



# Anti-inflammatory and Immunomodulatory Therapies in Atherosclerosis

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## Contents

1	Introduction .....	360
2	Targeting Innate Immune Responses .....	362
2.1	Targeting the NLRP3 Inflammasome .....	362
2.1.1	Targeting the IL-1 Signaling Pathway .....	365
2.1.2	NLRP3 Inhibitors .....	366
2.2	Targeting TNF and Interleukins .....	367
2.2.1	Tumor Necrosis Factor (TNF) .....	367
2.2.2	IL-6 .....	369
2.3	Targeting Chronic Inflammation Using Broad Anti-inflammatory Drugs .....	370
2.3.1	Methotrexate .....	370
2.3.2	Low-Dose Colchicine .....	372
2.4	Targeting Chemokine Signaling .....	374
2.4.1	Targeting the CCR5 Axis .....	374
2.4.2	Targeting the CCR2 Axis .....	375
3	Modulation of the Adaptive Immune Response .....	376
3.1	Strategies Targeting the Homeostatic Balance Between Regulatory and Effector T Cells .....	377
3.1.1	Low-Dose IL-2 Therapy .....	378
3.1.2	Alternative Means to Expand Regulatory T Cells .....	379
3.2	Strategies Targeting B Cells and Humoral Immunity .....	379
3.2.1	Anti-CD20 Mediated B Cell Depletion (Rituximab) .....	380
3.2.2	Other B Cell Depleting Antibodies .....	382
3.2.3	B Cell Depletion via Targeting of the BAFF/APRIL System .....	382
3.2.4	IgE Neutralization (Omalizumab) .....	382
3.3	Strategies Targeting the Interaction Between T Cells and Antigen-Presenting Cells .....	383
3.3.1	Targeting Co-stimulatory Pathways .....	383
3.3.2	Immunization Against LDL-Related Antigens .....	385
3.3.3	Tolerogenic Vaccination .....	386
3.3.4	Immunization Against Pathogen-Derived Antigens .....	386

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359

4 Conclusion .....	387
References .....	388

## Abstract

Hypercholesterolemia is a major risk factor in atherosclerosis development and lipid-lowering drugs (i.e., statins) remain the treatment of choice. Despite effective reduction of LDL cholesterol in patients, a residual cardiovascular risk persists in some individuals, highlighting the need for further therapeutic intervention. Recently, the CANTOS trial paved the way toward the development of specific therapies targeting inflammation, a key feature in atherosclerosis progression. The pre-existence of multiple drugs modulating both innate and adaptive immune responses has significantly accelerated the number of translational studies applying these drugs to atherosclerosis. Additional preclinical research has led to the discovery of new therapeutic targets, offering promising perspectives for the treatment and prevention of atherosclerosis. Currently, both drugs with selective targeting and broad unspecific anti-inflammatory effects have been tested. In this chapter, we aim to give an overview of current advances in immunomodulatory treatment approaches for atherosclerotic cardiovascular diseases.

## Keywords

Adaptive immunity · Anti-inflammatory therapy · Atherosclerosis · Cardiovascular diseases · Immunotherapy · Inflammation · Innate immunity

## 1 Introduction

Atherosclerosis is a lipid-driven disease that is characterized by the formation of plaques in the subendothelial space of arteries. Therefore, lipid-lowering drugs such as statins are considered the treatment of choice as their reduction of circulating low-density lipoprotein (LDL) can prevent plaque formation and progression in patients (FERENCE et al. 2017; Borén et al. 2020). A crucial step in disease initiation involves the retention of LDL and other ApoB-carrying lipoproteins within the vascular wall due to their ability to bind intimal proteoglycans. Subsequent enzymatic and non-enzymatic modifications of these lipoprotein particles result in the formation of damage-associated molecular patterns (DAMPs) that trigger an inflammatory response. This includes the activation of endothelial cells by oxidized phospholipids and other lipid species. In response, the endothelium secretes chemoattractant cytokines like monocyte chemoattractant protein-1 (MCP-1), growth factors (macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF)) and upregulates the expression of genes coding for adhesion molecules (VCAM-1 and ICAM-1), allowing the recruitment and differentiation of inflammatory monocytes. Monocytes recruited into the intima become lesional macrophages, which take up oxidized LDL and

aggregated LDL via scavenger receptors (CD36, SR-A), ultimately leading to foam cell formation – a rate-limiting step that greatly contributes to the development and progression of atherosclerotic plaques (Borén et al. 2020). Excess accumulation of modified lipoproteins, formation of foam cells, subsequent macrophage cell death, and impaired clearance of these apoptotic cells, all contribute to chronic inflammation in the vascular wall (Borén et al. 2020; Libby 2021). Studies over the past 20 years have focused on the chronic inflammatory nature of atherosclerosis and have demonstrated the detrimental role of inflammation in its pathophysiology. This work led to the consideration of markers of inflammation such as IL-6 and high-sensitivity C-reactive protein (hsCRP) as biomarker for risk stratification of cardiovascular patients (Ridker et al. 2000a, b, 2009). According to this concept, patients with signs of inflammation (i.e., elevated levels of hsCRP or IL-6) are considered at higher risk of experiencing a cardiovascular event during their lifetime compared to individuals without residual inflammation. Additionally, both innate and adaptive immunity have been found to play a crucial role in the initiation, progression, and rupture of atherosclerotic plaques (Gisterå and Hansson 2017; Wolf and Ley 2019; Libby 2021). Since the importance of the immune system in atherosclerotic plaque development is becoming increasingly clear, novel treatments that target both innate and adaptive immunity have been developed with the aim to complement already established lipid-lowering therapies (Bäck et al. 2019). This chapter aims to introduce the reader to these novel anti-inflammatory and immunomodulatory treatments, which range from small molecule drugs to antibodies and vaccination strategies.

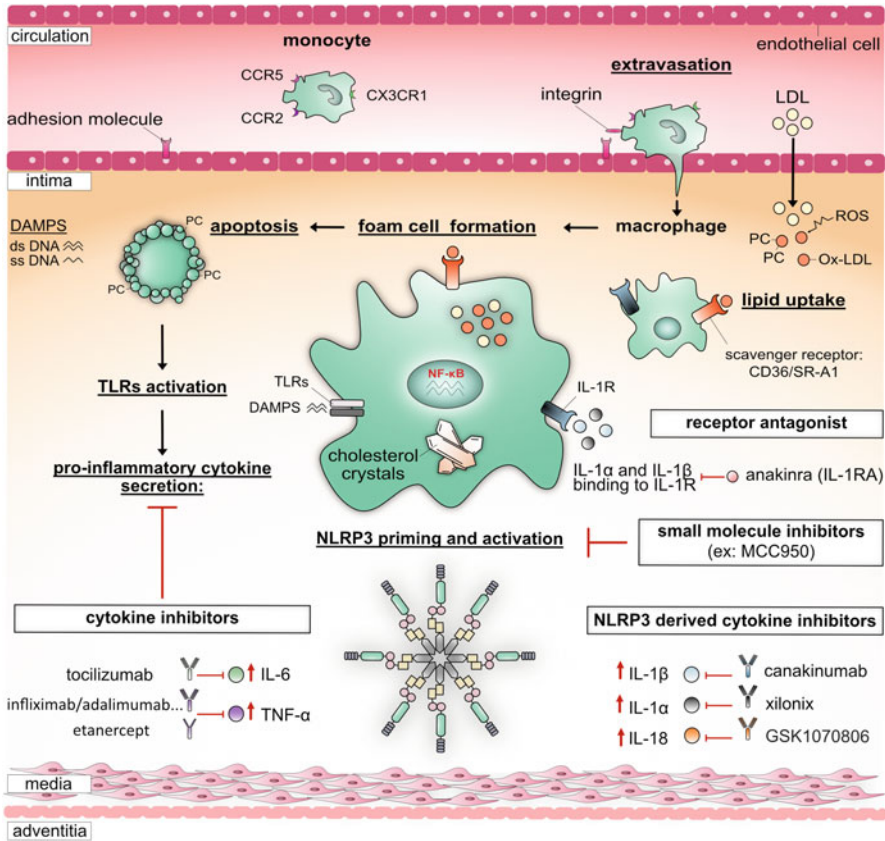
To optimally target the inflammatory component of atherosclerosis, one needs to understand the chronic inflammatory nature of this specific process. Chronic inflammation is typically the consequence of a non-resolving inflammation due to an impaired clearance and persistent presence of the inciting trigger combined with a hampered removal of freshly recruited inflammatory cells (Tabas and Glass 2013). In atherosclerosis the main triggers of inflammation are sterile DAMPs that result from the pathological accumulation of lipids in the vascular wall. Therefore, optimal strategies targeting chronic inflammation are either directed toward limiting the generation and activity of disease-specific DAMPs or the inflammatory response they incite. Other strategies aim at dampening inflammation or enhancing the resolution processes. Clearly, the more targeted anti-inflammatory treatments are to a specific pathophysiology, the lower the risk of adverse effects such as a reduction of the host's capacity to fight infections. Thus, careful assessment of the risks and benefits of each anti-inflammatory strategy is needed during the development of these treatments. Modulating both the innate and adaptive responses offers promising possibilities to limit chronic inflammation in the vascular wall.

## 2 Targeting Innate Immune Responses

### 2.1 Targeting the NLRP3 Inflammasome

Accumulating evidence shows that the nucleotide-binding oligomerization domain, leucine-rich repeat-containing receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome plays a central role in plaque formation, growth, and stability. Its activation is typically necessary for the secretion of proinflammatory cytokines, such as IL-1 $\beta$  and IL-18, which in turn activate the innate immune system. Ligation of both pattern recognition receptors (PRRs) and tumor necrosis factor receptor (TNFR) can activate the NF- $\kappa$ B pathway, which promotes the transcription of proinflammatory cytokines like tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin IL-6 while also initiating the priming of the NLRP3 inflammasome. The latter involves the production of cytosolic NLRP3 protein as well as the inactive form of IL-1 family cytokines: pro-IL-1 $\beta$  and pro-IL-18 (Kelley et al. 2019). Although several PRRs can trigger the formation of inflammasomes, such as NACHT, LRR, and PYD domains-containing protein 1 (NLRP1), NLR family CARD domain-containing protein 4 (NLRC4), and absent in melanoma 2 (AIM2), the NLRP3 inflammasome displays unique activation properties. A large variety of pathogen-associated molecular patterns (PAMPs) (microbial, viral, fungal molecules) and DAMPs (urate crystals) can activate NLRP3. In the context of atherosclerosis, cholesterol crystals (Duewell et al. 2010) have been suggested as key DAMPs driving NLRP3 activation. Importantly, NLRP3 also senses perturbations of cellular homeostasis such as lysosome rupture, ion channel disturbances (K<sup>+</sup> efflux), and mitochondrial stress resulting from various stimuli (Kelley et al. 2019). In its activated form, NLRP3 can recruit the apoptosis-associated speck-like protein which contains a caspase recruitment domain (ASC) and the inactivated form of caspase-1 (pro-caspase-1). The proximity of pro-caspase 1 proteins within the complex leads to caspase-1 activation, which proceeds to the proteolytic activation of pro-IL-1 $\beta$ , pro-IL-18, and the proapoptotic factor gasdermin D (GSDMD) involved in pyroptosis, a lytic cell death necessary for cytokine release.

