ESC European Society of Cardiology

European Heart Journal (2023) **44**, 2672–2681
https://doi.org/10.1093/eurheartj/ehad304

STATE OF THE ART REVIEW
Vascular biology and medicine

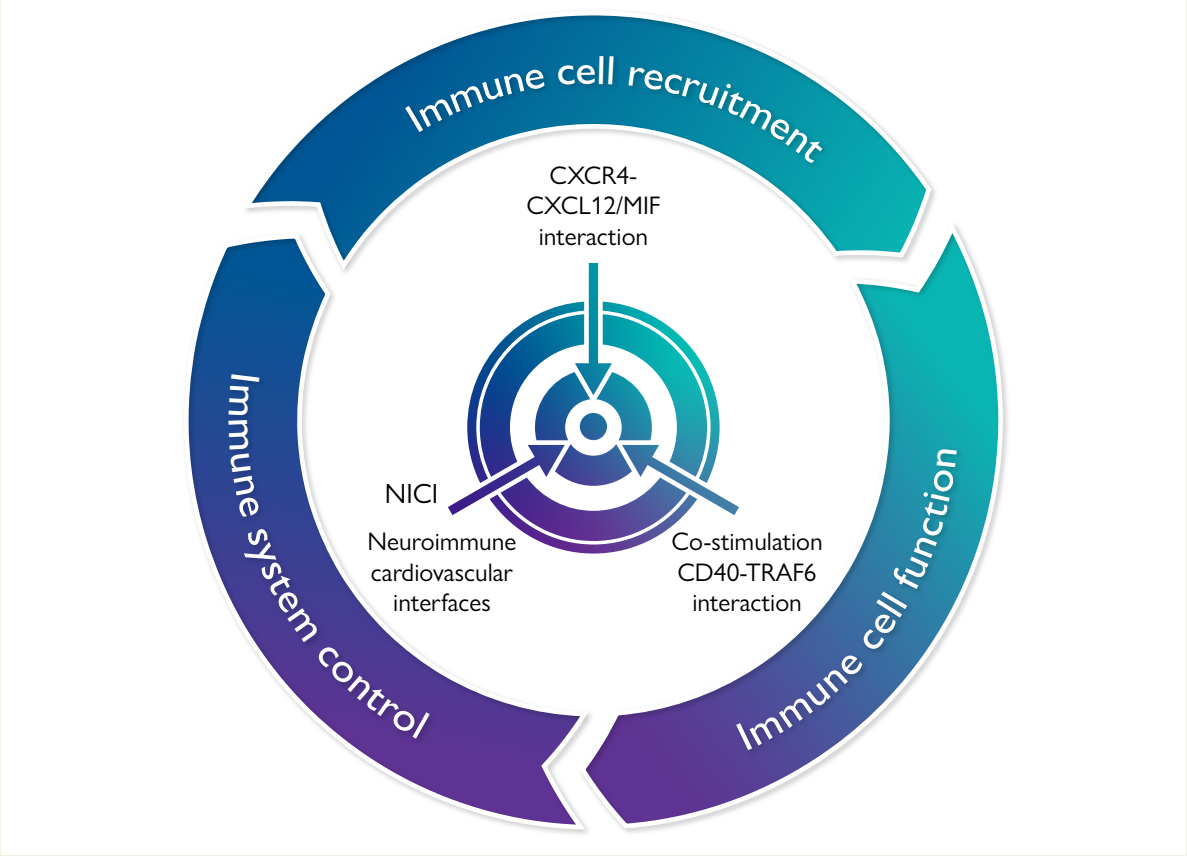
Novel mechanisms and therapeutic targets in atherosclerosis: inflammation and beyond

Christian Weber [1,2,3,4]*, Andreas J. R. Habenicht[1,2], and Philipp von Hundelshausen [1,2]

[1]Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-Universität München, Pettenkoferstraße 9, 80336 München, Germany; [2]German Centre for Cardiovascular Research (DZHK), partner site Munich Heart Alliance, Pettenkoferstraße 9, 80336 München, Germany; [3]Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Centre, Universiteitssingel 50, 6200 MD Maastricht, The Netherlands; and [4]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Received 25 January 2023; revised 4 April 2023; accepted 2 May 2023; online publish-ahead-of-print 20 May 2023

Graphical Abstract



To better treat the residual risk conferred by inflammation in atherosclerosis, a triad of three novel strategies can be envisioned. Immune cell recruitment can be targeted by specific interference with the CXC chemokine receptor (CXCR4) axis and its ligands CXCL12 or MIF. Immune cell function can be modulated by selective targeting the interaction between the co-stimulatory molecule CD40 and the TNF-receptor associated factor TRAF6. Recently identified neuroimmune cardiovascular interfaces regulating atherosclerosis and their connectivity in neuronal circuits could be specifically targeted by surgical, pharmaceutical, or bioelectronics methods.

* Corresponding author. Tel: +49-89-4400-54530, Fax: +49-89-4400-54532, Email: chweber@med.lmu.de

Abstract

This review based on the ESC William Harvey Lecture in Basic Science 2022 highlights recent experimental and translational progress on the therapeutic targeting of the inflammatory components in atherosclerosis, introducing novel strategies to limit side effects and to increase efficacy. Since the validation of the inflammatory paradigm in CANTOS and COLCOT, efforts to control the residual risk conferred by inflammation have centred on the NLRP3 inflammasome-driven IL-1β-IL6 axis. Interference with the co-stimulatory dyad CD40L–CD40 and selective targeting of tumour necrosis factor-receptor associated factors (TRAFs), namely the TRAF6–CD40 interaction in macrophages by small molecule inhibitors, harbour intriguing options to reduce established atherosclerosis and plaque instability without immune side effects. The chemokine system crucial for shaping immune cell recruitment and homoeostasis can be fine-tuned and modulated by its heterodimer interactome. Structure–function analysis enabled the design of cyclic, helical, or linked peptides specifically targeting or mimicking these interactions to limit atherosclerosis or thrombosis by blunting myeloid recruitment, boosting regulatory T cells, inhibiting platelet activity, or specifically blocking the atypical chemokine MIF without notable side effects. Finally, adventitial neuroimmune cardiovascular interfaces in advanced atherosclerosis show robust restructuring of innervation from perivascular ganglia and employ sensory neurons of dorsal root ganglia to enter the central nervous system and to establish an atherosclerosis-brain circuit sensor, while sympathetic and vagal efferents project to the celiac ganglion to create an atherosclerosis-brain circuit effector. Disrupting this circuitry by surgical or chemical sympathectomy limited disease progression and enhanced plaque stability, opening exciting perspectives for selective and tailored intervention beyond anti-inflammatory strategies.

