cytokines, including type I interferons[330]. In observational studies, hydroxychloroquine therapy was associated with a 72% decrease in the risk of cardiovascular events in patients with RA and a 68% reduction in thromboembolic events in patients with SLE[331,332]. Hydroxychloroquine is currently being tested in two clinical trials in patients with coronary artery disease[252,253] (TABLE 3).

The cytokine IL-2 is essential for the growth and survival of regulatory T (T_{reg}) cells, which have a role in the control of inflammation. Low-dose IL-2 therapy has been trialled in patients with SLE, RA and psoriasis[333]. The principle of using low doses of IL-2 for the treatment of inflammatory diseases is based on the differential sensitivity of distinct immune cell subsets to IL-2. Among all T cell and natural killer cell subsets, T_{reg} cells typically respond to the lowest concentrations of IL-2 owing to elevated surface expression of the IL-2 receptor subunit-α (also known as CD25) and the high-affinity IL-2 receptor complex in this cell subset. Low-dose IL-2 therapy increases the number of T_{reg} cells and the expression of functional markers, such as CD25, in patients with other inflammatory diseases[334,335]. High-dose IL-2 therapy administered to patients with cancer is associated with adverse effects, but this severe toxicity is not justifiable in the setting of autoimmune diseases[333]. The ongoing phase II LILACS trial[254,336] is testing low-dose IL-2 therapy in patients with stable ischaemic heart disease and patients with acute coronary syndrome, with preliminary results showing effective expansion of T_{reg} cells with the therapy. We look forward to the results of these and other exciting trials listed in TABLE 3.

Table 3 | **Ongoing randomized controlled trials targeting the immune system in atherosclerosis**

Trial name (number)	Agent	Drug target	Trial design	Patient cohort	Primary end point	Ref.
OXI (NCT02648464)	Hydroxychloroquine	Broad immunosuppression	Phase IV	125 patients with MI	Rate of cardiovascular adverse events (MI, death, hospitalization for unstable angina and heart failure)	252
CHANGAN (NCT02874287)	Hydroxychloroquine	Broad immunosuppression	Phase IV	35 patients with CAD and hsCRP >1 mg/l	Change in fasting hsCRP level	253
LILACS (NCT03113773)	Low-dose IL-2	Induces expansion of regulatory T cell numbers	Phase I–II	41 patients with a history of CAD or acute coronary syndrome	Safety, tolerability and circulating regulatory T cell levels	254
IVORY (NCT04241601)	Low-dose IL-2	Induces expansion of regulatory T cell numbers	Phase II	60 patients with ACS and hsCRP >2 mg/l	Change in vascular inflammation, as measured by FDG PET–CT	255
NCT04762472	Montelukast	Leukotriene receptor	Phase IV	200 adults asymptomatic for atherosclerotic disease and exposed to air pollution	Subclinical atherosclerosis (as measured by brachial flow-mediated dilatation, carotid intima–media thickness and blood inflammatory markers)	256
NCT04616872	Methotrexate delivered in LDL-like nanoparticles	Broad immunosuppression	Phase II–III	40 patients with multivessel CAD and hsCRP >2 mg/l	Reduction in plaque volume, measured by CTA	257
SARIPET (NCT04350216)	Sarilumab	Monoclonal antibody against IL-6 receptor	Phase IV	20 patients with active rheumatoid arthritis and CRP levels >1 mg/dl	Changes in carotid atheroma plaque assessed by ultrasonography	258
PAC-MAN (NCT04148833)	Paclitaxel	Proliferation	Phase II–III	40 patients with CAD	Low-attenuation plaque volume measured by CTA	259
GOLDILOX (NCT04610892)	MEDI6570	Antibody against LOX1 receptor (blocks uptake of oxidized LDL)	Phase IIb	792 patients with a history of MI	Non-calcified plaque volume measured by CTA	260
CLEAR-Synergy (NCT03048825)	Colchicine	Broad immunosuppression	Phase III	7,000 patients with MI	MACE	261
CONVINCE (NCT02898610)	Colchicine	Broad immunosuppression	Phase III	2,623 patients with ischaemic stroke or at high risk of transient ischaemic attack	Recurrence of non-fatal ischaemic stroke or non-fatal MACE, or vascular-related death	262
ZEUS (NCT05021835)	Ziltivekimab	Monoclonal antibody against IL-6	Phase III	6,200 patients with chronic kidney disease and CRP ≥2 mg/l	Time to first MACE	121

ACS, acute coronary syndrome; CAD, coronary artery disease; CRP, C-reactive protein; CTA, computed tomography angiography; FDG, fluorodeoxyglucose; hsCRP, high-sensitivity C-reactive protein; LOX1, lectin-like oxidized LDL receptor 1; MACE, major adverse cardiovascular events; MI, myocardial infarction.

