



The why and how of adaptive immune responses in ischemic cardiovascular disease

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Atherosclerotic cardiovascular disease is a major cause of disability and death worldwide. Most therapeutic approaches target traditional risk factors but ignore the fundamental role of the immune system. This is a huge unmet need. Recent evidence indicates that reducing inflammation may limit cardiovascular events. However, the concomitant increase in the risk of life-threatening infections is a major drawback. In this context, targeting adaptive immunity could constitute a highly effective and safer approach. In this Review, we address the why and how of the immuno-cardiovascular unit, in health and in atherosclerotic disease. We review and discuss fundamental mechanisms that ensure immune tolerance to cardiovascular tissue, and examine how their disruption promotes disease progression. We identify promising strategies to manipulate the adaptive immune system for patient benefit, including novel biologics and RNA-based vaccination strategies. Finally, we advocate for establishing a molecular classification of atherosclerosis as an important milestone in our quest to radically change the understanding and treatment of atherosclerotic disease.

Dyslipidemia and elevated low-density lipoprotein (LDL) cholesterol levels are causally involved in the chronic inflammatory response of the vascular wall¹. LDL oxidation in the intima and the sterile vascular inflammation associated with it engage adaptive immune responses that profoundly modulate atherosclerosis, from lesion initiation and progression to the occurrence of clinical events² (Box 1). Accumulating evidence also implicates adaptive immunity in the response to ischemic heart injury³ as a modulator of both post-ischemic cardiac remodeling and atherosclerosis progression⁴.

Recent studies have provided proof of concept that targeting inflammation may limit the occurrence of cardiovascular (CV) events^{5,6}. Here, we consider the intimate and complex interactions of the immune system with the heart and the vasculature in health and atherosclerotic disease, with a particular focus on adaptive immune mechanisms and their selective manipulation for patient benefit.

Evidence for adaptive immunity in ischemic heart disease

The association of adaptive immune responses with human atherosclerotic cardiovascular disease (ACVD) dates back to the 1980s and the discovery that abundant cells in atherosclerotic lesions expressed HLA-DR in association with activated T lymphocytes⁷ and immunoglobulins. This finding was further supported by the local presence of oxidized LDL (oxLDL), along with antibodies specific to it and T cells that recognize it^{8,9}. Subsequently, an overwhelming body of evidence has implicated the adaptive immune system in the development and progression of atherosclerosis^{2,10}. More recent studies harnessing the latest developments in mass cytometry and single-cell omics technologies have provided further support for a role of adaptive immunity in ACVD¹¹.

There is ample epidemiological evidence supporting a role for adaptive immunity in ACVD, as people with autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis and other rheumatic diseases, are at substantially increased risk of atherosclerosis and CV events. The levels of autoantibodies

often associate with a higher CV risk, suggesting a detrimental role. Moreover, induction of autoimmunity in mice enhances atherosclerosis¹², indicating a causal role.

Genome-wide association studies (GWASs)¹³ also highlight the causal role of inflammation in promoting ACVD¹⁴, and genome-wide network-driven systems-biology approaches have identified genes involved in B cell activation as potential key drivers¹³. Intriguingly, HLA genotypes show only weak association with ACVD¹⁵. However, this should not be interpreted as evidence for the lack of a causal role of autoimmune responses. It is likely that people with autoimmune disease are severely under-represented in ACVD case-control cohorts. Furthermore, as discussed in this Review, atherosclerosis does not manifest as a classic autoimmune disease with early breakdown of tolerance to self antigens, but instead involves autoimmune responses to altered or modified self antigens or stress-induced neo-self antigens. Breakdown of tolerance (Box 2), if it occurs, is a secondary and relatively late event in atherosclerosis. More generally, inflammatory responses are highly modulated by environmental factors and are likely to be more important in rapid ACVD acceleration than in linear slow progression, making it difficult to capture their causal role using GWASs¹⁶. Moreover, most GWASs have included participants with ACVD with a relatively high lifetime CV-risk burden, particularly a relatively high cholesterol load. Preclinical data indicate that T cells and B cells are much stronger drivers of atherogenesis in mice with low cholesterol levels than in mice with high cholesterol levels¹⁷. Thus, it is likely that in cohorts in which high cholesterol levels are not a strong driver, more immune-related variants could be identified. The growing understanding of a fragmentation of large disease entities into smaller ones with different molecular profiles suggests that individual patho-mechanistic differences may also be responsible for the residual CV risk despite optimal treatment of classic CV risk factors. In this context, a deeper understanding of the interplay of adaptive immunity with the major CV risk factors on an individual level is needed. Finally, the substantially increased risk