Keywords Chemokines • Checkpoint • Co-stimulatory molecule • CXCR4 • NICI

Introduction

Atherosclerosis is a chronic inflammatory disease of the arterial wall and the pathophysiological substrate of acute coronary syndromes and ischaemic strokes,[1] which account for the major cause of mortality and disability worldwide.[2,3] The clinical management of atherosclerosis and its complications has considerably improved due to recent developments in revascularization techniques and preventive strategies, mainly featuring effective lipid-lowering therapeutics (i.e. statins and PCSK9 inhibitors).[4] Notwithstanding, the prevalence of cardiovascular disease and the incidence of acute events (e.g. myocardial infarction) has increased over the last 30 years with a high burden of mortality[2], mandating a quest to identify novel targets to reduce the residual risk of cardiovascular events. This need is illustrated by the global burden of disease study.[2,3] Herein, cardiovascular disease, which is overwhelmingly caused by atherosclerosis as the underlying pathology, is the leading cause of mortality, claiming 15.6 million lives in 2010. Compared with other entities, this prevalence will continue to dominate, owing to an increasing life expectancy in western, but also in emerging societies. In the EU, atherosclerotic cardiovascular disease and associated thrombosis represent the most frequent cause of death, accounting for 40% or 2 million per year. The enormous socioeconomic costs imposed by coronary artery disease (CAD) on European healthcare systems are estimated at 110 billion Euro per year and continue to rise. Not only is atherothrombosis-based cardiovascular disease the underlying cause of heart disease and stroke, it also predisposes to an increased risk for lethal outcomes in patients with COVID-19.[5] Vice versa, COVID-19 increases the risk for disseminated thrombotic disease, due to excessive inflammation, platelet activation, and endothelial dysfunction.[6,7] Hence, anti-inflammatory therapeutic strategies may not only target atherosclerosis, atherothrombosis, and bone marrow homoeostasis as factors in cardiovascular disease, but they may also be of benefit to treat COVID-19-related complications.

Since the PROVE-IT and JUPITER trials, an additive prognostic value of high-sensitivity C-reactive protein (hsCRP) and the putative benefit of anti-inflammatory therapy has been postulated.[8] Given the importance of inflammation in atherosclerosis, anti-inflammatory agents have been tested in large randomized clinical trials. The positive outcome of the CANTOS trial, which randomized patients with previous myocardial infarction and elevated CRP to canakinumab, an antibody blocking the cytokine IL-1β, and resulted in a significant risk reduction, has validated the inflammatory pathogenesis of atherosclerosis. Likewise, the COLCOT and LoDoCo trials, which showed a CRP-independent effect of anti-inflammatory, low-dose colchicine in stable disease, constitute an important breakthrough in attempting to combat residual inflammation.[9–11]

These results were contrasted by the negative yet informative CIRT trial that tested low-dose methotrexate, a broad immunosuppressive agent, illustrating the complexity and specificity of atherosclerotic inflammation.[12] The moderate effect size, high cost, and noticeable side effects, namely in aged and diabetic patients, explained why efforts to pursue canakinumab for entry into clinical practice were discontinued, while related issues prevent colchicine despite being more affordable from becoming a blockbuster. Multiple studies to target the NLRP3 inflammasome, an integral activator of IL-1β production, which is implicated as a metabolic sensor and central relay in atherogenesis triggered by cholesterol crystals, disturbed flow, neutrophils extracellular traps (NETs), age/clonal haematopoiesis, or somatic mutations are underway.[13,14] Yet, blocking NLRP3 is expected to encompass considerable pleiotropy and immunological side effects, so that selective targeting of upstream stimulators may be more feasible and ideal. This will however require deeper exploration of immunomodulatory pathways more specific to atherosclerosis, in a persistent quest for alternative inflammatory targets and more specific therapeutics with limited side effects and improved precision in chronic treatment.[15] Our endeavour to identify some of these targets, to establish approaches for the development of novel modulatory agents, as well as the discovery of alternative pathways involving neuroimmune cardiovascular interfaces and thereby shaping the inflammatory pathogenesis of atherosclerosis—has been presented in the 2022 ESC William Harvey Lecture on Basic Science and is the topic of this article (*Graphical Abstract*).

Inhibiting the atherorelevant signalling part of CD40L–CD40 using tumour necrosis factor-receptor associated factor-STOPs

In addition to the IL-1β and inflammasome signalling pathways, co-stimulatory immune checkpoints profoundly determine the development of atherosclerotic lesions and their characteristic features. As a prototype for these co-stimulatory molecule axes, the tumour necrosis factor (TNF)-receptor-related signalling molecule CD40 and its ligand CD40L have been shown to be critical and powerful modulators of immune responses in atherosclerosis.[16]