The trial included 147 patients with stable atherosclerotic disease who were randomly assigned to receive a single dose of the anti-oxLDL antibody MLDL1278A, multiple doses of MLDL1278A or placebo. None of the MLDL1278A regimens had a significant effect on carotid plaque inflammation, possibly owing to the concomitant use of lipid-lowering medication, which might have masked the effect of passive vaccination with MLDL1278A[141]. This study also raises questions about the use of imaging as a surrogate end point for cardiovascular events. New PET–CT imaging tracers that can detect meaningful cardiovascular inflammation more accurately than FDG are needed[142]. An imaging technique developed in the past 4 years that is based on CT angiography showed that changes in the CT attenuation index of perivascular adipose tissue might be a marker of coronary perivascular inflammation associated with cardiovascular outcomes[143,144]. Further improvements in the imaging of atherosclerosis will facilitate the development of valid surrogate end points of cardiovascular outcomes. Although cardiovascular surrogate end points are at present not sufficiently specific and, therefore, have not reached the benchmark of a clinical trial, developments in the field of machine learning could be used to combine multiple surrogate end points for a more accurate prediction of clinical outcomes[145,146].

Considering the above-mentioned successes in therapeutic targeting of the immune system in atherosclerosis, the number of ongoing trials in this setting is surprisingly low. One reason could be the high costs of clinical trials in CVD, which make this area less attractive for industry investments. Trials in CVD are event-driven rather than symptom-driven and, therefore, require high patient numbers and long follow-up. Therefore, identifying reliable surrogate markers of vascular inflammation is crucial to facilitate the design of small proof-of-principle trials, allowing rapid innovation and reduced risks. One crucial need is the early identification of patients who are likely to respond to a specific treatment and patients who would not benefit

from the interruption of a specific inflammatory pathway. This concept is well exemplified by the CANTOS trial[32], which demonstrated that patients with the larger reductions in hsCRP levels with canakinumab therapy derived the largest clinical benefit from the treatment. Patients with a decrease in hsCRP levels greater than the median percentage reduction had a 27% reduction in cardiovascular events compared with a reduction of only 5% in those patients with a decrease in hsCRP levels that was lower than the median[96]. Moreover, the fall in hsCRP levels has so far gone hand in hand with outcome benefits in the majority of clinical trials of anti-inflammatory therapies in CVD. In the future, new surrogate end points that are based on immunophenotyping and/or imaging could be used in clinical trials, provided that an association with cardiovascular outcomes is demonstrable.

Looking to the future, the secondary effects of anti-inflammatory therapies should be carefully considered. Canakinumab administration was associated with a major reduction in the incidence of lung cancer compared with placebo in the CANTOS trial[98]. By contrast, in the CIRT trial[135,147], methotrexate was linked to a small increase in the incidence of skin cancer compared with placebo, emphasizing the complexity of the effects of immunotherapy on CVD and cancer. Immunosuppression and chronic inflammation can both increase the risk of cancer[147]. Furthermore, preclinical studies have spotlighted the existence of an immune-mediated link between MI and breast cancer that can accelerate cancer progression[148]. An increasing number of studies have also shown that immune checkpoint inhibitor therapies might increase the risk of CVD in patients with cancer[36,37], whereas inhibition of adaptive immunity increases the risk of cancer through disruption of antitumour immunity[149]. Now that anti-inflammatory therapies in CVD are close to implementation in clinical practice, unravelling the complex immunological relationship between cancer and CVD is crucial.