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Box 1 | OxLDL and OSE-specific immune responses

Once LDL is trapped in the artery wall, it becomes oxidized by enzymatic and non-enzymatic processes¹. The oxidation of LDL contributes to atherogenesis in many ways, including the generation of bioactive lipids that activate ECs as well as the formation of oxLDL particles that are taken up by macrophages, leading to foam-cell formation¹. OxLDL is also recognized by innate and adaptive immune responses. It can trigger NFκB-dependent expression of proinflammatory chemokines and cytokines, as well as components of the NLRP3 inflammasome, through TLR4/6 and CD36 (ref. 147). Following the uptake of oxidized and aggregated LDL, endosomal leakage of cholesterol crystals can activate the NLRP3 inflammasome¹⁴⁸. OxLDL uptake by APCs also leads to presentation of ApoB100-derived peptides to specific T cells². Oxidation of LDL leads to the generation of various OSEs¹³². Oxidation of the polyunsaturated fatty acids of LDL generates highly reactive breakdown products, including MDA, 4-hydroxynonenal (4-HNE) and the phosphocholine-containing oxidized phospholipids (PC-oxPLs). In turn, these can form covalent adducts with *ε*-amino groups of ApoB or other proteins with phospholipids. OSEs represent prototypic examples of stress-induced neo-self antigens and have the capacity to trigger proinflammatory responses in macrophages, such as the expression of cytokines and chemokines, but are also bound by specific antibodies in a hapten-specific manner. A series of studies has shown that OSEs represent a class of stress-induced DAMPs that are recognized by cellular pattern-recognition receptors, such as CD36 and SRA-1, as well as soluble pattern-recognition proteins, such as complement factor H and germline-encoded natural IgM antibodies, but also as antigens by specific class-switched IgG antibodies¹³². Importantly, the same OSEs that are present on oxLDL are also present on a subset of extracellular vesicles and on dying cells that accumulate in atherosclerotic plaques, but also in the context of many other acute and chronic inflammatory settings. Thus, immune responses targeting OSEs have an important role in identifying stress-induced self antigens and promoting their removal. In situations of increased oxidative stress when more OSEs are generated, endogenous clearance or housekeeping mechanisms may become overwhelmed, leading to a loss of homeostasis, which would trigger sterile inflammation. Therefore, an inadequate response to OSEs is considered an important factor contributing to pathological immune activation during atherogenesis.

of cardiovascular inflammation, atherosclerosis progression and CV events after immune-checkpoint inhibitor therapy¹⁸ is an important reminder of the major role of adaptive immunity in cardiovascular homeostasis.

Why and how adaptive immunity is involved in atherosclerosis

The immuno-vascular unit in health. Studies employing two-photon intravital microscopy, mass cytometry and single-cell transcriptomics have revealed the presence of several types of resident immune cells of lymphoid origin (T cells, B cells, natural killer cells and innate lymphoid cells) and myeloid origin (macrophages, dendritic cells (DCs) and mast cells) in the intima and adventitia of normal arteries¹¹ (Fig. 1). The adventitia is also a site of neuro-immune-vascular cell interactions¹⁹. The presence of such a vascular-associated lymphoid tissue (VALT) in healthy arteries²⁰ suggests that it may play a role in immune surveillance, and recent work indicates that it is also involved in maintaining vascular homeostasis and adaptation to hemodynamic cues.

Monocytes, macrophages and DCs. Circulating Gr1^{dim} (mouse) and CD14^{dim}CD16⁺ (human) monocytes that crawl on endothelial cells (ECs) and patrol the vessels for the presence of danger- or damage-associated molecular patterns (DAMPs) fulfill an immune-surveillance program²¹ (Fig. 1). The number of patrolling monocytes increases in hypercholesterolemia, and their absence is generally associated with increased atherosclerosis²², suggesting a protective role.