We thought that selective targeting of signalling molecules directly downstream of CD40 such as TNF-receptor associated factors (TRAFs) e.g. in macrophages may provide better specificity. To elucidate the role of CD40–TRAF signalling in atherosclerosis, we examined disease progression in transgenic mice with a deficiency in specific CD40–TRAF interactions and found that the absence of CD40–TRAF6 but not CD40–TRAF2/3/5 signalling abolished atherosclerosis and conferred plaque stability in ApoE-deficient mice by skewing the immune response toward an anti-inflammatory profile.[17] We further found that among the different TRAFs TRAF6, which is highly expressed in macrophages, was relevant for metabolic inflammation.[18] Using a structural model-informed synthetic library screen, virtual docking at the TRAF6-binding pocket, and validation in human and mouse cell-based assays, we were then able to identify two specific small molecule inhibitors (termed TRAF-STOPs) blocking CD40/TRAF6 signalling, which reduced even established atherosclerosis, limited plaque instability, and monocyte recruitment, while preserving classical CD40-mediated immune responses (*Figure 1*).[19,20] This is of particular importance as the CD40–CD40L dyad plays a role in several cell types of the innate and adaptive immune system, and thus co-stimulation by antigen-presenting cells, Ig isotype switching, and germinal centre formation remained unaffected. Besides leucocytes, platelets also express CD40 and CD40L, the latter being strongly up-regulated upon activation.[21] Therefore, exploring cell-specific roles and inventing cell-specific targeting mechanisms for CD40–CD40L appear crucial. Packaging TRAF-STOPs in rHDL nanoparticles facilitated their uptake in macrophages and was sufficient to reduce atherosclerosis in mice and non-human primates.[19,22] This nanotherapeutic concept enabled selective enrichment in myeloid cells but not lymphocytes, in organs that metabolize HDL such as the liver and in atherosclerotic lesions. Already a single week of therapy rapidly reduced macrophage infiltration in established atherosclerotic lesions.[22]

Likewise, we investigated the function of CD40L-expressing cell types most relevant to atherosclerosis. Indeed, CD40L-deficiency in CD4+ T cells reduced atherosclerosis in mice and impaired Th1 polarization and interferon-γ production, and this was phenocopied in mice with by CD40-deficiency in CD11c+ dendritic cells.[23] Thus, expression of CD40L in CD4+ T cells, likely through CD40 expressed on DCs, promotes atherogenesis by enhancing a Th1 signature.[23] On the other hand, not all cells expressing CD40L affect atherosclerosis, as platelet-specific deficiency of CD40L did not inhibit atherosclerosis.[23] Nevertheless, CD40L on platelets contributes to thrombus formation and atherothrombosis, possibly independent of CD40 but instead involving αIIbβ3.[23–25] We further found that the absence of CD40 signalling in myeloid cells limited atherosclerosis and systemic inflammation

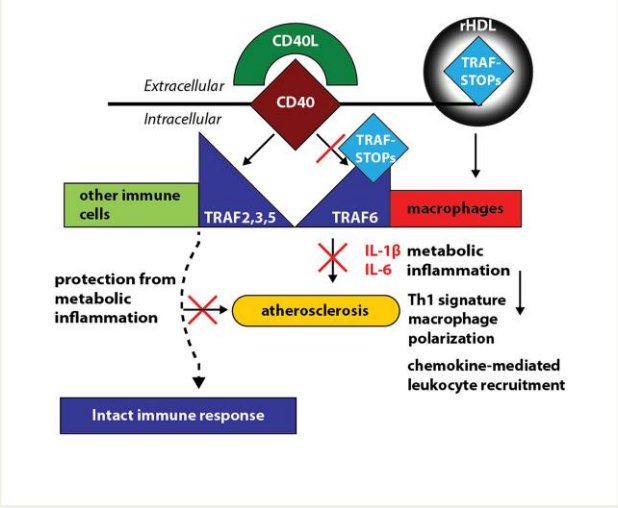


Figure 1 Proatherosclerotic CD40L signalling can be blocked by tumour necrosis factor-receptor associated factor-STOPs while maintaining intact immunity. Binding of CD40L to CD40 results in the recruitment of tumour necrosis factor-receptor associated factors, and propagation of signalling. CD40 anchors to proatherosclerotic TRAF6 highly expressed in macrophages whereas protective TRAF2/3/5 are found in other immune cells such as dendritic cells and B cells. Specifically targeting TRAF6 can be achieved by applying small molecules that prevent selectively the binding of TRAF6 to the cytoplasmic region of CD40 and by packaging into recombinant HDL nanoparticles that preferentially release their cargo when taken up by macrophages.

by preventing a shift in macrophage polarization towards pro-inflammatory states, thus confirming the value of macrophage-targeted CD40 inhibition as a valuable therapeutic strategy to combat atherosclerosis.[26] Overall, our results establish the divergent and cell-specific roles of CD40L–CD40 in atherosclerosis, harbouring important implications for the specific therapeutic targeting of this pathway.

Modulating the chemokine-interactome to treat atherosclerosis

Whereas the CD40–TRAF6 pathway drives atherosclerosis through the inflammatory capacities and functional interactions of T cells and antigen-presenting cells, namely macrophages, we pursued an alternative concept to limit atherosclerosis by targeting the recruitment of immune cells into nascent lesions. Several classes of molecules in the adhesion cascade are required to guide leucocytes from the circulation to their destination in different tissues.[27] Among these, chemokines and their heptahelical receptors provide the directional cues to orchestrate the trafficking of many leucocytes subtypes, and their activity must be finely regulated to ascertain proper homoeostasis.[28,29] To accomplish this, multiple regulatory elements exist: leucocytes express a specific chemokine receptor profile representing the binding places for chemokines. A respective chemokine usually binds several chemokine receptors with different affinities, and a respective chemokine receptor is activated by several chemokines. Most receptors trigger Gi activation, stimulating leucocyte chemotaxis but also other cellular functions.