Finally, the pathogenesis of CVD is multifactorial, and several types of coronary culprit lesions lead to the same clinical presentation and syndromes[17]. Different disease settings have distinct immune signatures, as illustrated by the different signatures in plaque erosion and rupture[150], which calls for the identification of the disease setting in which a therapy will be most successful. Implementing deep immunophenotyping strategies can improve the selection of patients with the highest likelihood of benefiting from a specific therapy and facilitate rapid identification of responders and non-responders to therapy[151]. Immunophenotyping of patients with CVD is still in its infancy; however, a few of the currently available markers could guide patient selection, such as hsCRP and IL-6 levels in the plasma[96,97,152]. The discovery of CHIP as a novel risk factor of atherosclerosis will potentially enable further risk stratification of patients[153]. For example, a re-analysis of CANTOS data suggested that anti-inflammatory treatment might be more effective in patients carrying CHIP-associated gene variants[154]. Taken together, extensive immunophenotyping and immune-based risk stratification might facilitate patient selection and stratification and identification of treatment responders, allowing efficient design of clinical trials and realizing the potential of targeted immunomodulatory therapies for CVD.

In summary, the challenges in addressing the low-grade inflammation associated with CVD are manifold and encompass the need for careful risk–benefit assessment, the existence of several coronary syndromes with potentially different endotypes and pathogenesis, our current inability to identify responders to treatment early, and our reliance on 'hard' clinical end points in trial design owing to the limitations of our current imaging techniques. Further understanding of the immune signature of CVD together with the evolution of cardiovascular imaging technologies will accelerate the translation of therapies targeting inflammation from the preclinical to the clinical arena.

New targets for clinical translation

Advances in our understanding of the pathogenesis of atherosclerosis have highlighted several potential cellular and molecular therapeutic targets. In this section, we focus on a selection of the most promising areas supported by the convergence of several lines of evidence from CVD and other diseases, and which are, therefore, closer to translation to patient therapies in the medium term (FIG. 3).

Immunometabolism and trained immunity

Targeting immunometabolic processes is a promising strategy for modulating inflammation and immunity. Atherosclerosis-associated changes in blood and bone marrow are regulated by immunometabolic events[155]. In mice, a Western diet and hyperglycaemia have been shown to induce epigenetic reprogramming of myeloid progenitors, which resulted in sustained monocyte and macrophage pro-inflammatory priming, thereby driving tissue inflammation and CVD[156–158]. These effects persisted even after restoring lipid and glucose levels to normal levels owing to the phenomenon of 'trained immunity', whereby transcriptomic, epigenetic and metabolic rewiring of innate immune cells leads to an altered response towards a second challenge[159].

Epigenetic regulation is of particular interest because of the potential for pharmacological inhibition. Histone deacetylases (HDACs) repress gene expression by removing open-chromatin acetylation marks. Broad HDAC inhibition in atherosclerotic mice showed mixed results[160–162], whereas inhibition or genetic deletion of HDAC3 or HDAC9 reduced atherosclerosis in mice[163–165]. Variants in *HDAC9* have been associated with abdominal aortic calcification and ischaemic stroke in genome-wide association studies in humans[166,167], highlighting the clinical potential of specific HDAC targeting in CVD.

Targeting metabolic rewiring is an alternative strategy because increased glucose metabolism in human and mouse haematopoietic stem and progenitor cells (HSPCs) dictates myeloid lineage commitment[168]. Glucose transporter 1 (GLUT1), a ubiquitously expressed glucose transporter, is a well-recognized target in other inflammatory conditions[169]. GLUT1 deficiency in bone

Box 4 | **Eliciting an innate response: TLRs and inflammasomes**

TLRs

Toll-like receptors (TLRs) are a family of ten proteins in humans that recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), triggering an innate immune response[10]. Whereas the intracellular receptors TLR3 (REF.[337]) and TLR7 (REF.[338]) are involved in anti-atherogenic processes, the extracellular sensors TLR2 and TLR4 are thought to initiate the immune response in the arterial wall by recognizing modified lipoproteins in concert with scavenger receptors[15,339], leading to cell activation and induction of pro-inflammatory cytokines, such as IL-1β, while also priming the inflammasome, which regulates IL-1β production[340]. TLR2 and TLR4 are significantly upregulated in human atherosclerotic tissue[341] and on circulating monocytes from patients with acute coronary syndrome[342], linking TLR levels to the risk of cardiovascular events[338,343]. Although TLR4 induces the most powerful responses of any TLR in pathogen-related situations, mutations and/or deletions of *Tlr4* in experimental models of atherosclerosis produced mixed results, with a varying degree of reduction or no effect on atherosclerotic lesion size[344]. By contrast, deletion of *Tlr2* consistently reduced lipid deposition and macrophage content in atherosclerotic lesions in mice[345]. Moreover, inflammation in human atherosclerosis has been shown to be driven by TLR2 via the signalling adaptor MyD88 (REF.[217]). Overall, this evidence strongly suggests that TLR2 is one of the most pro-atherogenic TLRs.