With respect to atherogenesis, important insights came from studies by Duewell and colleagues, who showed that lipid accumulation in the vessel wall leads to the formation of cholesterol crystals which are taken up by macrophages where they activate the NLRP3 inflammasome (Rajamaki et al. 2010; Duewell et al. 2010). In parallel, many studies have investigated the role of NLRP3-derived cytokines (i.e., the IL-1 cytokine family: mainly IL-1 $\beta$ , IL-1 $\alpha$ , IL-18, and IL-1R antagonist (IL-1Ra)), as well as proteins involved in NLRP3 activation (ASC, caspase-1) in murine atherosclerosis using transgenic mice. Targeting IL-1 signaling using recombinant IL-1Ra, which competes with IL-1 $\alpha$  and IL-1 $\beta$  for binding of IL-1 receptors, in Apoe<sup>-/-</sup> mice prone to atherosclerosis, resulted in reduced fatty streak formation. This indicates an important role of IL-1Ra in the suppression of lesion development during the early phase of the disease (Elhage et al. 1998; Isoda et al. 2004; Merhi-Soussi et al. 2005). Similarly, overexpression of IL-1Ra in Ldlr<sup>-/-</sup> mice, fed a high-cholesterol/high-fat diet (containing cholate) for 10 weeks led to a reduction of



**Fig. 1** Therapies targeting innate immune responses in atherosclerosis. Proinflammatory cytokines such as IL-6, TNF, IL-1, and IL-18 actively modulate atherosclerosis development. Therefore, several strategies aim at reducing their levels in circulation. The NLRP3 inflammasome greatly contributes to the secretion of such cytokines and several therapeutic approaches have been developed to prevent its deleterious effects. Drugs targeting NLRP3 are of three types: monoclonal antibodies targeting NLRP3 products (i.e., IL1- $\alpha$ -Xilonix, IL1- $\beta$ -Canakinumab, IL-18-GSK1070806), recombinant proteins (i.e., IL-1-receptor antagonist-Anakinra), and NLRP3 direct inhibitors (i.e., MCC950). While some of these therapies were assessed in large clinical trials (i.e., Canakinumab), others remained inconclusive due to the low number of patients involved in the study (i.e., Xilonix), or were not investigated in humans yet. The potential benefit of such therapies still requires to be evaluated in robust clinical trials

atherosclerotic lesion area (Devlin et al. 2002). Taken together, these studies suggest that targeting the NLRP3 inflammasome using small molecule inhibitors or targeting NLRP3 products represents promising therapeutic targets for cardiovascular diseases (Fig. 1).

Regarding NLRP3 products, the two IL-1 isoforms (IL-1 $\alpha$  and IL-1 $\beta$ ) share molecular similarities but are produced by different cellular sources and seem to display different release mechanisms. The release of IL-1 $\alpha$  can be inflammasome-

dependent or independent, according to the nature of the activating stimulus (Groß et al. 2012). In contrast to IL-1 $\beta$ , the precursor form of IL-1 $\alpha$  is active and acts as an alarmin. IL-1 $\alpha$  is constitutively expressed as a membrane-bound protein on the surface of various cell types including non-immune cells like epithelial cells. When cells expressing IL-1 $\alpha$  undergo cell death, IL-1 $\alpha$  is released and acts as an alarmin, inducing IL-1 $\beta$  production and contributing to local inflammation. Additionally, IL-1 $\alpha$  can be produced by macrophages, in an inflammasome-independent manner (Freigang et al. 2013). IL-1 $\beta$  on the other hand is mainly produced by hematopoietic cells like dendritic cells, monocytes, and macrophages with its activation requiring (in the majority of cases) the NLRP3 inflammasome. Importantly, IL-1 $\beta$  has been detected in human atherosclerotic plaques and its circulating levels correlate with the severity of coronary atherosclerosis (Galea et al. 1996; Dewberry et al. 2000). Despite these discrepancies, the two IL-1 isoforms share similar downstream effector functions as they both signal via the IL-1 receptor family (IL-1R). Mechanistically, IL-1 signaling impacts various cell types within the atherosclerotic lesion, affecting plaque size and stability. It also contributes to the activation status of endothelial cells, which express adhesion molecules (VCAM-1 and ICAM-1) for the recruitment of leukocytes (Wang et al. 1995). Furthermore, IL-1 $\beta$  induces MCP-1 secretion, which promotes monocyte recruitment and is strongly associated with cardiovascular risk (Jun et al. 2009).

The importance of IL-1 $\alpha$  has only recently been appreciated. A small study carried out in coronary artery disease (CAD) patients and healthy control subjects showed higher expression of IL-1 $\alpha$  in peripheral blood mononuclear cells (PBMC) of CAD patients (Wæhre et al. 2004). Additionally, IL-1 $\alpha$  has been described as the key driver of the senescence-associated secretory phenotype (SASP) of vascular SMCs that can promote vascular inflammation (Clarke et al. 2010; Gardner et al. 2015). Another study carried out by S. Freigang and colleagues also suggests that IL-1 $\alpha$  greatly contributes to vascular inflammation in atherosclerosis. Reconstitution of cholesterol fed Ldlr<sup>-/-</sup> mice with bone marrow from IL-1b<sup>-/-</sup>, IL-1a<sup>-/-</sup> and wild-type mice resulted in dramatically reduced atherosclerosis in IL-1 $\alpha$ -deficient chimeras but not IL-1 $\beta$ -deficient or wild-type chimeras (Freigang et al. 2013). Similarly, D. Gomez et al showed that antibody-mediated inhibition of IL-1 $\beta$  in Apoe<sup>-/-</sup> mice with established atherosclerosis failed to decrease lesion size but resulted in adverse plaque remodeling (Gomez et al. 2018). In contrast, other studies suggest beneficial effects of IL-1 $\beta$  targeting in murine atherosclerosis (Kirii et al. 2003; Bhaskar et al. 2011).

Thus, IL-1 $\alpha$  and IL-1 $\beta$  may affect plaque formation at different time points in lesion development (Kirii et al. 2003; Duewell et al. 2010; Bhaskar et al. 2011; Gomez et al. 2018; Vromman et al. 2019). While IL-1 $\alpha$  appears to be important for the early phase of atherosclerosis, IL-1 $\beta$  seems to be more involved in the progression of established plaques (Vromman et al. 2019). Additionally, Vromman et al. showed that the anatomical location of plaques matters in the response to therapies targeting IL-1 signaling. For example, neutralization of IL-1 $\alpha$  or both isoforms using either an anti-IL1 $\alpha$  and/or an anti-IL-1 $\beta$  monoclonal antibody reduces the lesion area in aortic roots but not in the brachiocephalic arteries in Apoe<sup>-/-</sup> mice. Moreover,

compensatory outward remodeling was more impaired in the brachiocephalic artery compared to the aortic roots during early atherosclerosis.

In summary, the NLRP3 inflammasome and inflammasome-derived cytokines represent attractive points for therapeutic intervention in cardiovascular diseases. Careful design of preclinical studies will allow better dissection of the mechanistic and physiological aspects of IL-1 inhibition and improvement of the development of new therapies.

### 2.1.1 Targeting the IL-1 Signaling Pathway

#### Anti-IL-1 $\beta$ (Canakinumab)

The recent CANTOS (Canakinumab. Anti-Inflammatory Thrombosis Outcome Study) trial has made a profound impact on the field of atherosclerotic cardiovascular disease. It showed for the first time in a large phase 3 clinical trial that therapeutic targeting of inflammation provides a clinical benefit to patients with stable atherosclerosis. The goal of the study was to evaluate the effect of canakinumab, a monoclonal antibody targeting IL-1 $\beta$ , on adverse cardiac events in patients with a history of MI and elevated hsCRP (hsCRP  $\geq 2$  mg/L). 10,061 patients, most of whom were on statin therapy, received either placebo or canakinumab at three different doses: 50 mg, 150 mg, or 300 mg, every 3 months. The best results were obtained with the intermediate dose (150 mg) which led to a 15% reduction in the primary endpoint of nonfatal MI, nonfatal stroke, or cardiovascular death. However, as IL-1 $\beta$  is also a crucial cytokine in the host immune response against bacterial infection, its inhibition also significantly increased the number of fatal infections (Ridker et al. 2017). Despite these non-negligible side effects, this study clearly demonstrates a beneficial effect of inhibiting IL-1 $\beta$  signaling.

To better predict positive outcomes of canakinumab therapy in patients, an additional analysis of the CANTOS trial was conducted (Ridker et al. 2018b). Within the canakinumab treated group, patients with hsCRP levels  $< 2$  mg/L following treatment had a 25% reduction in major adverse cardiovascular events while no significant benefits were observed in patients with levels  $\geq 2$  mg/L. Importantly, cardiovascular mortality and all-cause mortality were both reduced by 31% in patients with hsCRP levels  $< 2$  mg/L. This was not the case in patients with levels  $\geq 2$  mg/L where no benefits were shown. This analysis clearly documented the need to assess the successful impact on inflammation to optimally appreciate the protective effect of this intervention.

Another analysis published by the CANTOS group indicates that targeting IL-1 $\beta$  alone may not sufficiently alleviate CVD risk, as both IL-6 and IL-18 levels remain predictors of future cardiovascular events in patients receiving anti-IL-1 $\beta$  treatment. Briefly, in patients treated with canakinumab, the risk of major adverse cardiac events (MACE) increased by 15% and 42% for each tertile increase in IL-18 and IL-6 levels, respectively. This suggests a residual inflammatory component that needs to be considered for further cardiovascular risk reduction. Drugs targeting the IL-6 cytokine are currently tested in clinical trials and might benefit patients with



sustained elevated IL-6 levels by preventing recurrent CVD events (Biasucci et al. 2020; Ridker et al. 2020).