Other receptors, e.g. ACKR1/DARC, do not signal via G-proteins, yet provide important homoeostatic functions, which explain ethnic differences in haematopoiesis and CV risk[30], whereas CCL17 and CXCR31 beyond eliciting cell migration and control cell survival.[31,32] Besides their cognate receptors, binding of chemokines to the cell surface is conferred by various antenna-like glycosaminoglycans, which are attached onto proteoglycans and virtually bind all chemokines, however with varying affinities, resulting in their immobilization and accumulation on the cell surface. This feature of chemokines and their propensity to form dimers, tetramers, and in some cases higher order oligomers shapes their *in vivo* activity.[33,34]

Depending on the subfamily of a given chemokines, CC- or CXC-type, the homophilic interaction follows a characteristic structural pattern so that CC-type dimers associate primarily via their N-termini, and CXC-type dimers associate through extending their β1-strands in their core β-sheet to a six-stranded β-sheet. The next layer of regulation comprises that chemokines do not only tend to form homophilic complexes but also interact with other chemokines and even with other inflammatory mediators such as galectins to form heteromers.[35–37] In a given microenvironment such as atherosclerosis, some of the chemokine heteromers, as is the case for CCL5 which becomes more active when complexed with CXCL4, contribute to the pathogenesis of atherosclerosis by increasing arterial monocyte recruitment. Preventing the formation of these heterodimers by specific peptide inhibitors that compete with the interaction motif reduced formation of atherosclerotic lesions in mice.[38] However, a systematic evaluation of the chemokine interactome as a basis to understand how heteromerization of chemokines amplifies, inhibits, or modulates their activity and to leverage the potential for selective targeting had remained elusive. Employing two-way immunoblotting and surface plasmon resonance with either binding partner immobilised, we obtained a comprehensive map of all bivalent chemokine–chemokine interactions, identifying hotspots of heterodimeric interactions for inflammatory chemokines. Moreover, we provided a structure–function analysis of various prototypic heterodimers by NMR and accordingly modelled specific peptide interceptors to confer atheroprotection by blunting myeloid recruitment driven by CCL5–CXCL4 synergy, or by boosting CCL17-suppressed Tregs blocking CC-type heteromeric interactions with CCL5 through tailored intervention in the mouse and human system.[39] One cyclic peptide, $[VREY]_4$, designed to specifically target the interaction between CCL5 and CXCL12, behaved differently than expected because it competed with the interaction but also mimicked its inhibitory effects at the same time.[39] This novel principle can be exploited to inhibit platelet activation as detailed below.

Targeting of CXCR4 as a versatile receptor with atheroprotective and atherogenic properties

CXCL12–CXCR4 in atherothrombosis

Atherothrombosis defines the clinical syndrome consisting of atherosclerotic plaque rupture combined with inflammatory and prothrombotic changes, both local and systemic, and its acute manifestations in coronary, cerebral, or peripheral arteries. Ruptured atherosclerotic plaques expose prothrombotic molecules such as collagen I, which trigger platelet activation and the coagulation cascade to form a clot and block arterial blood flow, causing atherothrombosis and acute coronary syndromes (ACS). Heparin and dual antiplatelet therapy (DAPT) using aspirin and a $P2Y_{12}$ inhibitor such as prasugrel have become the standard for first-line treatment of ACS and after percutaneous coronary intervention. Targeting different pathways involved in thrombotic processes can exert additive effects to counteract acute and long-term complications of ACS.[40] However, more potent platelet inhibition so far always came at the cost of an increased bleeding risk. After revascularization or stent implantation, it is state-of-the-art to treat ACS patients with DAPT for 12 months to protect from recurrence of ischaemia due to plaque rupture or stent thrombosis[41], before continuing life-long monotherapy for secondary prevention. The PEGASUS-TIMI 54 trial showed that a prolonged DAPT beyond 12 months protects from ischaemic events by an absolute 1%. But increased TIMI major bleeding more than two-fold to an absolute 2.6% during the follow-up interval of 3 years cancelled out benefits of an extended therapy. Antiplatelet drugs exerting rapid and reliable antithrombotic efficacy without increasing the risk of bleeding thus represent an important unmet clinical need but our understanding of the platelet machinery that drives arterial thrombosis remains incomplete. Various stimuli exposed by endothelial denudation as well as prothrombotic components of atherosclerotic plaques can activate platelets to release chemokines including CCL5 and CXCL12.[42,43] Whereas CXCL12 derived from activated platelets leads to an autocrine forward loop by activating platelets via CXCR4[44], CCL5 competitively blocks this effect.[45] This effect is unlikely to play a major role in a physiological scenario with low chemokine concentrations, and as the affinity of CCL5 for CXCL12 is relatively low[39], this concept of an inhibitory chemokine–chemokine interaction can be applied therapeutically. We found that platelet-derived CXCL12 contributes significantly to atherosclerotic platelet activation and arterial thrombosis in mice. A scaffolded and improved version of a CCL5-mimicking helical peptide, $i[VREY]_4$, binds to CXCL12 in a complex with CXCR4 on activated platelets with high affinity and can be used to block CXCL12-induced platelet activation in the mouse and human system by modulating the CXCL12–CXCR4 interaction[46] and thereby prevented platelet CXCL12-dependent vessel occlusion in a model of arterial thrombosis. Unlike antiplatelet therapies such as aspirin or P2Y12 inhibition, $i[VREY]_4$ reduced CXCL12-induced platelet aggregation and arterial thrombosis without prolonging bleeding time. The compound also inhibits CXCL12-induced activation of Bruton's tyrosine kinase (BTK) as a converging hub for collagen and FcRγ-signalling. Beyond atherothrombosis, this may also explain the effectiveness of BTK inhibitors in blocking platelet activation, involving anti-CXCL4 antibodies in patients with vaccine-induced immune thrombotic thrombocytopaenia.[47–49]

CXCL12/CXCR4 in atherosclerosis

Chemokines and their receptors have emerged as key players and possible therapeutic targets in atherosclerosis but their role in vascular biology extends far beyond immune cell recruitment. This is epitomized by the chemokine receptor CXCR4, which is essential for embryonic angiogenesis and for preserving vessel integrity in adulthood,[50,51] and its cognate ligands CXCL12 and the atypical chemokine ligand MIF (*Figure 2*).[1] The important role of CXCR4 and CXCL12 in CAD and atherosclerosis in humans is strongly supported by genetic association studies.[52,53] Integrating these studies in humans with studies interrogating this ligand-receptor pair in mouse models has revealed a complex pattern with differential effects of CXCL12 and CXCR4 in coronary artery disease and atherosclerosis and has been instrumental to dissect