Inflammasomes

Inflammasomes are multimeric protein complexes that form in response to endogenous and exogenous danger signals, and promote pro-inflammatory cytokine production (including IL-1β and IL-18) and pyroptotic cell death[346]. The NLRP3 inflammasome is an important driver of lipid-driven vascular inflammation and atherosclerosis. Triggers for the activation of the NLRP3 inflammasome include potassium efflux, mitochondrial reactive oxygen species and cathepsin B[346]. All these triggers are likely to be present in the atheroma; however, a crucial driver of NLRP3 inflammasome activation in the arterial intima is cathepsin B activation downstream of cholesterol crystal, oxidized LDL and calcium phosphate crystal accumulation in phagocytic cells[347,348].

A crucial role for the absent in melanoma 2 (AIM2) inflammasome in atherosclerosis is emerging. In atherosclerotic mice, AIM2 inflammasome activation resulted in the production of IL-1β and IL-18 accompanied by an unstable plaque phenotype[349]. By contrast, *Aim2* deletion or pharmacological inhibition with an AIM2-antagonizing synthetic oligonucleotide increased plaque stability. Interestingly, AIM2 activation was also shown to be involved in atherosclerosis driven by clonal haematopoiesis[179,350]. AIM2-dependent inflammasome formation depends on the detection of cytosolic double-stranded DNA[179], possibly downstream of atherosclerosis-associated necrosis and apoptosis. AIM2 activation then leads to the release of cellular contents into the extracellular space, thereby driving inflammation.

Under normal conditions, the levels of NLRP3 or AIM2 inflammasome complexes in cells are minimal[351], and the inflammasomes remain in an inactive state through ubiquitination[352]. Both, NLRP3 and AIM2 inflammasomes require an initial priming signal, which promotes the expression of proteins involved in inflammasome signalling in a nuclear factor-κB-dependent fashion and stimulates inflammasome deubiquitination[351,352]. Subsequently, activation signals promote the assembly and activation of the inflammasome, enabling the proteolytic function of caspase 1 (REF.[346]). In addition to the processing of IL-1 cytokines, inflammasome activation facilitates the cleavage of gasdermin D, thereby inducing pore formation in the plasma membrane and pyroptosis, which is critical for the release of IL-1β and IL-18 to further promote inflammation in atherosclerosis[353].

marrow cells resulted in reduced HSPC proliferation, myelopoiesis and atherogenesis in mice[170]. However, further investigation of the effects of GLUT1 inhibition in humans is necessary, because patients with GLUT1 deficiency syndrome have neurological symptoms, such as epilepsy[171].

Targeting CHIP

The discovery of CHIP has led to the identification of new potential targets. The most commonly occurring variants associated with CHIP are loss-of-function variants in *DNTM3A*, *ASXL1* and *TET2* and gain-of-function variants in *JAK2* (*JAK2*^{V617F}), that all result in growth and survival advantages in the cells carrying the gene variant[172]. Mice with TET2 deficiency or carrying the *Jak2*^{V617F} variant showed accelerated atherogenesis[30,153,173,174]. Both macrophages from *Tet2*-knockout mice and peripheral blood monocytes from patients with aortic valve stenosis carrying a *DNTMA3* or *TET2* variant produce high levels of IL-1β and show NLRP3 inflammasome priming[30,153,175]. NLRP3 inflammasome inhibition by administration of MCC950 prevented TET2-dependent atherosclerosis progression in mice in vivo[30,153]. Similarly, clonal haematopoiesis driven by TET2 deficiency aggravated heart failure, cardiac dysfunction and obesity in mice, whereas NLRP3 inhibition with MCC950 protected against the development of heart failure and insulin sensitivity[176–178]. Activation of the absent in melanoma 2 (AIM2) inflammasome has been associated with *Jak2*^{V617F}-driven atherosclerosis in mice. In a mouse model of *Jak2*^{V617F}-driven atherosclerosis, deletion of the genes encoding for essential components that act downstream of the AIM2 inflammasome, such as caspase 1, caspase 11 and gasdermin D, induced a more stable plaque phenotype[179] (BOX 4). Taken together, the findings of these studies highlight the potential of targeting CHIP-driven inflammation with the use of NLRP3 or AIM2 inflammasome inhibitors.