Resident arterial macrophages originate from embryonic CX3CR1⁺ precursors and, postnatally, from definitive hematopoiesis, and are maintained through local proliferation. They accumulate preferentially at branch points characterized by intimal thickening, suggesting a role for hemodynamic stresses. Their location shapes their resident phenotype and leads to distinct gene signatures for intimal versus adventitial macrophages²³. The latter maintain arterial tone and regulate arterial remodeling in response to blood flow, in part through MMP9- and FXIIIa-dependent regulation of collagen accumulation^{24,25} (Fig. 1). Intimal macrophages maintain a non-thrombogenic intravascular state²⁶ and are the earliest foam cells that accumulate in plaques²³. In mice, intimal macrophages have previously been confounded with vascular-associated DCs, but it is unclear whether this holds true for human vascular DCs. Resting immature adventitial DCs have been described in healthy human arteries²⁷ and may have a role in maintaining immune tolerance (Fig. 1). Spatial single-cell sequencing will be useful for characterizing the various macrophage/DC subtypes and examining whether different subtypes populate distinct vascular beds and perform specific functions.

T cells, B cells and fat-associated lymphoid clusters. In human arteries, T cells occur mainly at sites of intimal thickening, whereas adventitial T cells are found both in human arteries and in mouse arteries and are thought to home constitutively to the adventitia, in part through an L-selectin-dependent mechanism²⁸ (Fig. 1). Most of these T cells are αβ CD4⁺ cells, along with a few γδ CD4⁺ cells, as well as CD8⁺ T cells. Some express CD25, suggesting that they are in an activated state. However, their characterization remains incomplete.

B cells are largely absent from the intima but are present in the adventitia of both healthy and atherosclerotic arteries (Fig. 1). The fact that adoptively transferred splenic B cells also home to the adventitia of recipient non-atherosclerotic aortas²⁸ further underscores the artery wall as a physiological niche for B cells (Fig. 1). Arterial homing of B cells also depends in part on L-selectin²⁸. A substantial proportion of T cells and B cells is located in the perivascular adipose tissue (PVAT) and may be organized within fat-associated lymphoid clusters (FALCs) around stromal cells. Unlike the formation of secondary lymphoid organs (SLOs), FALC formation is independent of lymphoid tissue-inducer cells and the lymphotoxin-β receptor pathway, but requires TNFR signaling and the presence of commensal flora. PVAT and FALCs contain a substantial amount of innate B1 cells that secrete natural immunoglobulin M (IgM)²⁹, including IgM with specificity for oxidation-specific epitopes (OSEs)³⁰. Part of this B1 cell activation may be driven by innate lymphoid cells type 2 (ILC2)³¹, which are major producers of atheroprotective interleukin 5 (IL-5) and IL-13 (refs. 31–33) (Box 2 and Fig. 1). The fundamental triggers that initiate immune-cell accumulation in normal arteries remain poorly understood (Box 3).

Medial immunoprivilege revisited. The medial layer of the artery wall is highly protected from invasion by effector immune cells. Several mechanisms of medial immunoprivilege have been proposed³⁴. Passive mechanisms include the presence of elastic laminae and the avascular nature of the media. However, these mechanisms are not entirely consistent with the sustained medial immunoprivilege of advanced plaques, even in the presence of

Box 2 | Mechanisms of immune-cell accumulation in healthy arteries

Most resident intimal macrophages accumulate at sites of altered hemodynamics (for example, reduced shear stress). However, how macrophages accumulate in the adventitia is unclear. We speculate that sudden increases in both blood pressure and shear stress shortly after birth could direct monocyte recruitment through the adventitial microvessels (low shear stress) rather than the intimal side (high shear stress). This could be further promoted by blood-pressure-mediated activation of the vessel wall to express chemokines (for example, CX3CL1 or CXCL10), known to be involved in adventitial macrophage accumulation^{25,159}. Could resident macrophages also sense hemodynamic stress (for example, pressure) or the consequences thereof, and contribute to the recruitment of other monocyte-derived macrophages?

Similar questions apply to DCs, T cells and B cells that accumulate in healthy arteries. Do they sense components of the artery wall, particularly those from the immunoprivileged media that might be released in response to physiological (for example, hemodynamic) stresses, and be presented to maintain peripheral tolerance? Do they detect and present lipoprotein components, like ApoA, that cross the arterial wall through outward convection and are removed from the tissue by arterial lymphatics? Could this contribute to the maintenance of a T_{reg} cell phenotype and limit the conversion of T_{reg} cells into pathogenic T cells⁵⁸? Do resident B cells in the adventitia and PVAT mediate housekeeping functions by secreting natural antibodies that prevent the accumulation of damaged vascular wall components? We have recently identified a non-canonical role for the B cell cytokine APRIL, which limits lipoprotein retention and acts as a key molecule in vascular homeostasis⁶⁰. Could APRIL be also involved in the immune homeostasis of arteries?