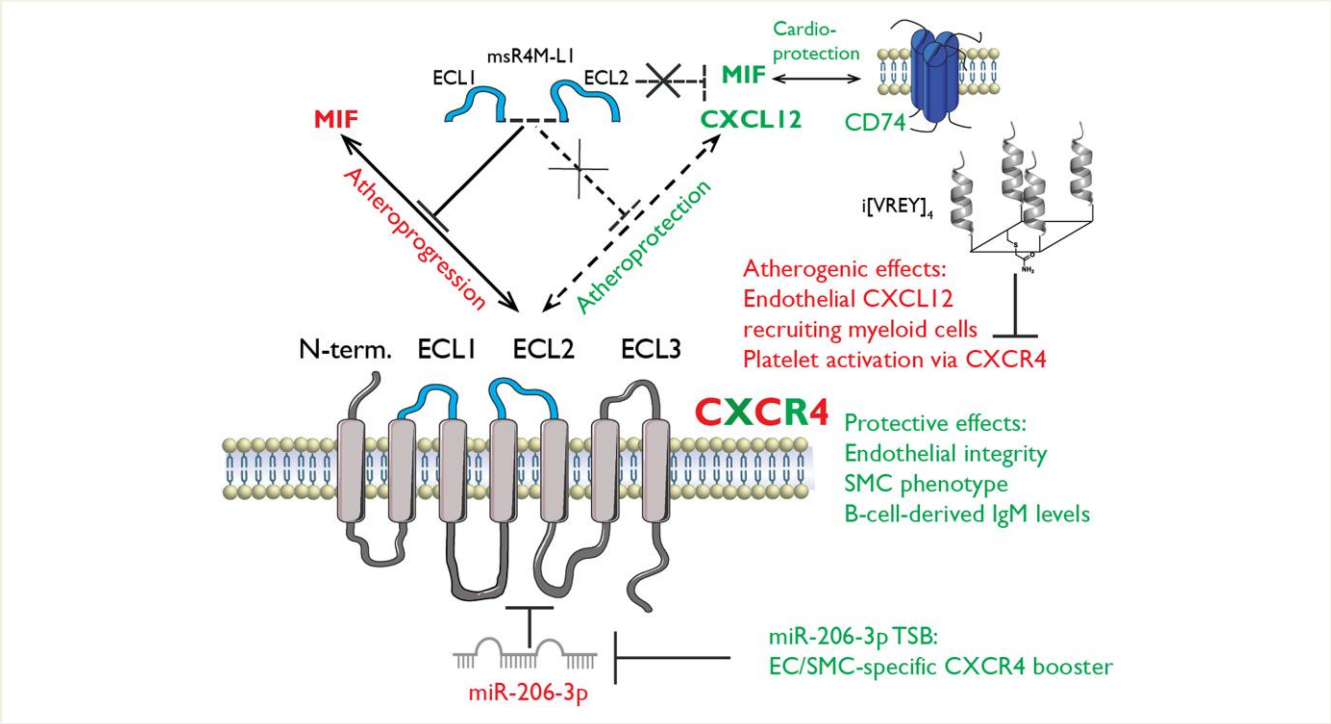


Figure 2 Therapeutic strategies targeting the functions of CXCR4 and its ligands CXCL12 and MIF. Distinct binding sites in the CXC chemokine receptor CXCR4 for CXCL12 and MIF comprising the N-terminus (CXCL12) vs. extracellelular loops (ECL) 1 and 2 (MIF) differentially target and therapeutically distinguish between atherogenic and atheroprotective functions of CXCR4 and spare effects of MIF mediated via invariant chain CD74 (msR4M-L1 ectodomain mimic). The scaffolded helical peptide inhibitor i[VREY]$_4$ modulates the CXCL12/CXCR4 interaction. The target site blocker to the vascular cell-specific miR-206-3p repressing CXCR4 can boost protective CXCR4 expression. EC, endothelial cell; SMC, smooth muscle cell.

and better establish the directionality and mechanisms behind their association with coronary artery disease. Since both CXCL12 and CXCR4 are almost ubiquitously expressed in numerous cell types to different degrees, it is important to understand how the cellular source determines how this receptor–ligand pair affects homoeostasis and atherosclerosis.

The genetic variant rs2322864 is associated with lower CXCR4 expression in atherosclerotic plaques and a higher prevalence of coronary heart disease.[50] Consistently, conditional deletion of *Cxcr4* in arterial endothelial cells aggravates atherosclerosis in mice by impairing Wnt/β-catenin-driven endothelial integrity causing leaky arteries with increased leucocyte infiltration and also impaired reendothelialization after vascular injury.[50,54] Similarly, deletion of *Cxcr4* in vascular smooth muscle cells (SMCs) promoted atherosclerosis, as expression of CXCR4 by SMCs was required for maintaining a contractile and non-secretory phenotype, normal cholesterol-efflux, and prevented differentiation into macrophage-like SMCs.[50] Thus, these data clearly supported a protective role of vascular CXCR4 in mouse and human atherosclerosis (*Figure 2*).