### **IL-1 Receptor Antagonist (Anakinra)**

Anakinra is an antagonist of the IL-1 receptor, blocking the action of both IL-1 $\alpha$  and IL-1 $\beta$  isoforms. Since 2001, it has been used as a treatment in patients with rheumatoid arthritis (RA), who are also known to be at high risk of cardiovascular disease (Nurmohamed 2009; Avina-Zubieta et al. 2012; Primdahl et al. 2013). Anakinra was assessed in small clinical trials in patients with myocardial infarction: The “Virginia Commonwealth University-Anakinra Remodeling Trial 1-2-3” (VCU ART1-2-3) and the “Markers of inflammation in non-ST elevation acute coronary syndromes” (MRC ILA-HEART) study (Abbate et al. 2010, 2013, 2020; Morton et al. 2015). In VCU ART, this type of interleukin-1 blockade significantly reduced the systemic inflammatory response with a strong reduction of hsCRP levels in patients with ST-Elevation Myocardial Infarction (STEMI) as well as decreasing the incidence of heart failure. In the MRC ILA-Heart trial, a reduction of IL-6 and hsCRP levels was also observed in non-STEMI (NSTEMI) patients who received Anakinra daily for 2 weeks. However, by day 30, the hsCRP levels in patients treated with Anakinra were significantly higher than in the placebo group, and an unexpected increase of late recurrent ischemic events was observed. Interestingly, a recent study carried out by the Interleukin-1 Genetics Consortium supports the hypothesis that long-term IL-1 inhibition may be associated with an increase in cardiovascular events. In this study, individuals who carried four IL-1Ra raising alleles were found to have an increased odds ratio for coronary heart disease (1.15) compared to those carrying no IL-1Ra raising alleles. Additionally, the concentration of proatherogenic lipids, including LDL cholesterol, increased with each extra allele present (Freitag et al. 2015). However, the VCU-ART study showed no effect on recurrent ischemic events, while there was an increase of MACE upon Anakinra treatment after 1 year in the MRC ILA-Heart trial.

In summary, the relationship between IL-1 targeting and clinical outcomes seems more complex than originally thought. Currently attempted therapeutic strategies include targeting IL-1 $\beta$  (Canakinumab) or both IL-1 $\alpha$  and IL-1 $\beta$  with the human recombinant IL-1RA (Anakinra). Further understanding of the distinct roles of the different IL-1 isoforms would enhance our understanding of clinical trial data (CANTOS, VCU ART-1/2/3, MRC ILA-HEART) and improve future trial design.

### **2.1.2 NLRP3 Inhibitors**

Despite the importance of the NLRP3 inflammasome in many inflammatory diseases, there is currently no approved inflammasome inhibitor for the treatment or prevention of atherosclerosis. Nevertheless, many small molecule compounds targeting the NLRP3 inflammasome have been developed, with promising results. Importantly, both canonical and non-canonical NLRP3 activation pathways can be targeted.



## MCC950

MCC950 is a small molecule inhibitor of the NLRP3 pathway, with a mechanism which is yet to be fully understood (Coll et al. 2015). Nevertheless, it was found to reduce atherosclerotic lesion formation in Apoe<sup>-/-</sup> mice. This was associated with decreased expression of the adhesion molecules ICAM-1 and VCAM-1, as well as reduced macrophage infiltration within plaques and no change in necrotic core size (Van Der Heijden et al. 2017). This early data suggests that MCC950 represents an interesting candidate to target NLRP3 induced inflammation in atherosclerosis.

## Tranilast

Tranilast, which was originally used to treat allergy, was shown to inhibit NLRP3 by facilitating its ubiquitination and impairing ASC oligomerization (Huang et al. 2018; Chen et al. 2020). The effect of Tranilast on atherosclerosis was assessed in Watanabe heritable hyperlipidemic rabbits and resulted in decreased atherosclerosis upon treatment (Matsumura et al. 1999). Recently, another study carried out in mice showed that Tranilast decreased the initiation and progression of atherosclerosis in both Ldlr<sup>-/-</sup> and Apoe<sup>-/-</sup> prone atherosclerotic mice (Chen et al. 2020). Thus, pharmacologic manipulation of NLRP3 ubiquitination offers a new strategy for protection against atherosclerosis.

Despite encouraging results from animal models, many questions remain to be answered concerning the safety of NLRP3 inhibitors. It will be critical to further examine how these compounds affect the activity of other inflammasomes and how the host immune response is affected in the long run. The initiation of new clinical trials evaluating the benefit of NLRP3 inhibitors in the context of cardiovascular diseases will also depend on these factors.

## 2.2 Targeting TNF and Interleukins

### 2.2.1 Tumor Necrosis Factor (TNF)

Tumor necrosis factor (TNF) is a proinflammatory cytokine with a broad spectrum of functions and targets. It controls inflammatory responses by regulating leukocyte activation, maturation, and cytokine/chemokine release. TNF secretion can be triggered by the recognition of PAMPs and DAMPs, but also by cytokines and interferon such as IL-1, GM-CSF, TGF- $\beta$ , TNF- $\alpha$  itself (autocrine loop), and IFN- $\gamma$  (Zelová and Hošek 2013). It is mainly produced by macrophages and T cells, but many other cell types, including non-immune cells such as endothelial cells and smooth muscle cells (SMCs), can also secrete TNF. Importantly, TNF seems to be highly expressed in human atherosclerotic plaques (Rus et al. 1991; Canault et al. 2006). Ohta et al showed that TNF enhances the expression of ICAM-1, VCAM-1, and MCP-1 in endothelial cells, favoring monocyte recruitment into the vascular wall. Additionally, an increase of scavenger receptor A dependent uptake of oxidized LDL by macrophages has been described, as well as decreased IL-1 $\beta$  and INF- $\gamma$  mRNA expression, which together strongly support a deleterious role of TNF in murine atherosclerosis (Ohta et al. 2005; Xiao et al. 2009). Interestingly, recent

studies suggest that TNF might also be involved in the regulation of the NLRP3 inflammasome (Sode et al. 2014; Bauernfeind et al. 2016; McGeough et al. 2017).

TNF is a central mediator of the inflammatory response and its signaling mechanism is well described. Many biological agents interfering with TNF signaling have been developed and tested in clinical trials in the context of inflammatory diseases associated with high cardiovascular risk, such as rheumatoid arthritis (RA), psoriatic arthritis, and Crohn's disease. For example, treatment with TNF blockers (infliximab or etanercept) in RA is associated with a lower incidence of cardiovascular events (Jacobsson et al. 2005). Two meta-analyses also confirmed that RA patients treated with anti-TNF therapy have a lower risk of cardiovascular events in comparison with patients treated with disease-modifying antirheumatic drugs (DMARDs) (Barnabe et al. 2011; Roubille et al. 2015). Currently, five different anti-TNF drugs are approved for clinical use, which include soluble TNF-receptor (etanercept) and TNF monoclonal antibodies (adalimumab, infliximab, golimumab, and certolizumab pegol). The evidence for their use in atherosclerotic cardiovascular disease will be discussed below.

### **Adalimumab**

While adalimumab has been used as a safe therapy in a myriad of inflammatory diseases (Burmester et al. 2020), no large-scale trial regarding its effect on atherosclerotic cardiovascular disease exists. A small study in RA patients showed that treatment with adalimumab improves endothelial-dependent vasodilatation (Gonzalez-Juanatey et al. 2006), findings which were confirmed in a second study by the same authors. In this latter study, a comparison of baseline and post-treatment (12 months) carotid artery wall thickness (intima-media) measurement in RA patients showed no statistical difference (Gonzalez-Juanatey et al. 2012). Nevertheless, these observations suggest that adalimumab may improve the state of subclinical atherosclerosis in RA patients. Improvement of endothelial cell function was also observed in a study involving 14 psoriatic patients (Avgerinou et al. 2011). After 12 weeks of adalimumab treatment, no significant change on hsCRP level compared to baseline was observed, but ICAM-1 expression was decreased and the flow-mediated dilation (FMD), used as a marker of endothelial dysfunction, was improved. A larger study (NCT01722214) included 107 psoriatic patients randomized to receive either adalimumab for 52 weeks or placebo for 16 weeks followed by adalimumab for 36 weeks (per-protocol setting). At 16 weeks, the adalimumab treatment group had no difference in vascular inflammation compared to the placebo control group. At the 52-week time point, there was no difference observed in the ascending aorta with adalimumab, but a modest increase in vascular inflammation in carotids was described (Bissonnette et al. 2017). Thus, in lieu of a large clinical trial, the effect of adalimumab in ASCVD remains unclear.

### **Golimumab**

Golimumab is a fully-humanized monoclonal anti-TNF antibody approved for the treatment of RA, ankylosing spondylitis (AS), psoriatic arthritis, and ulcerative colitis. Golimumab has a greater affinity for the soluble (sTNF) vs. the

transmembrane form (mTNF). While no data exist regarding the therapeutic benefit of golimumab in atherosclerosis, a pilot study in patients with ASCVD provided encouraging results (Tam et al. 2014). This randomized, double-blind, placebo-controlled trial aimed to assess the efficacy of Golimumab in preventing atherosclerosis progression and arterial stiffness in AS patients. A total of 20 patients received 50 mg of Golimumab monthly, with 21 receiving placebo treatment, for 1 year. After 6 months, no significant change in vascular parameters (i.e. aortic stiffness, carotid intima/media thickness) was observed between the two groups. However, a significant progression of the mean intima-media thickness (IMT) was only seen in the placebo group and not in the golimumab group. Maximum IMT, pulse wave velocity (PWV), and augmentation index (Aix) remained unchanged (paired t-test analysis). Nevertheless, no significant difference concerning vascular parameters was demonstrated between the two groups after 1 year of treatment. Further large-scale studies are needed to fully validate the potential effects seen in this study.

In addition to these findings in AS patients, TNF inhibitors were found to improve the overall pathologic profile of RA and psoriasis patients at high cardiovascular risk. Therapeutic targeting of TNF has shown benefit in the prevention of atherosclerosis in RA (Del Porto et al. 2007), which might also be the case in psoriasis (Sattar et al. 2007). Interestingly, a recent meta-analysis that includes 7,697 coronary artery disease (CAD) patients and 9,655 control patients has investigated the association of *TNF* gene polymorphisms and CAD susceptibility (Huang et al. 2020). The authors concluded that there was no association of *TNF* 308G/A, 857C/T, 863C/A, and 1031 T polymorphisms with CAD susceptibility. However, the *TNF* 238G/A genotype showed a significant association with higher CAD susceptibility in the subgroup of Europeans and North Asians, which suggests a direct role of TNF in CAD. Additionally, several studies described an adverse effect of TNF inhibitors on lipid profiles and described an increase of total cholesterol and triglycerides levels (Curtis et al. 2012; Hassan et al. 2016). In summary, further large-scale studies are required to evaluate the potential benefits of anti-TNF agents.