JAK2 inhibitors could represent an alternative strategy for targeting inflammation in atherogenesis. Ruxolitinib and fedratinib are FDA-approved drugs for the treatment of myeloproliferative neoplasms and are currently being tested for use in other inflammatory conditions, such as RA[180]. Both drugs were effective in reducing inflammation and atherosclerosis in mouse and rabbit models of atherosclerosis[174,181]. Although treatment with the JAK1–JAK2 inhibitor ruxolitinib reduced atherosclerotic plaque size in mice with *Jak2*^{V617F}-dependent atherosclerosis[174,179], the treatment also increased necrotic core size and reduced cap thickness, resulting in an unstable plaque phenotype[179]. Therefore, a more specific JAK2 inhibitor, such as fedratinib, might be of interest in CVD.

Targeting monocyte recruitment

Monocyte recruitment in atherosclerosis depends on the CCR2, CCR5 and CX3CR1 chemokine receptors[182]. Genetic deletion of *Ccr2* or its ligand *Ccl2* reduced bone marrow-derived monocytosis and atherosclerotic lesion size in mice[42,183–185]. Similarly, mice with MI treated with a small interfering RNA (siRNA) targeting *Ccr2* had decreased monocyte recruitment to the infarct area and reduced disease severity[186]. In humans, genetic predisposition to elevated plasma CCL2 levels is associated with an increased risk of stroke, MI and CAD, and increased CCL2 levels in the blood and atherosclerotic plaques correlate with a higher risk of stroke and with markers of plaque destabilization[187]. MLN1202, a CCR2-blocking antibody, reduced hsCRP levels in patients at risk of atherosclerotic CVD[188]. Pharmacological inhibition of CCR5 with the FDA-approved CCR5 antagonist maraviroc reduced atherosclerosis in *Ldlr*$^{-/-}$ mice[189,190]. Interestingly, treatment with maraviroc also led to reduced atheroprogression in patients with HIV infection and high risk of CVD compared with baseline,

as assessed by intima–media thickness[191,192]. However, given that circulating monocytes traffic into tissues during homeostasis, inflammation and inflammation resolution[47,193], the effect of targeting monocyte recruitment on these processes will need monitoring.

Reprogramming inflammatory macrophages

Macrophage polarization is orchestrated by key master regulators, including nuclear factor-κB, the STAT family, peroxisome proliferator-activated receptor-γ (PPARγ) and the interferon regulatory factor (IRF) family[194]. Reprogramming pro-inflammatory macrophage populations that drive vascular inflammation towards homeostatic pro-resolving phenotypes could reduce disease burden. Pioglitazone is an FDA-approved PPARγ agonist that induces a pro-resolving macrophage phenotype by reducing pro-inflammatory cytokine production and promoting monocyte differentiation into alternatively activated macrophages[195–197]. In mice with atherosclerosis, administration of pioglitazone reduced macrophage content and increased plaque stability[198,199]. Clinical studies investigating the role of pioglitazone in patients with CVD and/or type 2 diabetes mellitus showed atheroprotective effects and a reduction of cardiovascular events with pioglitazone therapy[200–203], highlighting the therapeutic potential of this drug in CVD.

In mouse models of CVD, global or myeloid-specific IRF5 deficiency reduced atherosclerosis and improved plaque stability[204,205], and IRF5 inhibition with nanoparticles decreased myocardial infarct size[206]. The transcription factor IRF5 induces a pro-inflammatory phenotype in mouse and human macrophages[207]. Therefore, IRF5 is a promising therapeutic target in CVD. Inhibitors of IRF5 have proven to be therapeutically effective in mouse models of systemic lupus erythematosus[208,209].