Because the circulating number of neutrophils correlates with atherosclerosis, and these cells contribute to lesion development and destabilization of plaques, e.g. by promoting lytic cell death of vascular SMC through release of NETs that contain lytic histone H4,[55] it was to be expected that blocking CXCR4 by AMD3465 resulted in larger atherosclerotic lesions through enhanced leucocyte mobilization, and transplantation of CXCR4-deficient bone marrow yielded a consistent effect.[56] This atheroprotective function of leucocyte CXCR4 thus relates to bone marrow homoeostasis and retention of leucocytes by CXCR4 that overall appears to prevail over the role of leucocyte CXCR4 for the subsequent recruitment of leucocytes from the circulation into the arterial wall. A further protective effect of BM CXCR4 is conferred by B-1a cells, a B cell subset shuttling between bone marrow and peritoneal cavity, which produces large amounts of protective IgM directed against oxidized epitopes including those in LDL particles.[57]

Of note, the lack of an atherogenic phenotype upon post-natal global *Cxcr4* deletion strongly indicates that atherogenic properties of CXCR4 are likely to occur in other cell types (e.g. platelets, haematopoietic, or myeloid cells)[58,59] and may thus counterbalance the protective function of CXCR4 in ECs, VSMCs, and B-cells, yielding an overall neutral phenotype (*Figure 2*). This could be related to the crucial role of the CXCR4–CXCL12 axis in maintaining the bone marrow microenvironment with implications for haematopoiesis and the mobilization of stem/progenitor cells[60,61] but is more likely due to an involvement in the recruitment of leucocytes, e.g. myeloid cells.[50,56–58,62] This is further supported by genome-wide association studies (GWAS) on the CXCR4 ligand CXCL12, which have established a link between the genomic locus 10q11 hosting the CXCL12 gene, and the risk for CAD.[52] Although CAD risk alleles downstream of CXCL12 were associated with higher plasma levels of CXCL12, the directionality of this association remained elusive.[63] Following a Mendelian randomization study identifying genetic determinants of biomarkers in the ORIGIN and CARDIoGRAM cohorts, which implicated CXCL12 as a causal mediator of CAD,[64] we detailed the association between CXCL12 and CAD in a conditional GWAS meta-analysis of the EPIC-Norfolk and PROMIS cohorts. We

found the intergenic variant rs2802492 near CXCL12 to be independently associated with CXCL12 plasma levels, arterial CXCL12 expression, and increased risk for CAD, corroborating increased CXCL12 levels as a driver of CAD. These data were validated in mouse models dissecting effects of cell-specific deficiency where CXCL12 derived from arterial endothelial cells (and not bone marrow cells or SMCs) promotes atherosclerosis to drive CAD, possibly by facilitating leucocyte recruitment.[65]

The complex functions of CXCR4/CXCL12 outlined above require a tailored manipulation in specific cell types, imposing obstacles for the exploitation of this pathway for therapeutic purposes in atherosclerosis. Hence, a development of strategies of selectively boosting CXCR4 for targeting this receptor for therapeutic purposes is warranted. Such a possibility to increase CXCR4 specifically only in the vascular wall arose from the finding that miR-206-3p acts as a vascular-specific CXCR4 repressor (Ismail et al., unpublished data) Therefore, we developed an antisense oligonucleotide target-site blocker (CXCR4-TSB) specifically disrupting this interaction to therapeutically increase CXCR4 only in vascular cells. Indeed, CXCR4-TSB in ApoE-deficient mice enhanced CXCR4 expression in ECs and vascular SMCs in the vessel wall, reduced vascular permeability and monocyte adhesion to endothelium, and attenuated the development of diet-induced atherosclerosis. The disruption of cell-specific microRNA-dependent regulatory pathways, as epitomized by the miR-206-3p-CXCR4 axis, reveals a novel therapeutic approach and paves the way for a tailored use of TSBs in the treatment of atherosclerosis and other diseases (*Figure 2*).

Furthermore, a targeted interference with functionally relevant heteromeric interactions of the CXCR4 ligand CXCL12 that may occur in a cell-specific context could be a therapeutic solution to this problem, as exemplified by peptides such as CKEY or i[VREY]$_4$ (*Figure 2*).[38,46] Likewise, targeting specific chemokine/receptor axes involved in different mechanisms of the atherogenic process could be an option but remains challenging. To this end, Kontos et al. collaborated to develop an entirely different therapeutic principle to target atherosclerosis via CXCR4, which relies on the fact that macrophage inhibitory factor (MIF) promotes atherosclerosis by activating CXCR2 and CXCR4 through a binding site non-congruent to that of CXCL12, so that MIF acts as a partial agonist.[66–68] Based on structural information,[69] synthetic peptides harbouring varying sequences of the extracellular loops of CXCR4 were engineered, thus resulting in *M*IF-specific CXC*R4 m*imics ('msR4Ms'). One of these ectodomain mimics, msR4M-L1, showed a desirable behaviour, as it selectively prevented the activation of CXCR4 by binding and deactivating CXCR4-MIF signalling but did not affect CXCL12-induced signals nor the cardioprotective MIF signalling via CD74.[70] The msR4M-L1 accumulated at MIF deposits in human and mouse atherosclerotic lesions, reduced arterial leucocyte recruitment, and reduced inflammation. These results provide a new peptide prototype that discriminates between disease-exacerbating and -protective pathways of a chemokine GPCR in atherosclerosis (*Figure 2*).

While most of these approaches await final validation for suitability and use in humans and clinical trials, late pre-clinical development of compounds covered herein, e.g. TRAF-STOPs, msR4M-L1, and i[VREY]$_4$ derivatives is underway and well advanced to lead discovery and optimization for potential translation to clinical therapies. For instance, TRAF-STOP candidates have encountered some obstacles during the late stage of detailed multi-route pharmacokinetics and medicinal chemistry analysis and are currently undergoing an extended high-throughput screen applying additional filters for successful hit-to-lead development, while the peptide strategies have entered further validation for pharmacokinetics (absorption, distribution, metabolism, and excretion (ADME), toxicity, off target analysis, etc.) and in other animal models. Notably, many other agents deserve to enter the translational pipeline in order to sufficiently increase the number of opportunities to successfully bridge the valley of death to clinical use and practice.