### 2.2.2 IL-6

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in different arms of the immune system. It can result in both pro- and anti-inflammatory outcomes which can be explained by the different signaling pathways engaged: the classical pathway, the trans-signaling pathway, and the blockade pathway. The classical pathway occurs in a few cell types such as T cells, hepatocytes, and monocytes. It involves the IL-6 receptor (IL-6R) and glycoprotein 130 (gp130), a signal transducer sub-unit of the IL-6 receptor. In the trans-signaling pathway, the mechanism involves the signal transducer gp130 as well as soluble IL-6 receptor (sIL-6R). In contrast to the classical pathway, trans-signaling can occur in any cell type expressing a membrane-bound gp130 protein. In this scenario, sIL-6 binds to sIL-6R and forms a complex that can activate signaling through gp130 resulting in a greater magnitude of proinflammatory state. IL-6 signaling can also be blocked via the soluble form of gp130 which acts as a natural inhibitor of trans-signaling by binding sIL-6/IL-6R complex (Jostock et al. 2001).

Despite inconclusive data from experimental models (Elhage et al. 2001; Song and Schindler 2004; Schieffer et al. 2004; Schuett et al. 2012), shreds of evidence from translational studies identify IL-6 as a key regulator of atherogenesis. Expression of IL-6 was detected in human atherosclerotic plaques (Seino et al. 1994; Rus et al. 1996; Schieffer et al. 2000). Several studies found that high levels of IL-6 are associated with increased cardiovascular risk in human (Mendall et al. 1997; Ridker et al. 2000b; Fisman et al. 2006; Zakai et al. 2007; Danesh et al. 2008; Lefkou et al. 2010). Among them, a study from J. Danesh and colleagues, who compared serial measurements of serum IL-6 between patients with a history of coronary heart disease and controls. Results showed that long-term exposure to circulating IL-6 was associated with an increased risk of coronary artery disease (Danesh et al. 2008). However, follow-up studies of the CANTOS trial showed that patients with elevated IL-6 levels after canakinumab treatment have no significant benefit as opposed to patients with lower IL-6 levels (Ridker et al. 2018a, 2020).

Collectively, these studies and the studies based on the CANTOS trial suggest an important role of IL-6 in cardiovascular diseases. Targeting the IL-6 axis may be crucial to reduce the residual inflammation responsible for the increased risk of cardiovascular events.

Several clinical trials tested the therapeutic benefit of targeting the IL-6 axis using Tocilizumab (Table 1). However, higher-powered studies are required to better evaluate the safety and efficiency of current IL-6 targeting agents, and appreciate their benefit in preventing atherosclerosis.

### **2.3 Targeting Chronic Inflammation Using Broad Anti-inflammatory Drugs**

Given the importance of inflammation in atherosclerosis, the usage of broad anti-inflammatory drugs could potentially reduce the risk of atherosclerotic events. Well known for their anti-inflammatory properties, methotrexate and colchicine are widely used as main treatments in inflammatory diseases with a high risk of CVD, notably RA, psoriasis, and other rheumatologic disorders (Saag et al. 2008; Singh et al. 2012). The repurposing of these two drugs as a therapeutic approach against atherosclerosis was evaluated in several clinical trials discussed below.

#### **2.3.1 Methotrexate**

Treatment with low-dose methotrexate (LD-MTX) reduces the levels of circulating CRP, IL-6, TNF-alpha and cardiovascular events in RA patients (Choi et al. 2002; Wessels et al. 2008; Westlake et al. 2010; Roubille et al. 2015). An important feature of LD-MTX is its ability to increase adenosine production and stimulate the adenosine A<sub>2A</sub> receptor, which has been shown to promote the expression of several proteins involved in reverse cholesterol transport, thus potentially reducing foam cell formation (Reiss et al. 2008). The potential benefit of LD-MTX in the treatment of atherosclerosis was recently assessed in the cardiovascular inflammation reduction trial (CIRT) which is discussed below.

**Table 1** Tocilizumab therapy and consequences on CVD risk factors

Drug	Target	Approval	Cohort	Trial design	Effect	References
Tocilizumab	IL-6R	RA patients	RA patients	Non-randomized pilot study	<ul style="list-style-type: none"> <li>Improved endothelial function and aortic stiffness</li> <li>↑ CRP level</li> <li>Modification of total cholesterol levels</li> </ul>	Protergerou et al. (2011)
			RA patients	Non-randomized pilot study	<ul style="list-style-type: none"> <li>Side effect on lipid profile: consequences on cardiovascular risk were not assessed</li> </ul>	Strang et al. (2013)
			RA patients	Randomized, double-blind placebo-controlled	<ul style="list-style-type: none"> <li>Side effect on lipid profile: consequences on cardiovascular risk were not assessed</li> </ul>	McInnes et al. (2015)
			Non-ST-elevation myocardial infarction (NSTEMI) patients	Randomized, double-blind placebo-controlled	<ul style="list-style-type: none"> <li>Inflammation and primary percutaneous coronary intervention related to high-sensitivity troponin T release</li> <li>↑ CRP level</li> </ul>	Kleveland et al. (2016)

### Cardiovascular Inflammation Reduction Trial (CIRT)

The CIRT trial was performed in parallel with the CANTOS trial and aimed to determine if LD-MTX could reduce cardiovascular events in patients with stable atherosclerosis and high cardiovascular risk (Everett et al. 2013). The double-blind trial included 4,786 patients with a history of myocardial infarction or multivessel coronary disease, as well as either type 2 diabetes or metabolic syndrome. Patients were randomized and treated with either LD-MTX (2,391) or placebo (2,395). The final primary endpoint was a composite of nonfatal myocardial infarction, nonfatal stroke, cardiovascular death, and hospitalization for unstable angina that led to urgent revascularization. After a median follow-up of 2.3 years, the primary endpoint occurred in 201 patients in the methotrexate group and 207 patients in the placebo group (HR 0.96, 95% CI 0.79–1.16). Also, treatment with LD-MTX did not reduce plasma levels of CRP, IL-1 $\beta$ , IL-6, TNF- $\alpha$ . No significant difference between the two groups for any component of the primary endpoint was found.

Considering the success of the CANTOS trial and the beneficial effect seen with LD-MTX in RA patients, the CIRT trial results are disappointing. While CANTOS targeted a specific and well-defined pathway, the CIRT trial used a drug with a large spectrum of anti-inflammatory effects with molecular consequences which are not yet understood. Besides the different mechanisms of action, an additional explanation for the discrepant results between CIRT and CANTOS may be differences in the study design of the two studies. Patients recruited to the CIRT trial had a lower residual inflammatory profile (median baseline CRP: 1.6 mg/L) compared to patients from the CANTOS trial, where patient recruitment was based on hsCRP levels  $\geq 2$  mg/L as a marker of high residual inflammation (median baseline CRP: 4.2 mg/L). In support of this explanation, treatment of RA patients with LD-MTX led to a reduction of hsCRP (Westlake et al. 2010) and decreased cardiovascular risk.

These data demonstrate that the design of future clinical trials needs to consider inclusion criteria that target individuals with a high inflammatory profile.

#### 2.3.2 Low-Dose Colchicine

Colchicine is an inexpensive anti-inflammatory drug used in patients with gout, familial Mediterranean fever, and pericarditis. By preventing microtubule assembly, colchicine can disrupt inflammasome activation, microtubule-based inflammatory cell chemotaxis, phagocytosis, and other host immune mechanisms. The first benefit of colchicine in ischemic heart disease (IHD) has been observed in familial Mediterranean fever patients (Langevitz et al. 2001). Patients diagnosed with FMF show a sustained inflammatory response which puts them at a higher risk of suffering a heart attack in comparison with the general population. Langevitz and colleagues showed that treatment with colchicine lowered the risk of IHD in FMF patients compared to untreated patients with other inflammatory conditions and similar rates of risk factors. Also, patients taking colchicine achieved a frequency of IHD comparable to the general population. To assess the potential benefit of colchicine in atherosclerosis, several clinical trials such as the low-dose colchicine trial (LoDoCO), or more recently the colchicine cardiovascular outcomes trial (COLCOT), have been carried out and will be discussed below.

### LoDoCo-MI and LoDoCo-1/2 Trials

In 2007, a small pilot study had demonstrated the potential of low-dose colchicine to reduce hsCRP levels, independently of lowering cholesterol (Atorvastatin), in patients with stable coronary artery disease (Nidorf and Thompson 2007). However, in the randomized and placebo-controlled LoDoCo (Low-Dose Colchicine)-MI study (237 patients), colchicine at 0.5 mg/day for 30 days failed to reduce hsCRP levels ( $\leq 2$  mg/L) in patients with recent MI (Hennessy et al. 2019).

Subsequently, the LoDoCo (Low-Dose Colchicine) trial was designed as a prospective study to assess the efficiency and safety of long-term low-dose colchicine usage in patients with stable coronary disease. 532 patients on lipid-lowering medication and antithrombotic therapy were randomized and treated with a daily dose of colchicine (0.5 mg/mL) or no colchicine. After a median follow-up of 3 years, the primary outcome (acute coronary syndrome, out-of-hospital cardiac arrest, or non-cardioembolic ischemic stroke) had occurred in 5.3% of patients in the low-dose colchicine group, as opposed to 16% in the non-treated group (Nidorf et al. 2013). To validate the observations from the LoDoCo study, a large-scale study (LoDoCo 2) involving 5,522 patients was conducted. LoDoCo 2 confirmed the significant treatment benefit of low-dose colchicine in reducing CVD risk in patients with stable coronary disease. The primary endpoint was a composite of cardiovascular death, spontaneous (non-procedural) myocardial infarction, ischemic stroke, or ischemia-driven coronary revascularization. The secondary endpoint was a composite of cardiovascular death, spontaneous MI, or ischemic stroke. Primary endpoints occurred in 6.8% of patients in the low-dose colchicine group as opposed to 9.6% in the placebo-treated group, and secondary endpoints occurred in 4.2% in the colchicine group and 5.7% in the placebo group. However, an increase in death from non-cardiovascular diseases was observed in the colchicine treated group, which further justifies the need for more targeted therapies that limit undesirable side effects (Nidorf et al. 2019, 2020; Pradhan 2021).

### COLCOT Trial

Another recent double-blind randomized placebo-controlled colchicine intervention study, named COLCOT (colchicine cardiovascular outcomes trial), aimed to assess the benefit of colchicine in patients with a recent acute MI (Tardif et al. 2019). In total, 4,745 patients were divided into two groups that were given oral colchicine at 0.5 mg/day or a placebo. The primary efficacy endpoint was a composite of death from cardiovascular causes, myocardial infarction, stroke, urgent hospitalization for angina leading to coronary revascularization, or resuscitated cardiac arrest. At a median of 23 months, the primary composite endpoint had occurred in 5.5% of the treatment group and 7.1% in the placebo group (hazard ratio, 0.77). Remarkably, the hazard ratio for stroke (HR = 0.26) and hospitalization for angina (HR = 0.50) indicate an important benefit in the colchicine treated group for these two particular primary endpoints. Overall, colchicine showed a good safety and tolerability profile despite a slight but significant increase in pneumonia occurrence (0.9%) in the colchicine group compared to the placebo group (0.4%). There were no differences in peripheral blood counts and CRP levels among the treated group compared to the



placebo. Recent follow-up analysis suggests that the main benefit of colchicine therapy occurs when treatment is initiated in the first 3 days post-MI (Bouabdallaoui et al. 2020).