Targeting the inflammasomes

Selective inhibition of the NLRP3 inflammasome with MCC950 reduced atherosclerosis in hypercholesterolaemic or diabetic mice[210,211]. MCC950 has been tested in phase II trials in patients with RA, but the trials had to be discontinued owing to liver toxicity[212]. The interest in using NLRP3 inflammasome inhibitors for the treatment of chronic inflammatory and neuroinflammatory diseases is increasing and these agents are being tested in clinical trials[213]. The NLRP3 inflammasome inhibitor OLT1177 has been assessed in phase I–II clinical trials in patients with osteoarthritis[214], acute gout[213] or heart failure[215] and has shown high tolerability. OLT1177 is also currently being tested in a study in patients with COVID-19 (REF.[216]).

Alternative approaches to targeting the inflammasome in atherosclerosis include the prevention of inflammasome priming with the use of TLR inhibitors[217], targeting the AIM2 inflammasome[179] and inhibition of caspase 1 (BOX 4). The catalytic activity of caspase 1 is required to convert pro-IL-1β into its active form downstream of NLRP3 and AIM2. The caspase 1 inhibitor VX-765 reduces atherosclerosis in mice[218]. However, phase II trials of the caspase 1 inhibitors VX-740 and VX-765 in patients with psoriasis or epilepsy revealed drug-induced hepatotoxicity and further development was stopped[219], highlighting the challenges presented by inhibition of inflammasomes.

Targeting the adaptive immune system

Immune recognition of LDL and oxLDL moieties leads to the generation of autoantibodies and oxLDL-reactive T cells[14,220]. Immunization with ApoB-derived antigens induces atheroprotective effects in mice and rabbits via diverse mechanisms including the induction of a humoral antibody response, T_{reg} cell activation, suppression of $CD4^+$ T cells and reduction of dendritic cell numbers in the plaque[221–223]. However, passive immunization with MLDL1278A, an anti-oxLDL antibody, added to lipid-lowering therapies did not reduce cardiovascular events in patients with stable atherosclerotic disease, as discussed above[141]. To improve the translation of ApoB-based immunization therapies from the preclinical to the clinical setting, Wolf and colleagues used in silico prediction methods to identify ApoB peptides that would bind to various major histocompatibility complex class II variants[81]. Using the in silico methods, the investigators identified 30 ApoB peptides that successfully induced a response in human T cells in vitro[81].

Another approach to targeting adaptive immune cells is the direct targeting of atherogenic B cell subsets[224]. B cell depletion therapies are already in clinical use for the treatment of RA and multiple sclerosis, and studies in mice have shown that preferential B2 cell depletion with the use of an anti-CD20 antibody reduces atherosclerosis[225,226]. A single dose of rituximab, a B cell-depleting anti-CD20 antibody, was safe and efficiently depleted B cells in patients with acute STEMI[227]. Antibodies for B cell depletion targeting CD19 (blinatumomab and inebilizumab), CD22 (inotuzumab ozogamicin) or B cell maturation antigen (belantamab mafodotin and AMG420) have been approved or are currently in clinical development for the treatment of multiple sclerosis and cancer. Other promising strategies targeting B cells include: impairment of B cell survival and proliferation (with atacicept, belimumab, blisibimod and ianalumab), modulation of B cell receptor signalling (with acalabrutinib, epratuzumab and ibrutinib), antibody neutralization (with omalizumab), and the modulation of B cell co-stimulation (with abatacept)[224,228].

Targeting co-stimulation pathways

Immune checkpoints are immune regulatory co-stimulatory molecules that provide stimulatory or inhibitory signals to adaptive and innate immune cells[229]. Immune checkpoints modulate the immune response in CVD[229]. In vivo studies in mice identified crucial co-stimulatory axes in atherosclerosis with the use of genetic deletion and agonistic and antagonist antibodies: activation of CD27–CD70, B and T lymphocyte attenuator (BTLA), CD200 receptor (CD200R)–CD200 and CD80/CD86–CTLA4 (cytotoxic T lymphocyte antigen 4) pathways or inhibition of CD40–CD40 ligand, OX40–OX40 ligand and CD30–CD30 ligand pathways might be beneficial therapeutic strategies in atherosclerosis[230–237]. Multiple immune checkpoint