Neuroimmune cardiovascular interfaces form an atherosclerotic artery brain circuit

As plaques lack innervation, the impact of direct control on atherosclerosis via the nervous system remained—until recently—unknown. However, our work on the adventitia, which forms the outer connective tissue-coat of arteries, led to a series of unexpected observations: as the immune system responds to plaques by forming leucocyte-infiltrates in the adventitia and because the peripheral nervous system (PNS) uses the adventitia as their principle conduit to reach distant targets, we postulated that the PNS may directly interact with diseased arteries. Curiously, wide-spread neuroimmune cardiovascular interfaces (NICIs) arose in murine and human atherosclerosis: adventitia segments showed extensive axon networks including growth-cones at axon endings forming junctions with immune cells and media smooth muscle cells of large and intermediate-sized arteries.[71–73] Moreover, NICIs established atherosclerosis-brain circuits in mice: abdominal-adventitia nociceptive afferents entered the central nervous system (CNS) through dorsal root ganglia; multiple sensory afferent CNS neurons were traced to the brainstem, parabrachial and central amygdala neurons (*Figure 3A*);[74] and sympathetic efferents projected from medullary and hypothalamic neurons to the adventitia through spinal cord intermediolateral neurons and celiac ganglia (*Figure 3B*).[74] Furthermore, PNS components of the atherosclerosis-brain circuit were activated: splenic sympathetic and celiac vagus nerve activities increased during disease progression while celiac ganglionectomy led to disintegration of adventitial NICIs, reduced disease progression, and enhanced plaque stability. Thus, the PNS employs NICIs to assemble atherosclerosis-brain circuits and its therapeutic interruption attenuates atherosclerosis (*Figure 3*), opening a completely new chapter in atherosclerosis research.

This series of experiments provided evidence for a new and unexpected communication network of atherosclerotic artery segments and the brain.[71] The delineation of a NICI-triggered atherosclerosis-brain circuit rooted in multiple independent lines of evidence: (i) pioneering work of the founder of modern-day anatomy, the Belgian physician Andreas Vesalius, in 1543 described the close and—indeed striking—macroanatomical proximity of the PNS and the adventitia, of all arteries (reviewed in Carmeliet et al.[75]); however, these studies —while turning out to be functionally important in our work, remained silent for centuries, as the nervous system was believed to use the adventitia only as conduit to reach distant targets rather than innervating the arteries themselves and thereby affecting the physiology and pathophysiology of the adjacent arteries; and (ii) in the observation that adventitia segments burdened by atherosclerotic plaques in the intima of hyperlipidaemic mice and human diseased arteries—but not adventitia segments without plaques—develop leucocyte aggregates including macrophages, dendritic cells and T cells.[71–74,76–81] These data indicated that the immune system is capable of sensing atherosclerotic plaques via the adventitial leucocyte aggregates; as early as 2004 some

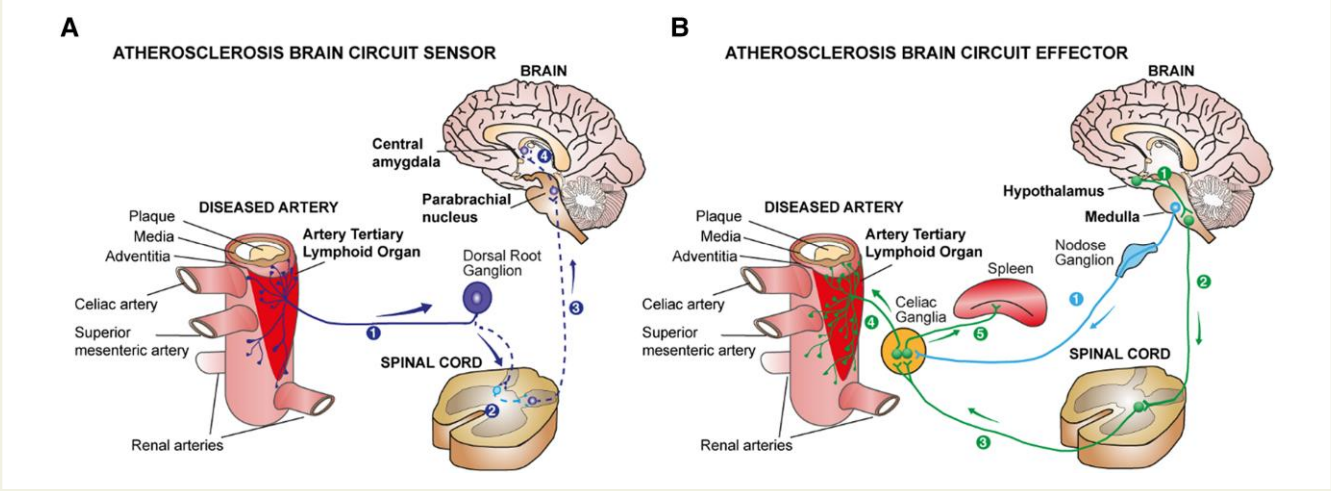


Figure 3 Neuroimmune cardiovascular interfaces create an atherosclerosis-brain circuit. Atherosclerotic arteries talk to the brain via a sensor, and the brain responds via an effector. (*A*) Adventitial neuroimmune cardiovascular interfaces initiate the atherosclerosis-brain circuit using sensory neurons of dorsal root ganglia to enter the central nervous system via the spinal cord dorsal horn and—from there—projects to the brain stem medulla oblongata. (*B*) Sympathetic nervous system efferents project from hypothalamic and brainstem nuclei to the spinal cord and—from there—to the adventitia via the ciliary ganglion, while vagal efferents originating in the medulla oblongata project to the ciliary ganglion—after traversing the nodose ganglia in the neck—to create an atherosclerosis-brain circuit effector.
Reproduced from Mohanta et al.[71]

of these leucocyte aggregates had indeed been observed to form well defined artery tertiary lymphoid organs (ATLOs), which contained separate T-cell areas and activated germinal centres involved in both T-cell responses and B-cell responses;[72,81] our more recent data then revealed that the adventitial NICI forms a biologically active anatomically discernable structure in which the immune system interacts with both the diseased artery and the nervous system in a tripartite complex tissue network.[71,73,80]