Additionally, the repurposed usage of low-dose colchicine has shown multiple benefits for the treatment of stable coronary artery diseases. A recent pilot study carried out in 80 patients with recent acute coronary syndrome suggests that low-dose colchicine uptake on a regular basis can efficiently stabilize plaques on top of optimal medical therapy (Vaidya et al. 2018). Further upcoming data from large-scale trials such as CLEAR-Synergy (4,000 patients with ST-elevation myocardial infarction undergoing percutaneous coronary intervention (NCT03048825)) will help to validate its efficiency and tolerability in coronary diseases.

## 2.4 Targeting Chemokine Signaling

Considerable work has been carried out on the identification of inflammatory mediators that actively participate in the progression of atherosclerosis which has increased the number of potential new therapeutic targets. Among them, chemokines were identified as key players in leukocyte recruitment/adhesion and activation as well as promotion of local inflammation in arteries. The contribution of chemokines to plaque formation/destabilization and thrombus formation has been extensively reviewed elsewhere (Zernecke and Weber 2014).

Despite the proven relevance of specific chemokines in atherogenesis, the clinical evaluation of drugs targeting specific chemokines or chemokine pairings has not progressed much. This is mainly due to the complexity of the “working network” within which chemokines operate and their critical role in fundamental host defense.

Several chemokines and their receptors such as CCL5/CCR5 (including heterodimeric interactions), CCL2/CCR2, CX3CL1/CX3CR1 are an active part of disease progression (Combadière et al. 2008). Drugs targeting those axes were developed in the context of other diseases like human immunodeficiency virus infections and facilitate the establishment of new clinical studies involving patients with cardiovascular diseases. Currently, drugs used to disrupt chemokine–receptor interactions rely on four main strategies: the modification of the chemokine N-terminal domain, the synthesis of small molecules used as a receptor antagonist, the usage of specific antibodies targeting chemokines, and other drugs interfering with chemokines’ heterodimeric interactions. Here, we will focus on promising chemokine targeting therapies with available clinical data in the context of atherosclerosis.

### 2.4.1 Targeting the CCR5 Axis

Expression of CCL5 and CCR5 has been identified in atherosclerotic plaques more than 15 years ago (Wilcox et al. 1994; Pattison et al. 1996; Veillard et al. 2004). Atheroprotective effects resulting from the perturbation of CCR5/RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) interactions

were described in several mouse studies. The genetic inactivation of CCR5 in chow or high-fat diet-fed Apoe<sup>-/-</sup> mice was shown to be protective in advanced atherosclerosis, but not in the early phase of the disease. The atheroprotective effect was associated with a decrease of macrophage numbers in plaques and reduced levels of circulating IL-6. The plaque quality was also affected by an increased content of SMCs, promoting plaque stabilization (Kuziel et al. 2003; Braunersreuther et al. 2007; Quinones et al. 2007). Moreover, a natural variant of CCR5 (CCR5Δ32 polymorphism) which reduces the cell surface expression of CCR5, is associated with a reduced risk of coronary artery diseases and MI (Szalai et al. 2001; González et al. 2001). Thus, the CCR5 pathway appears to be an interesting potential target for antiatherosclerotic therapy.

### Maraviroc

The CCR5 inhibitor maraviroc is one of the few small molecules targeting a chemokine/receptor interaction approved by the US Food and Drug Administration and the European Medicines Agency as a preventive treatment for HIV (2007). HIV-associated inflammation increases HIV patients' risk of cardiovascular disease (Shah et al. 2018), which suggests a potential role of maraviroc in modulating the atherogenic risk of these patients. This was confirmed preclinically as the treatment of atherosclerosis-prone Apoe<sup>-/-</sup> mice with maraviroc has been found to reduce atherosclerotic lesion development (Cipriani et al. 2013).

A recent pilot study in HIV patients showed that maraviroc treatment decreases carotid intima-media thickness, reflecting potential anti-atherogenic effects (Francisci et al. 2019). Additionally, an anti-human CCR5 monoclonal antibody (HGS004-Human Genome Sciences), tested in a clinical trial in HIV patients, showed a good safety profile and could be an interesting candidate for atherosclerosis therapy (Lalezari et al. 2008). Finally, a phase 4 clinical trial evaluating the efficacy of maraviroc in modulating atherosclerosis in HIV patients has been completed (NCT03402815) and results are forthcoming. This data will provide crucial information regarding the future of CCR5 axis targeting therapy in atherosclerosis.

### 2.4.2 Targeting the CCR2 Axis

Many pieces of evidence from experimental models demonstrate the proatherogenic role of CCR2 and its ligand CCL2/MCP-1 (Monocyte chemoattractant protein 1) (França et al. 2017). Notably, CCR2 signaling can promote monocyte recruitment to atherosclerotic lesions and foam cell formation (Boring et al. 1998; Bobryshev 2006). A recent discovery by Winter et al showed that the recruitment of myeloid cells within atherosclerotic lesions is subject to circadian regulation (Winter et al. 2018). This establishes a new concept of chrono-pharmacology-based therapy for the treatment of atherosclerosis, which is particularly relevant for the usage of CCR2 antagonists given their role in the promotion of monocyte recruitment (Winter et al. 2018).

### **MNL1202**

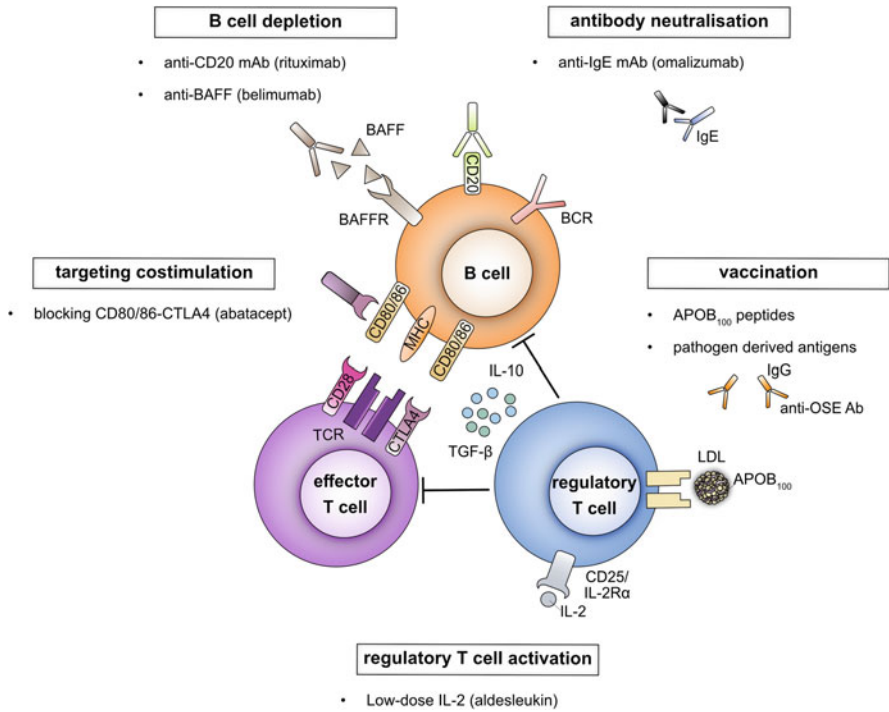
In an effort to develop a specific antagonist of CCR2, a humanized antibody MNL1202 has been generated and tested in a phase 2 clinical trial in patients at risk for CVD (with at least 2 risk factors for atherosclerotic CVD, hsCRP level >3 mg/L, and receiving or not receiving lipid-lowering drugs). The direct blocking of CCR2 with MLN1202 was well tolerated and significantly reduced hsCRP levels in the antibody-treated group compared to the placebo-treated group (Gilbert et al. 2011). Thus MLN1202 appears to be a promising candidate to target atherosclerosis-associated inflammation.

Despite encouraging preclinical results, few molecules targeting chemokines and their receptors have been approved for clinical use yet. The fundamental role of chemokine signaling during both innate and adaptive immune response suggests that its targeting likely critically impairs the host immune response. For example, treatment with the CCL5 variant Met-RANTES or deficiency in CCL5 delays viral clearance by macrophages and impairs T-cell function (Makino et al. 2002). In this regard, the specific targeting of chemokine heteromerization represents a good alternative as it poses fewer risks of immunological side effects than chemokine antagonists. Up to now, 11 heteromeric chemokine pairs have been reported (Koenen and Weber 2010), expanding the therapeutic possibilities. Importantly, preclinical studies are carried out in a pathogen-free context, which is not representative of human pathogen exposure. Thus, future studies must assess the risk of long-term exposure to such chemokine targeting therapies. The complexity of the chemokine network, the lack of data concerning the toxicity of their targeting agents, and the availability of other, safer therapeutic approaches have drastically slowed down the translation of chemokine targeted therapies to human cardiovascular disease. Future studies should include a parallel assessment of risks and benefits provided by molecules targeting chemokine interactions to better appreciate the net effect on patients with cardiovascular diseases.

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## **3 Modulation of the Adaptive Immune Response**

Adaptive immunity plays a key role in driving and modulating the pathogenesis of atherosclerosis (Sage et al. 2018; ; Wolf and Ley 2019; Libby 2021). The adaptive branch of immunity consists primarily of T and B cells, both of which have the capacity to induce immune responses that are specifically targeted to disease-associated antigens. Adaptive immunity therefore represents an attractive therapeutic target as its modulation could allow for a more specific response, potentially reducing the risks of weakened host defenses associated with broad anti-inflammatory therapies (Fig. 2).