inhibitors and agonists targeting the above pathways are in clinical development for the treatment of cancer and RA[238,239]. In preclinical models, specific inhibition of tumour necrosis factor receptor-associated factor 6 (TRAF6), downstream of the pro-inflammatory CD40 signalling pathway, with small-molecule inhibitors resulted in plaque stabilization without inducing adverse effects and sparing host defence[240]. Similarly, CD200R expression is restricted to the myeloid compartment, making the CD200–CD200R pathway amenable for selective targeting of the monocyte–macrophage axis locally and in the bone marrow in CVD[234].

Targeting the atherosclerotic plaque

Long-term immunosuppression might disrupt cardiovascular homeostasis and host defence[241]. Local delivery of drugs has been used in the clinic in the vascular field for many years with the use of drug-eluting stents containing sirolimus or paclitaxel, both of which have anti-inflammatory properties[242]. Furthermore, microneedle injections of dexamethasone in the adventitia prevents restenosis in patients who have undergone percutaneous transluminal angioplasty[243].

An alternative strategy for minimizing the systemic adverse effects of off-target cell activation with systemic immunosuppressive approaches and improving accessibility to the cell type of interest is the use of cell-targeted delivery approaches. Nanoparticles have a high engagement with myeloid cells and can be modified to target specific subsets with ligand-decorated nanomaterials[244]. Nanoparticles have been used to target macrophages in several trials in patients with CVD[245,246]. Flores and colleagues used PEGylated, single-wall carbon nanotubes to deliver a downstream inhibitor of the anti-phagocytic CD47 pathway to lesional macrophages in mice, which resulted in a reduced plaque burden without toxic effects[247]. Administration of macrophage-targeted nanoparticles carrying siRNA against *Camk2g* increased plaque stability in mice owing to improved efferocytosis, leading to rebalancing of the immune system in atherosclerosis[248] (BOX 2). Nanoparticles decorated with collagen type IV accumulate in the atherosclerotic lesion shoulder and the use of these nanoparticles for the targeted delivery of IL-10 or the anti-inflammatory annexin A1 biomimetic Ac2-26 peptide stabilized atherosclerotic lesions in mice[249,250]. TRAF6 inhibitors or pioglitazone delivered with nanoparticles was also effective in increasing plaque stability in atherosclerotic mice[198,240]. These studies highlight the potential of modulating the immune system in CVD by specifically targeting atherosclerotic plaques to avoid toxic effects associated with systemic immunosuppression approaches.

Conclusions

Cardiovascular research lags behind oncology and rheumatology in recognizing the effects of chronic inflammation on CVD and translating inflammatory targets to human cardiovascular therapy. Although our understanding of the role of inflammation in atherosclerosis has improved substantially over the past two decades, the nuanced balance between pro-inflammatory and anti-inflammatory cells required for homeostasis remains elusive. To identify new therapeutic targets in atherosclerosis, we need to improve our interpretation of the determinants of this equilibrium. Single-cell biology approaches can accelerate clinical translation by facilitating the examination of immune signatures in patients with CVD. Identification of culprit cell types with the use of multiomics approaches could help identify the most suitable patient population for clinical trials and support target selection and informed decision-making in a clinical setting, moving towards personalized medicine. In addition, it is imperative to determine the window of opportunity for anti-inflammatory therapy in atherosclerosis, in which the benefits of immune system inhibition outweigh the systemic immunosuppressive effects. More targeted approaches using biologics or vaccination might allow specific targeting of atherosclerotic inflammation and thus minimize off-target effects. The development of mRNA vaccines has revolutionized the field of RNA-based therapeutics, extending the toolkit for vaccines against atherosclerosis and for previously 'undruggable' targets[251]. The association of CHIP with CVD risk exemplifies the importance of patient stratification beyond the use of traditional risk factors to define the patient population that will benefit from treatment. It is time to take inflammation seriously as a pathogenic driver of CVD and direct resources towards mechanistic and translational studies to find the cause of and a remedy for inflammation in this context. There has never been a more exciting time for research in cardiovascular inflammation.

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