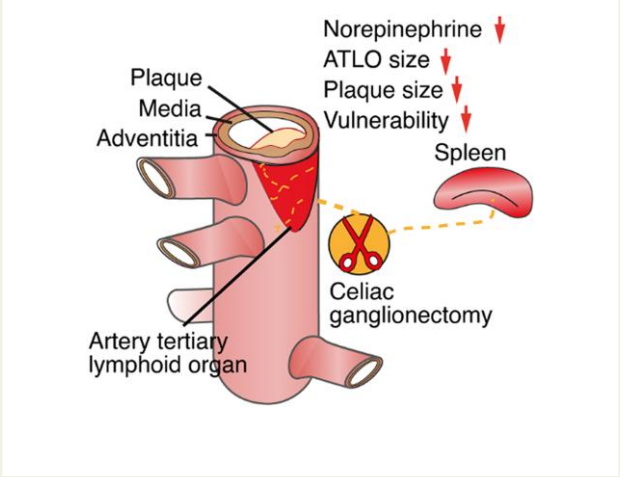


Figure 4 Celiac ganglionectomy attenuates atherosclerosis progression in mice. Surgical intervention into the adventitia neuroimmune cardiovascular interface in mice attenuates atherosclerosis progression providing a proof-of-concept type evidence that the atherosclerosis-brain circuit may be directly involved in atherosclerosis.
Adopted from Mohanta et al.[71,74]

Our data on ATLOs in human cardiovascular tissues suggest a new disease paradigm as it connects the arterial tree with both the immune system and the nervous system.[71] This tripartite interaction of three systemically organized biological systems offers mechanistic insights into new principles controlling the pathogenesis of atherosclerosis, as chemical or surgical disruption of the sympathetic nervous system that specifically innervates the adventitia led to attenuation of atherosclerosis progression over time in hyperlipidaemic mice (*Figure 4*).[71] Thus, surgical intervention into the PNS provided proof-of-concept evidence that disruption of components of the atherosclerosis-brain circuit may hold promise for other types of interventions including pharmaceutical and bioelectronics approaches.[71,74] Compared with the late pre-clinical development of the strategies outlined above, these options for targeting neurovascular interfaces and control are still in their infancy and require substantial validation.

Perspectives

The delineation of a NICI-triggered atherosclerosis-brain circuit calls for a better understanding of this so far unique definition of the structural connectivities of atherosclerotic arteries and the brain: we will have to identify the nuclei and neuron subtypes across the brain that are specifically and differentially activated in aged hyperlipidaemic mice burdened with advanced atherosclerosis. Once these neuronal areas have been defined and their secondary brain centres characterized, neuron-specific activation and inactivation tools should test the feasibility of interference approaches in the brain on atherosclerosis outcomes during different time windows of the lifespan of targeted mice. A series of advanced neurobiological tracing tools and imaging protocols will allow to achieve progress on these imminent goals. Furthermore, single-cell RNA sequencing of distinct brain areas as well as PNS ganglia are likely to identify the genes and molecules within the nervous system and the immune system that may play important

roles in the control of atherosclerosis progression driven by the nervous system. This work may lead to two types of nervous system maps: one that involves the PNS and the other that involves the CNS, and will add to recent work applying paired single-cell RNA analysis and T-cell antigen receptor profiling to diseased aorta revealing a breakdown of T-cell tolerance checkpoints in advanced atherosclerosis to indicate that clinically relevant stages of the disease also comprise a T cell-dependent autoimmune component.[82] These transcriptome and protein maps derived from unbiased genome-wide analyses will yield blueprints to develop hypothesis-driven experimental interference strategies to advance our understanding of the inner workings of the NICI-triggered atherosclerosis-brain circuit.

Acknowledgements

We wish to thank Dr Sarajo Mohanta and Dr Jürgen Bernhagen for their support with the illustrations. C.W. is Van de Laar professor of atherosclerosis.

Data availability

No data were generated or analysed for this manuscript.

Conflict of interest

All authors declare no conflict of interest for this contribution.

Funding

The research highlighted herein was supported by the Deutsche Forschungsgemeinschaft (SFB1123-A1/A10 to C.W. and –A2 to P.v.H.; excellence cluster SyNergy-C6 to C.W.), European Research Council (ERC AdG 0692511 to C.W.) and Federal Ministry of Education and Research (BMBF, German Centre for Cardiovascular Research—DZHK partner site Munich Heart Alliance to C.W. and ERA-CVD PLAQUEFIGHT 01KL1808 to A.J.R.H.).

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Corrigendum

https://doi.org/10.1093/eurheartj/ehad406
Online publish-ahead-of-print 3 July 2023

Corrigendum to: Heart failure, peripheral artery disease, and dapagliflozin: a patient-level meta-analysis of DAPA-HF and DELIVER

This is a corrigendum to: Jawad H Butt, Toru Kondo, Mingming Yang, Pardeep S Jhund, Kieran F Docherty, Muthiah Vaduganathan, Brian L Claggett, Adrian F Hernandez, Carolyn S P Lam, Silvio E Inzucchi, Felipe A Martinez, Rudolf A de Boer, Mikhail N Kosiborod, Akshay S Desai, Lars Køber, Piotr Ponikowski, Marc S Sabatine, Sanjiv J Shah, Natalia Zaozerska, Ulrica Wilderäng, Olof Bengtsson, Scott D Solomon, John J V McMurray, Heart failure, peripheral artery disease, and dapagliflozin: a patient-level meta-analysis of DAPA-HF and DELIVER, *European Heart Journal*, 2023;**44**:2170–2183, https://doi.org/10.1093/eurheartj/ehad276

In the originally published version of this manuscript, the note indicating that authors Jawad H Butt and Toru Kondo contributed equally to the manuscript was inadvertently omitted.

This error has been corrected.