**Fig. 2** Targeting adaptive immune responses in atherosclerosis. Regulatory T cells ( $T_{regs}$ ) play a protective role in atherosclerosis via their immunosuppressive capacities such as the secretion of IL10 and TGF $\beta$ . They can suppress the activity of proatherogenic effector T cells such as TH1 cells.  $T_{reg}$  numbers can be increased in a non-specific manner via the use of low-dose IL2 or in an antigen-specific manner by immunization with ApoB100-derived peptides. Furthermore, blocking effector T cell activation by inhibiting the interaction between co-stimulatory CD80/CD86 on antigen-presenting cells such as B cells and CD28 on T cells has been shown to reduce atherosclerosis in mice. B2 cells and many of their effector functions including some proatherogenic antibody isotypes such as IgE have been shown to promote atherogenesis. Thus, B cell depleting antibodies such as anti-CD20 or anti-BAFF antibodies have been shown to protect mice from atherosclerosis. Additionally, the depletion of proatherogenic antibodies such as IgE may have beneficial effects. On the other hand, immunization with modified LDL has been shown to induce protective antibody responses (both IgG and IgM) against oxidation-specific epitopes (OSE) that ameliorate atherosclerosis in preclinical studies

### 3.1 Strategies Targeting the Homeostatic Balance Between Regulatory and Effector T Cells

Although no therapies targeting T cells are currently in clinical use for the treatment and prevention of cardiovascular disease, some therapeutic approaches have shown promise in preclinical models and are being evaluated in clinical trials. In atherosclerosis-prone mice, regulatory T cells ( $T_{regs}$ ) have been shown to be beneficial both in the progression and regression of atherosclerosis (Ait-Oufella et al. 2006; Mor et al.

2007; Klingenberg et al. 2013; Sharma et al. 2020). In contrast, many effector T cells are thought to be proatherogenic, particularly IFN $\gamma$ -secreting T helper 1 (Th1) (Saigusa et al. 2020; Buono et al. 2005). In humans, cardiovascular disease is associated with an imbalance in T<sub>reg</sub> and effector T cells, where T<sub>regs</sub> are decreased in patients with acute coronary syndrome and low numbers are associated with increased rates of acute coronary events (Mor et al. 2006; Han et al. 2007; Cheng et al. 2008; Ammirati et al. 2010; Wigren et al. 2012).

Therefore, the aim of T cell-targeted therapeutic approaches in atherosclerosis is to change the homeostatic balance of different T cell subsets by expanding atheroprotective, immunosuppressive T<sub>regs</sub> while suppressing putatively proatherogenic effector T cell populations (Foks et al. 2015). This could be achieved on the one hand by the stimulation of non-specific T<sub>regs</sub> by targeting pathways such as interleukin-2 (IL-2). On the other hand, specific T<sub>regs</sub> can be expanded with the use of tolerogenic vaccines against atherosclerosis-relevant antigens.

### 3.1.1 Low-Dose IL-2 Therapy

Interleukin-2 is a cytokine that plays a key role in the regulation of T cell activity and survival. Although IL-2 has the ability to regulate all T cells, it appears to be particularly important in the development and survival of T<sub>regs</sub> (Boyman and Sprent 2012). The activation threshold for T<sub>regs</sub> is lower than for other effector T cells (Yu et al. 2009), which may explain the somewhat paradoxical effect of IL-2 therapy. While high-dose IL-2 therapy is thought to promote effector immunity, low-dose IL-2 stimulates immunosuppressive T<sub>regs</sub> (Klatzmann and Abbas 2015). A potential added benefit of this therapy could be the IL-2 mediated expansion of innate lymphoid cells 2 (ILC2) (Van Gool et al. 2014). ILC2 cells represent a rare subset of immune cells that likely exert atheroprotective functions via the secretion of type-2 cytokines such as IL-5 and IL-13 (Binder et al. 2004; Cardilo-Reis et al. 2012; Newland et al. 2017). Preclinical studies have shown that administration of recombinant IL-2 complexed with monoclonal antibodies against IL-2 increases the levels of T<sub>regs</sub> and reduces atherosclerotic plaque burden in mice (Foks et al. 2011; Dinh et al. 2012; Proto et al. 2018).

The IL-2 analogue aldesleukin is currently approved for the treatment of advanced renal cell carcinoma and metastatic melanoma. In cardiovascular disease, low-dose IL-2 therapy is being evaluated in clinical trials with the aim to increase T<sub>reg</sub> numbers, thereby limiting post-ischemic inflammatory responses and promoting myocardial healing in the setting of ischemic heart disease and acute coronary syndrome. The low-dose Interleukin-2 in patients with stable ischemic heart disease and acute coronary syndromes (LILACS) study is a randomized, placebo-controlled, double-blind phase I/IIa clinical trial that aims to assess the safety and tolerability of escalating doses of recombinant IL2, as well as its ability to alter T<sub>reg</sub>, effector T cell and other immune cell populations (NCT03113773; expected completion date 06/2021; Zhao et al. 2018). Preliminary results indicate the treatment is well tolerated and induces robust increases in T<sub>regs</sub> without affecting effector T cells. Interestingly, a dose-dependent reduction in B cells was also seen, which may have further beneficial effects on atherosclerosis (Zhao et al. 2020a). The subsequent

low-dose IL-2 for the Reduction of Vascular Inflammation In Acute Coronary Syndromes (IVORY) phase II trial will assess changes in vascular inflammation in patients with acute coronary syndromes (NCT04241601; expected completion date 01/2024; Zhao et al. 2020b).

One limitation of low-dose IL-2 therapy are potential off-target effects, which include mild increases in NK cells and eosinophils (Zhao et al. 2020a). Furthermore, initially protective  $T_{\text{regs}}$  may undergo a phenotypic switch toward a proinflammatory  $T_{\text{H1}}$ -like phenotype with progressing disease in mice (Li et al. 2016; Butcher et al. 2016; Wolf et al. 2020). It remains to be addressed whether this switch can occur in humans.

### 3.1.2 Alternative Means to Expand Regulatory T Cells

Alternative options to harness the immunosuppressive capacity of  $T_{\text{regs}}$  is by ex vivo expansion of  $T_{\text{regs}}$ , where autologous naïve Tregs are isolated from the blood and expanded in vivo using a cytokine cocktail or antigenic stimuli. The adoptive transfer of  $T_{\text{regs}}$  has been tested in humans in the setting of transplantation (Trzonkowski et al. 2009; Brunstein et al. 2011; Di Ianni et al. 2011; Mathew et al. 2018) and type 1 diabetes (Marek-Trzonkowska et al. 2014), but not cardiovascular disease. Another way to expand  $T_{\text{regs}}$  is using tolerogenic non-FcR binding anti-CD3 antibodies such as teplizumab, which have shown promise in clinical trials for the treatment and prevention of type 1 diabetes (Herold et al. 2013, 2019). However, the effect of anti-CD3 on atherosclerosis has so far only been addressed in preclinical murine studies alone (Steffens et al. 2006; Kita et al. 2014) or in combination with IL-2 complex (Kasahara et al. 2014), where it was associated with reduced atherosclerosis progression or increased  $T_{\text{reg}}$ -dependent plaque regression.

## 3.2 Strategies Targeting B Cells and Humoral Immunity

B cells play a well-established role in the pathogenesis of atherosclerosis, with different B cell subsets having distinct effects on the pathogenesis of atherosclerosis (Sage et al.). B cells exert their function primarily via the generation of humoral immunity in the form of highly specific antibodies, but they also have the capacity to act as antigen-presenting cells to T cells and to secrete cytokines. The most abundant B cell type, follicular B2 cells, as well as B cell-derived germinal center B cells that arise upon interaction with follicular T helper cells, are generally considered proatherogenic (Clement et al. 2015; Tay et al. 2018; Centa et al. 2019; Bagchi-Chakraborty et al. 2019). In contrast, marginal zone B2 cells may protect from atherosclerosis by suppressing T follicular helper cells (Nus et al. 2017) and potentially via the secretion of atheroprotective IgM (Grasset et al. 2015). B1 cells, which can be further subdivided into B1a and B1b cells, represent an innate-like B cell type that can secrete germline-encoded natural antibodies that are typically of the IgM class in a T cell-independent manner. Their atheroprotective function, which is dependent on their ability to secrete IgM, is well-established in mice (Lewis et al. 2009; Kyaw et al. 2012; Rosenfeld et al. 2015; Gruber et al. 2016; Tsiantoulas et al.

2017). Other minor B cell subsets include B1 cell derived innate response activator (IRA) B cells that can promote atherosclerosis via the secretion of GM-CSF and the subsequent induction of Th1-differentiation (Hilgendorf et al. 2014) and IL-10-secreting B regulatory cells (Bregs), whose role in atherosclerosis remains controversial (Strom et al. 2015; Sage et al. 2015; Douna et al. 2019).

Although most evidence on the role of B cells in atherosclerosis stems from preclinical studies, B cells also appear to be key players in human cardiovascular disease. Patients suffering from autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis have an increased risk of cardiovascular disease independently of other risk factors (Hollan et al. 2013). A genome wide association study (GWAS) and transcriptome analysis of the Framingham Heart Study cohort recently revealed genes associated with B cell activation and differentiation to be the most deregulated genes between patients with and without coronary heart disease, suggesting a key function for B cells in modulating atherosclerosis (Huan et al. 2013). B cells are present in the vascular wall of human atherosclerotic plaques (Hamze et al. 2013; Kortelainen and Porvari 2014), where they may undergo antigen-driven clonal expansion ((Burioni et al. 2009). However, it is currently not clear how findings from murine B cell subsets and their functions in cardiovascular disease translate to human B cells. For example, although B1 cells are a well-described atheroprotective subset in mice, the nature and existence of the human equivalent of murine B1 cells remain controversial (Griffin et al. 2011; Upadhye et al. 2020). McNamara and colleagues have recently found a subset of CXCR4-expressing B1 cells that correlated with the levels of IgM directed against MDA-LDL and that inversely correlated with plaque volume and stenosis (Upadhye et al. 2019). Furthermore, increased levels of CD86-expressing CD19+ B cells were found to associate with an increased risk of stroke, while CD40-expressing CD19+ B cells showed a negative association (Mantani et al. 2014).

Overall, the aim of B cell-targeted therapy is to reduce the proatherogenic effects of follicular B2 cells, ideally while preserving atheroprotective IgM-secreting B1 cells (Porsch and Binder 2019). Currently, B cell depletion therapy is primarily used in the treatment of B cell malignancies and in the treatment of autoimmune diseases. An alternative therapeutic target is humoral immunity. On the one hand, putatively proatherogenic antibody classes such as IgE may be neutralized. On the other hand, passive and active immunization strategies may be harnessed to specifically target atherosclerosis-associated antigens such as oxidation-specific epitopes.

### **3.2.1 Anti-CD20 Mediated B Cell Depletion (Rituximab)**

Given the strong evidence for a proatherogenic role of follicular B2 cells, the major B cell type in the body, some high-risk patients may benefit from their depletion. Indeed, preclinical studies suggest that anti-CD20 mediated B cell depletion could ameliorate the development of several cardiovascular pathologies including atherosclerosis (Ait-Oufella et al. 2010; Kyaw et al. 2010), myocardial infarction (Zougari et al. 2013), myocardial infarction-accelerated atherosclerosis (Kyaw et al. 2021), abdominal aortic aneurysm (Schaheen et al. 2016), and hypertension (Chan et al. 2015).