VALT formation, remodeling and function may also be influenced by the lymphatic system, which controls the drainage of macromolecules and may affect the emigration of myeloid and lymphoid cells. Finally, beyond its role in controlling vascular tone, arterial innervation by sympathetic adrenergic and parasympathetic cholinergic nerves could also affect VALT formation and function through adrenergic and cholinergic receptors expressed on immune cells.

extensive neoangiogenesis, abnormal lymphangiogenesis and disrupted elastic laminae. Proposed active mechanisms include the relatively low expression of major histocompatibility complex (MHC) and costimulatory molecules, the high expression of coinhibitory molecules and the production of immunosuppressive factors, mainly IDO1 and transforming growth factor- β (TGF- β), by medial vascular smooth muscle cells (VSMCs). However, VSMCs express high levels of MHC molecules and inflammatory mediators during atherosclerosis, and medial immunoprivilege is preserved in mice with IDO1 deficiency or disruption of TGF- β signaling. Thus, medial immunoprivilege remains a mystery.

Peripheral lymphoid stromal cells, particularly follicular dendritic cells (FDCs), have a critical role in peripheral tolerance. Their expression of self antigen induces both deletion T cell tolerance and non-deletional self-tolerance that maintains antigen-specific regulatory T cells (T_{reg} cells)³⁵. Self-antigen expression by FDCs also controls the elimination of self-reactive B cells, particularly those generated during secondary diversification in the germinal center (GC)³⁶. Interestingly, these stromal cells share the same perivascular precursor with VSMCs and thus express many similar transcripts, both during differentiation and at the mature stage³⁷. Here, we propose that the common ontogenic origin of VSMCs and lymphoid

stromal cells is the primary mechanism that ensures medial immunoprivilege. A breakdown in lymphoid stromal-cell-dependent tolerance or a substantial alteration or loss of VSMCs would be required to disrupt the immunoprivilege of the arterial media.

The immuno-vascular unit in atherosclerotic disease. Given the immunoprivilege of the media, it is unlikely that pathogenic adaptive immune cells are activated in response to arterial media components. Intimal and adventitial T cell accumulation most likely indicates an adaptive response toward altered components of, or present in, these arterial layers. The oligoclonal T cell repertoire, as well as the limited sets of hypermutated variable heavy-chain regions and the inverted κ/λ light-chain ratio of B cells found in atherosclerotic lesions^{38,39}, suggest an active expansion of antigen-specific T cell and B cell clones. The autoimmune responses most studied in this context are those directed toward lipoprotein components trapped in the artery wall^{2,40} and toward proteins expressed by stressed ECs²⁰.

Adaptive T cell immunity in atherosclerosis. How do activated T cells accumulate in inflamed arteries? Naive $CD4^+$ T cell priming in the artery wall⁴¹ is probably a marginal phenomenon in the early stages of atherosclerosis. Adventitial resident DCs are more likely than intimal macrophages to migrate to the draining lymph nodes (LNs) for T cell activation. Therefore, activated T cells in early lesions are mostly likely recruited as pre-activated (in draining LNs and SLOs) effector memory T cells (T_{EM} cells) or T_{reg} cells, transmigrating through activated (and potentially antigen-presenting) ECs. Chemokine-chemokine receptor pairs involved in T_{EM} cell recruitment to the inflamed vasculature (for example, CX3CL1-CX3CR1) are likely to be different from those involved in T_{reg} cell recruitment (for example, CCL5-CCR5 (ref. 42) and CCL1-CCR8 (ref. 43)) (Fig. 1). Once in the artery wall, some of these T cells may adopt a resident memory phenotype with low levels of S1PR1 and high expression of CD103 and CD69 (ref. 41). The latter binds oxLDL, limiting type 17 helper T cell (T_H17 cell) differentiation while favoring atheroprotective T_{reg} cells⁴⁴.