In mice, anti-CD20 treatment preferentially depletes B2 cells, while preserving atheroprotective B1 cells (Hamaguchi et al. 2005). In line with that, anti-CD20 treatment of *Ldlr*<sup>-/-</sup> and *Apoe*<sup>-/-</sup> mice potently decreases B2 cell numbers and IgG levels and induces significant reductions in atherosclerosis (Ait-Oufella et al. 2010; Kyaw et al. 2010). In the context of acute myocardial infarction, Zougari et al. could show that anti-CD20 mediated B2 cell depletion was associated with reduced inflammation and CCL7-mediated monocyte mobilization while improving cardiac function, reducing infarct size and fibrosis (Zougari et al. 2013). Furthermore, anti-CD20 mediated B2 cell depletion significantly reduced myocardial infarction-accelerated atherosclerosis (Kyaw et al. 2021).

Rituximab is a CD20-targeted monoclonal antibody that has been approved for the treatment of some B cell malignancies (NHL, CLL) and autoimmune diseases (rheumatoid arthritis, granulomatosis with polyangiitis, pemphigus vulgaris) for more than two decades. Given its long clinical use, there is increasing evidence on its effect on cardiovascular parameters. Although a recent meta-analysis could not show an effect on short-term cardiovascular adverse events in randomized placebo-controlled trials involving rituximab, long-term follow-up data is lacking (Morris-Rosenfeld et al. 2014). Similarly, a study assessing the effects of biologic and conventional disease-modifying antirheumatic drugs on incident cardiovascular disease in rheumatoid arthritis patients with a mean follow-up of 4 years could find no beneficial effects of rituximab on cardiovascular risk, although the number of patients on rituximab in this study was relatively low (Ozen et al. 2020). However, several small human studies could show reductions in carotid intima-media thickness (Kerekes et al. 2009; Benucci et al. 2013; Novikova et al. 2016), improved flow-mediated dilation (Kerekes et al. 2009; Benucci et al. 2013; Hsue et al. 2014) and decreased arterial thickness (Provan et al. 2015) in patients receiving rituximab.

The Rituximab in patients with acute ST-elevation myocardial infarction (RITA-MI) trial was a prospective, open-label, single-arm phase I/IIa trial testing a single intravenous injection of rituximab in patients with STEMI within 48 h of symptom onset with a 6-month follow-up. Preliminary data suggests the treatment is well tolerated at all doses tested and resulted in a rapid, robust reduction of circulating B cells by a mean 96.3% that persisted throughout the follow-up period in a dose-dependent manner. Interestingly, immunoglobulin levels were not affected at that point. Additionally, clinical cardiac parameters including ejection fraction and (cardiac) biomarkers such as C-reactive protein (CRP) and B-type natriuretic peptide (BNP) were improved at follow-up (NCT03072199; Zhao et al. 2021). A subsequent phase IIb trial, RITA-MI 2, will be carried out in the form of a placebo-controlled randomized trial, which will allow further assessment of whether anti-CD20-mediated B cell depletion provides clinical benefits compared to placebo treatment (Zhao et al. 2021). The ICFER-RITU2 trial is another phase II clinical trial assessing the safety of rituximab and the effect of rituximab on cardiac fibrotic remodeling in patients with chronic heart failure with reduced ejection fraction (HFrEF; NCT03332888; Sánchez-Trujillo et al. 2019). Furthermore, the risk of atherosclerotic cardiovascular disease was reduced in patients receiving rituximab following

kidney transplantation (Kim et al. 2019), further supporting the potential of anti-CD20 mediated B cell depletion in the treatment of cardiovascular disease.

### 3.2.2 Other B Cell Depleting Antibodies

In addition to rituximab, there are emerging targets on B cells such as CD19 (inebilizumab, blinatumomab) and CD22 (inotuzumab ozogamicin) that are being targeted to deplete B cells in cancer and autoimmune disease (Hofmann et al. 2018). However, these receptors are also expressed on B1 cells, and may thus interfere with atheroprotective B cell effects. Additionally, there are therapies targeting receptors expressed on antibody-secreting plasma cells such as CD38 (daratumumab) and SLAMF7 (elotuzumab), which are used in the treatment of multiple myeloma (Radocha et al. 2021). However, the effect of such B cell depleting agents on cardiovascular disease has not been assessed directly.

### 3.2.3 B Cell Depletion via Targeting of the BAFF/APRIL System

An alternative means to deplete B cells is via the targeting of the B cell activating factor (BAFF)/A Proliferation-Inducing Ligand (APRIL) system, which is made up of soluble mediators that are required for B cell survival. BAFF appears to be particularly important in the survival of B2 cells (Rauch et al. 2009). In preclinical studies, BAFFR deficiency and anti-BAFFR mAb administration have been shown to robustly and selectively deplete B2 cells, thus decreasing atherosclerotic lesion formation (Kyaw et al. 2012; Sage et al. 2012) and improving recovery from myocardial infarction (Zougari et al. 2013). However, there is also evidence that BAFF may promote Breg differentiation (Yang et al. 2010) and that BAFF overexpression might protect from atherosclerosis (Jackson et al. 2016). BAFF may also exert atheroprotective functions via inhibition of TLR9-IRF7-dependent proinflammatory signaling in macrophages. Indeed, BAFF neutralization promoted lesion formation in atherosclerosis-prone mice and increased lesional CXCL10 content, suggesting potential adverse effects of BAFF-targeted therapies (Tsiantoulas et al. 2018). Belimumab is a BAFF-targeting antibody that is approved for the treatment of SLE, which robustly depletes B cells, plasma cells, and immunoglobulins. So far, the effect of belimumab on cardiovascular disease has not been assessed in human patients.

### 3.2.4 IgE Neutralization (Omalizumab)

The primary effector function of B cells is the generation of humoral immunity in the form of antigen-specific antibodies. There is ample epidemiological and preclinical experimental evidence suggesting an important role for humoral immunity and different antibody classes in atherosclerosis (Khamis et al. 2016; Sage et al. 2017; Tay et al. 2018; Centa et al. 2019; van den Berg et al. 2019). IgE is a class-switched, typically follicular B cell-derived antibody class that is associated with allergic responses. It can trigger proinflammatory responses via interaction with FcεRI receptors on mast cells, basophils, macrophages, and vascular cells (Wu and Zarrin 2014). Plasma IgE levels correlate with coronary heart disease in humans (Kounis

and Hahalis 2016). Interestingly, patients suffering from asthma are also at increased risk for cardiovascular disease (Tattersall et al. 2015). Preclinical studies suggest a proatherogenic role for IgE in Apoe<sup>-/-</sup> and Ldlr<sup>-/-</sup> mice (Wang et al. 2011; Wezel et al. 2015; Tsiantoulas et al. 2017; Zhang et al. 2020). Antibody-mediated IgE neutralization was found to reduce atherosclerotic lesion formation in Ldlr<sup>-/-</sup> mice in the context of secreted IgM deficiency (Tsiantoulas et al. 2017).

Omalizumab is an IgE-neutralizing antibody that interferes with the interaction of IgE and its receptors, resulting in the reduction of IgE levels. It is approved for the treatment of severe asthma and chronic idiopathic urticaria. Although a clinical trial suggested potential cardiovascular side effects of omalizumab treatment, this clinical trial was biased by the dramatically increased cardiovascular disease burden at baseline in the patients receiving omalizumab (Iribarren et al. 2017a). A subsequent pooled analysis of randomized, double-blind placebo-controlled trials involving omalizumab did not show an increased cardiovascular risk (Iribarren et al. 2017b). Therefore, the effect of IgE neutralization on cardiovascular disease remains to be evaluated in a clinical trial setting.

### 3.3 Strategies Targeting the Interaction Between T Cells and Antigen-Presenting Cells

Efficient T cell activation is crucially dependent on the engagement of co-stimulatory pathways. It has been hypothesized that T cells require two signals in order to become fully activated, one being antigen-presentation via MHC-molecules, the other being co-stimulation by receptors expressed on the surface of antigen-presenting cells such as dendritic cells, macrophages, and B cells. Inhibition of such co-stimulatory pathways may therefore interfere with the activation, proliferation, and differentiation of T cells, which may have beneficial effects on atherosclerosis. In contrast, targeting co-inhibitory pathways with the use of immune checkpoint inhibitors in cancer therapy may enhance T cell activity, which could have detrimental effects on cardiovascular disease.

#### 3.3.1 Targeting Co-stimulatory Pathways

##### Abatacept

CD80 (B7-1) and CD86 (B7-2) are expressed on activated antigen-presenting cells, where they can interact with both the co-stimulatory CD28 receptor and the inhibitory CTLA4 receptor on T cells (Sharpe and Freeman 2002).

Abatacept is a recombinant biologic consisting of the Ig-domain of CTLA4 and a human IgG1 Fc region. It binds CD80/CD86 with high affinity, thus blocking the interaction of CD80/CD86 with the co-stimulatory CD28 receptor and activation of T cells. Preclinical studies suggest a primarily proatherogenic role for CD80/CD86. However, CD80/CD86 may also be required for the efficient induction of T<sub>regs</sub> (Ait-Oufella et al. 2006). However, abatacept was shown to reduce atherosclerosis in different murine models of atherosclerosis (Ma et al. 2013; Ewing et al. 2013).

Abatacept is currently clinically approved for the treatment of rheumatoid, juvenile idiopathic, and psoriatic arthritis, while the highly similar belatacept is used in patients receiving kidney transplants. Some clinical trials suggest slightly reduced cardiovascular disease risk in patients receiving abatacept compared to TNF inhibitors (Zhang et al. 2016; Kang et al. 2018; Jin et al. 2018; Hsieh et al. 2020).

### **CD40-CD40L**

Another important co-stimulatory signal is provided by the CD40-CD40L interaction, a member of the tumor necrosis factor receptor (TNFR) family. Targeting this pathway reduced atherosclerosis in mice (Lutgens et al. 2000, 2010; Schönbeck et al. 2000), but not in others (Zirlik et al. 2007). Additionally, CD40-signaling intermediates, the so-called TNF-receptor associated factors (TRAFs), have been targeted successfully by a small molecule inhibitor in mouse models (Lutgens et al. 2010; Chatzigeorgiou et al. 2014). Importantly, no immunosuppressive side effects were observed with this inhibitor (Seijkens et al. 2018) and a newly developed delivery system, linking a TRAF6 inhibitor to a HDL particle, was found to be safe in non-human primates (Lameijer et al. 2018). Based on these initial findings, further assessment of CD40-TRAF inhibition needs to be carried out to verify its role in human atherosclerosis. While CD40-CD40L targeting therapeutics have not been approved for clinical use yet, several antagonistic antibodies are currently being evaluated in clinical trials for the treatment of inflammatory conditions (Karnell et al. 2019).

### **Co-inhibitory Pathways**

Recent years have seen the emergence of cancer immunotherapy and the widespread use of immune checkpoint inhibitors. Immune checkpoint inhibitors are used clinically with the aim to enhance anti-tumor immunity by T cells. Many of these drugs target pathways that have been described to play a proatherogenic role. Given the prominent role of effector T cells and these immunomodulatory pathways in cardiovascular disease, such T cell-stimulating therapy is expected to have detrimental effects on atherosclerosis (Simons et al. 2019). Importantly, a recent study by Drobni et al. could demonstrate a threefold increased risk for cardiovascular events and more than threefold increased aortic plaque volumes in patients receiving immune checkpoint inhibitor therapy (Drobni et al. 2020). Additionally, atherosclerotic mice which underwent short-term check point inhibition therapy developed a more vulnerable, proinflammatory atherosclerotic phenotype due to an excess of effector T cells, endothelial activation, and increased CD8<sup>+</sup> T cell recruitment (Poels et al. 2020). This study highlights the importance of cardiovascular risk awareness in cancer patients receiving checkpoint inhibitor immunotherapy.

### **Vaccination Strategies**

Vaccines represent an attractive future therapeutic avenue, as they may provide a durable, antigen-specific approach, thus limiting adverse effects on the host immune response. However, vaccination approaches remain challenging, as they require the targeting of specific self-antigens that may trigger unwanted autoimmune responses.

Unlike traditional vaccines that aim at enhancing the activity of the adaptive immune system against pathogens, the idea behind immunization strategies against atherosclerosis is either to induce immunological tolerance or to neutralize atherosclerosis-specific antigens without inducing a considerable inflammatory response. Additionally, passive immunization strategies offer the opportunity to directly administer antibodies that may modulate inflammatory and metabolic pathogenic mechanisms in atherosclerosis, although their effect is transient.

### 3.3.2 Immunization Against LDL-Related Antigens

LDL and particularly its modified forms represent well-established immunogenic epitopes that have previously been shown to play a role in atherosclerosis and to be recognized by various components of the immune system. LDL can undergo oxidative modifications that result in the formation of a variety of lipid peroxidation-derived oxidation-specific epitopes (OSE), including phosphorylcholine (PC)-containing oxidized phospholipids and malondialdehyde (MDA), which are highly immunogenic and trigger inflammation in the vascular wall. Notably, OSE are targeted by specific circulating antibodies that have been shown to neutralize their proinflammatory activities. Thus, several experimental studies have addressed the effect of immunization with modified LDL or parts of its main lipoprotein component, Apolipoprotein B (ApoB).

Palinski and colleagues were able to show that immunization with malondialdehyde (MDA)-modified LDL reduced atherogenesis in LDLR-deficient rabbits (Palinski et al. 1995), which was further confirmed by several groups using modified LDL in rabbits (Ameli et al. 1996; Nilsson et al. 1997; Asgary et al. 2007) and mice (George et al. 1998; Freigang et al. 1998; Zhou et al. 2001; Binder et al. 2004; Van Puijvelde et al. 2006). Specific advanced MDA adducts have been shown to be particularly immunogenic, which may further impact the effect of vaccination on atherogenesis (Gonen et al. 2014).

Although the mechanisms responsible for the atheroprotective effects observed in these studies have not been fully elucidated, several pathways play a role, including the induction of neutralizing antibodies that interfere with the proinflammatory effects of these OSE. However, even though several preclinical studies have shown a prominent induction of humoral immunity upon immunization (George et al. 1998; Freigang et al. 1998; Zhou et al. 2001; Binder et al. 2004) and the inverse association between plaque size and antibody levels against OSEs (Zhou et al. 2001), it is currently not clear how these findings translate to the clinic.

Passive immunization via administration of antibodies against OSE has shown promise in preclinical models (Schiopu et al. 2004, 2007; Poulsen et al. 2016). The phase II GLACIER trial failed to show reduced vascular inflammation following administration of a recombinant IgG1 antibody against MDA-modified ApoB100 (Lehrer-Graiwer et al. 2015). However, the relatively short follow-up period, the use of FDG-PET instead of coronary artery assessments, and the patient selection criteria may have been a limitation of this study. Given the proposed atheroprotective role of IgM against oxidation-specific epitopes, it will be interesting to assess the effect of passive administration of IgM antibodies (or single chain and other class switch

variants thereof) on human atherosclerosis in the future. One attractive candidate is the IgM antibody E06, which binds the phosphocholine (PC) headgroup of oxidized phospholipids (oxPLs), thus blocking the proinflammatory properties of oxPL and the uptake of oxLDL by macrophages (Shaw et al. 2000; Que et al. 2018).

### 3.3.3 Tolerogenic Vaccination

Another proposed atheroprotective mechanism is tolerization, which is associated with the induction of immunosuppressive  $T_{\text{regs}}$  that may limit inflammation via IL10, TGF $\beta$  and the suppression of T effector functions. Several preclinical studies found the reduction in atherosclerotic lesion formation upon administration of modified LDL or ApoB100-derived peptides to be associated with significant increases in  $T_{\text{reg}}$  numbers (Fredrikson et al. 2008; Wigren et al. 2011; Herbin et al. 2012).

In addition to lipid-derived antigens, peptide-based vaccination strategies based on ApoB100-derived peptides, many of which are recognized by autoantibodies from sera of patients with coronary heart disease, have also shown promise in preclinical studies (Fredrikson et al. 2003). Administration of several ApoB100 peptides, including P210, P45, P74, (Fredrikson et al. 2003, 2005, 2008; Dunér et al. 2021), P2 (Chyu et al. 2005), P3 (Tse et al. 2013), P18 (Kimura et al. 2018), P265 and P295 (Gisterå et al. 2017), has been shown to reduce lesion formation in murine atherosclerosis

However, several challenges remain before the clinical implementation of inflammation-restraining tolerogenic T cell vaccine is possible. Given that the interaction of TCRs with their cognate peptide is dependent on the efficient presentation on MHC type II molecules, HLA types need to be matched. The necessity for HLA matching has resulted in tetramer-based approaches to allow the identification of antigen-specific T cell populations (Kimura et al. 2017, 2018). The considerable heterogeneity of the human HLA locus and the need to adapt peptides to individual HLA types present a major challenge in the design of T-cell based peptide vaccines. Furthermore, the efficient induction of tolerance critically depends on dose, choice of adjuvant, and the route of administration (Van Puijvelde et al. 2006, 2007; Klingenberg et al. 2010; Czerkinsky and Holmgren 2012).

### 3.3.4 Immunization Against Pathogen-Derived Antigens

Some studies suggest that vaccination against common diseases such as influenza and pneumonia can reduce cardiovascular risk (Ren et al. 2015; Barnes et al. 2015). A double-blind randomized placebo-controlled clinical trial (Influenza Vaccination After Myocardial Infarction, IAMI; NCT02831608) is currently underway to assess the effect of influenza vaccination on cardiovascular outcomes in patients with preexisting cardiovascular disease (Fröbert et al. 2017). While it cannot be excluded that inflammation associated with infections may affect the plaque, the potential beneficial effect of pneumococcal vaccination may be due to its ability to induce antibodies that react with the oxidation-specific epitope, PC (Binder et al. 2003; Caligiuri et al. 2007). Initial studies have evaluated the consequences of the molecular mimicry between oxidized LDL and the CPS of pneumococci and demonstrated that immunization of  $Ldlr^{-/-}$  mice with pneumococcal extracts induced PC-specific

IgM and reduced lesion formation (Binder et al. 2003). A similar effect has been observed when Apoe<sup>-/-</sup> mice were immunized with PC-conjugated KLH (Caligiuri et al. 2007).

These studies suggested the possibility that existing pneumococcal vaccines could be repurposed to trigger anti-atherogenic immunity in humans. However, PC is not a major antigenic component of pneumococcal vaccines due to poor immunogenicity, and several studies indicated that unlike natural infections vaccination with existing vaccines does not trigger robust anti-OxLDL antibody titers. Small-scale clinical trials of the 13-valent conjugated pneumococcal vaccine Pevnar-13 did not find an induction of antibodies against oxLDL or phosphorylcholine in a small cohort of patients with metabolic diseases (Grievink et al. 2020; Shiri-Sverdlov et al. 2021), which may be due to the fact that PC is only a minimal constituent of Pevnar-13. The AUSPICE trial to assess the effect of the pneumococcal polysaccharide vaccine Pneumovax 23 on antibody levels against oxidation-specific epitopes and several cardiovascular disease markers in 4725 patients is currently underway (ACTRN12615000536561; Ren et al. 2016).

Heat shock proteins (HSPs) represent a group of highly conserved stress-associated proteins that can act as autoantigens either due to cross-reactivity against microbial-derived HSPs or following their expression during endothelial injury (Wick et al. 2014). Preclinical studies show that administration of microbial HSPs or HSP-related peptides protects mice from atherosclerosis (Maron et al. 2002; Van Puijvelde et al. 2007; Jing et al. 2011; Klingenberg et al. 2012; Grundtman et al. 2015). The atheroprotective mechanism may involve induction of cross-reactive antibodies against MDA-LDL (Kyrklund et al. 2020) or tolerogenic activation of T<sub>regs</sub> (Van Puijvelde et al. 2007).

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## 4 Conclusion

The immune system represents a versatile and essential part of human homeostasis which harbors great potential for the treatment and prevention of atherosclerosis. While the identification of relevant therapeutic targets remains challenging, it has greatly improved over the last decade as various anti-inflammatory and immunomodulatory approaches have been studied. These range from modulation of the innate immune system by interfering with cytokines and their production, chemokines and general inflammation, to the adaptive immune system where B cells, T cells, their interaction and their potential in immunization are utilized. While these approaches have proven promising in multiple preclinical studies, with some translation into human trials, a wider application and breakthrough into the clinic is yet to occur. One factor which slows the translation of preclinical findings into the clinic is the immune system's essential role in fighting infections. Unspecific modulation or downregulation of the immune system can lead to unwanted side effects such as increased infections. It is thus important to conduct further research in order to harvest the positive potential while minimizing side effects. Research into potential biomarkers such as hsCRP and IL-6 – but with even greater specificity for



the atherogenic disease process – represents an important step toward solving this issue, as they allow the identification of patients who are most likely benefiting from immunomodulatory treatment. Moreover, highly specific biomarkers would also be needed to monitor a successful response to anti-inflammatory or immunomodulatory treatments. Additionally, further research into the immune system's role in human atherosclerosis, combined with large-scale clinical trials will pave the way for immunomodulatory treatments to become a realistic addition to standard atherosclerosis therapies.

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