LDL particles can also reach SLOs systemically and, in the absence of systemic inflammation (that is, early atherosclerosis), apolipoprotein B (ApoB) peptides are likely to be presented in a tolerogenic manner, generating antigen-specific T_{reg} cells rather than effector T cells (T_{eff} cells) (Fig. 1). This would explain why most ApoB-specific T cells display a regulatory phenotype in the absence of overt atherosclerosis⁴⁵. It also provides a plausible explanation for the initial surge in T_{reg} cells rather than T_{eff} cells in response to hypercholesterolemia in mice⁴⁶. This scenario suggests that the first (ApoB-specific) T cells that accumulate in lesions are likely to be T_{reg} cells, which may subsequently switch to helper and effector-memory phenotypes with the increased burden of local and systemic inflammation⁴⁷. It is also plausible that ApoB-specific T_{reg} cells are instructed in the liver, where ApoB is produced⁴⁸.

Different scenarios may arise with different antigens, either sequentially or concomitantly. Among these scenarios is the example of autoimmunity to heat-shock proteins (HSPs), whereby pre-existing immunity to ubiquitous HSP60 (a highly immunogenic microbial antigen) could lead to cross-reactivity with autologous HSP60 (overexpressed on stressed ECs), triggering intimal inflammation²⁰. Wick et al. have shown that T cells from early human atherosclerotic lesions are interferon- γ (IFN γ)-producing memory effector $CD4^+$ cells and respond to HSP60 and HSP60-derived peptides *in vitro*⁴⁹. This recall response was associated with the generation of pathogenic anti-HSP60 antibodies. However, the early lesions assessed in that study were from individuals with 'inflammatory storm' conditions. The latter may have induced an activated T cell phenotype, and therefore it remains unclear whether HSP-specific T_{eff} cells are truly predominant in early atherosclerosis. Furthermore, most of

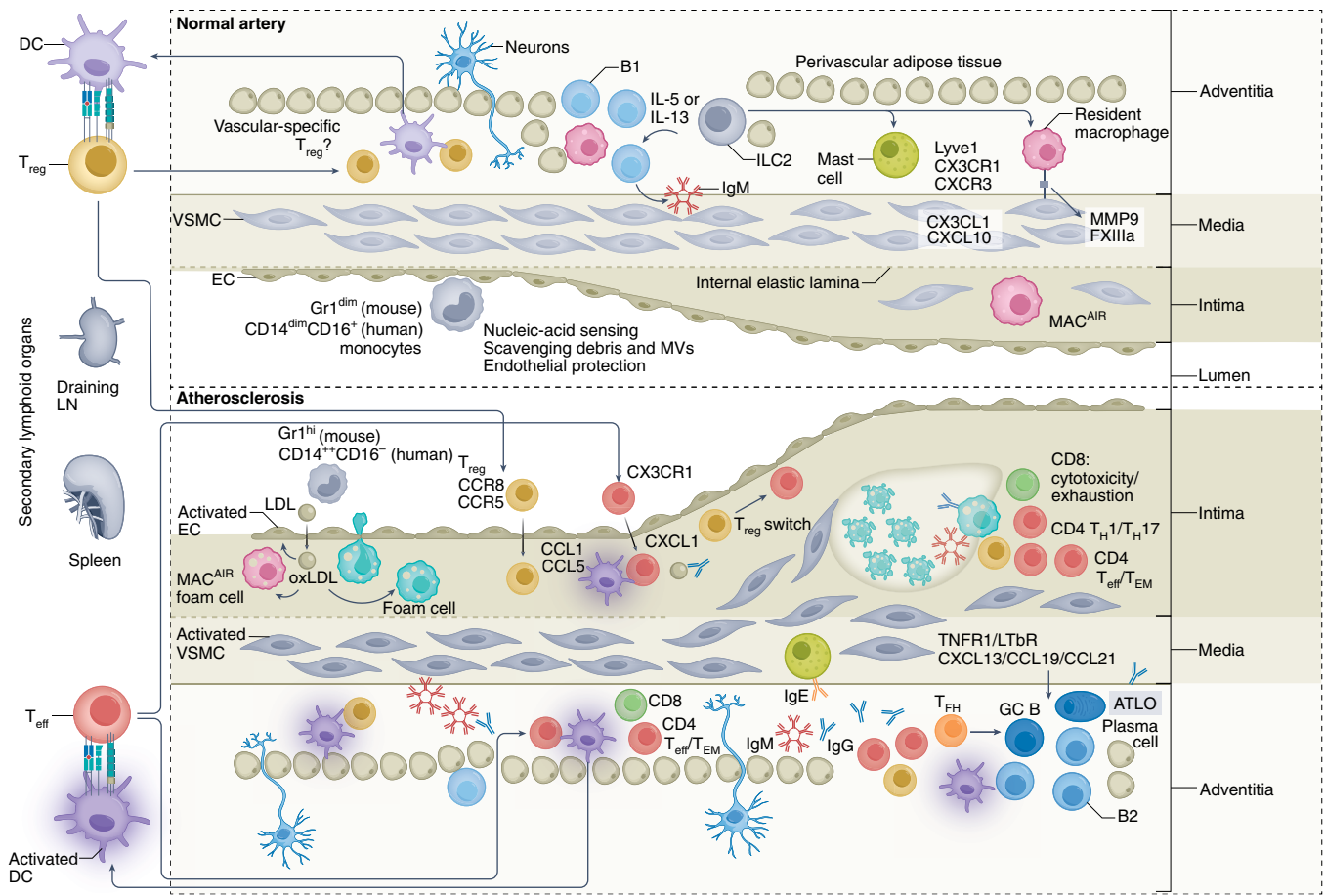


Fig. 1 | The immuno-vascular unit in health and atherosclerotic disease. VALT is present in normal, healthy arteries and comprises a variety of immune cells that serve essential roles in immune surveillance and vascular homeostasis. Crawling monocytes ($Gr1^{dim}$ in mice, $CD14^{dim}CD16^{+}$ in humans) sense nucleic acids, and those released after EC damage scavenge debris and microvesicles (MVs) and ensure EC protection. Intimal macrophages (MAC^{AIR}) accumulate at sites of intimal thickening (upper right, normal artery) and are the first myeloid cells that scavenge oxLDL in the initial stages of atherosclerosis (middle left, atherosclerotic plaque). This further drives vascular inflammation and leads to the recruitment of inflammatory monocytes ($Gr1^{hi}$ in mice; $CD14^{++}CD16^{-}$ in humans) that give rise to a variety of macrophage subsets. Adventitial macrophages, particularly Lyve1+ macrophages (upper right, normal artery), regulate arterial stiffness, in part through the production of MMP9 and FXIIIa. Their specific contribution to atherosclerosis is currently unknown. The adventitia and perivascular adipose tissue form a permissive niche for the accumulation of atheroprotective ILC2 and innate-like B cells, organized in FALCs (upper middle, normal artery). ILC2-derived type 2 cytokines (for example, IL-5 and IL-13) promote B1 cell activation and the production of natural IgM antibodies, and contribute to an anti-inflammatory macrophage phenotype. Most DCs and T cells of normal arteries are found in the adventitia. T cells of normal arteries are likely to be enriched for a regulatory phenotype¹⁴⁶, instructed by DCs presenting vascular-associated antigens (in draining lymphoid organs) and maintaining peripheral tolerance. The inflammatory milieu of developing lesions promotes DC maturation, which favors the generation of T_H cells, T_{eff} cells and T_{EM} cells (mostly in draining lymphoid organs) and their recruitment into both the intima and adventitia of atherosclerotic arteries (bottom left). Sustained stimulation of autoreactive T_{reg} cells may downregulate Foxp3, promoting their conversion into T_H1 cells and T_{eff} cells. Some of the T_{reg} cells and T_{eff} cells acquire a resident memory phenotype. T_H1 cells, T_{eff} cells and T_{EM} cells predominate in advanced lesions (bottom right), and activated cytotoxic $CD8^{+}$ T cells may acquire an exhausted phenotype. GC activation in secondary lymphoid organs leads to the production of affinity-matured class-switched (IgG, IgE) antibodies, which accumulate in lesions. Medial VSMCs of advanced lesions produce CXCL13 and CCL21 and may adopt features of lymphoid tissue organizer-like cells, leading to ATLO formation (bottom right). ATLOs are conducive to the generation of T_{reg} cells, which serve a counter-regulatory, atheroprotective role. The diseased adventitia also establishes neuro-immune vascular interactions, which affect lesion progression.

these studies have focussed on intimal T cells, and the antigens that trigger T_{eff} cell responses in the adventitia remain to be identified.

A shift from regulatory to pathogenic T cell immunity. Depletion of $CD4^{+}$ T cells accelerates early atherosclerosis in mice⁵⁰, supporting the concept that early $CD4^{+}$ T cell responses are mostly regulatory and atheroprotective. So, what shifts T cell responses from regulatory to effector? There is not a particular type of antigen-presenting cell (APC) that has been shown to selectively promote a T_{eff} cell phenotype versus a T_{reg} cell phenotype in atherosclerosis^{51,52}. Thus, the

activation state of the APC and the microenvironment of antigen presentation are paramount. Sustained local inflammation certainly has a role, activating macrophages and DCs and changing their metabolism and signaling pathways to upregulate costimulatory molecules (for example, CD40) and proinflammatory cytokines (for example, IL-1, IL-6) and chemokines (for example, CCL17) while downregulating coinhibitory molecules (for example, PDL-1) and anti-inflammatory mediators (for example, IL-10 and TGF- β)⁵³⁻⁵⁶. In addition, changes in T cell metabolism, driven by hypercholesterolemia, alterations in lipid-synthesis pathways, hypoxia and

Box 3 | Innate-like B cells and natural IgM antibodies

Innate-like B cells represent a special subset of B cells with important atheroprotective functions in atherosclerosis (reviewed in refs. ^{10,81,149}). These cells include B1 cells, as well as MZ³⁹ B cells, and have an important role in host defense and immune homeostasis. B1 cells, which in mice can be divided into B1a and B1b cells, differ from conventional B cells in their developmental origin, activation requirements and antibody repertoire. On the basis of these properties, equivalent B cell populations have been suggested to exist in humans, but their exact identity is under debate. Although B1 cells are typically located in serosal cavities, such as peritoneum and pleura, the spleen seems to be critically important for their maintenance. Adoptive transfer of B1a cells or B1b cells into splenectomized or B cell- and T cell-deficient mice, respectively, protected the mice against lesion formation^{150,151}. This effect was dependent on the capacity of B1 cells to secrete IgM antibodies. Indeed, B1 cells are the main source of germline-encoded natural antibodies, which have an important role in the first line of defense against infections, as well as in housekeeping functions by promoting the clearance of dying or damaged cells. The spleen and bone marrow are major sites of B1 cell-derived plasma cells and antibody production, although PVAT has been described as another potentially relevant site³⁰. B cell-specific Id3 deficiency in ApoE-knockout mice results in increased B1b cells in PVAT but not in the spleen, suggesting local recruitment. The latter is enhanced by CCR6 expression in B1 cells¹⁵². A large part (>30%) of B1 cell-derived natural IgM has specificity for different types of OSE⁷², which explains its involvement in atherosclerosis by neutralizing oxidized lipids of oxLDL, promoting apoptotic cell clearance and inhibiting the pathogenic activities of OSE⁺ microvesicles^{153,154}. Also, it is possible that local production of OSE-specific IgM in the aorta is triggered by chronic inflammation

and aids the removal of oxidized lipids and dying cells. However, excessive oxLDL generation and increased OSE accumulation during atherogenesis may overwhelm the homeostatic functions of OSE-specific IgM. As a consequence, OSE can trigger inflammatory responses, which may result in inappropriate activation of adaptive immune responses against these same antigens. Several cytokines (IL-5, IL-33), chemokines (CXCR4) and (co-)receptors (Siglec-G, Tim1) have been identified that control B1 cell numbers and plasma levels of OSE-specific IgM, and thereby modulate atherosclerosis^{32,155–157}. Another set of innate-like B cells are MZ B cells, which reside in the MZ of the spleen, where they survey antigens that pass through the red pulp. They are also considered atheroprotective, in part through their secretion of OSE-specific IgM antibodies¹⁵⁸. Whether these IgM antibodies differ functionally from B1 cell-derived IgM remains to be investigated. A large part of MZ B cell-derived hybridomas has been suggested to have specificity for OSEs. Therefore, the spleen may participate in the sensing of increased circulating levels of OSEs generated during atherosclerosis and in turn produce more neutralizing IgM antibodies. Indeed, inflammasome activation associated with hypercholesterolemia has been shown to occur in the MZ, where it can trigger B cell activation¹⁵⁸. Thus, the involvement of both types of innate-like B cells and the natural IgM they secrete reflects a response to chronic inflammation that is needed to maintain tissue homeostasis by neutralizing stress-induced self antigens and their accumulation. Another facet of natural IgM may be its ability to modulate B cell receptor-dependent differentiation of B cells expressing the low-affinity IgE-clearing receptor CD23, thereby preventing the accumulation of proatherogenic IgE antibodies⁹¹. Increased production of self antigens targeted by natural IgM could interfere with this activity.

other microenvironmental cues, may substantially alter the T_{reg} cells' suppressive phenotype^{57,58}. Interestingly, sustained activation of T_{reg} cells by self antigens in an inflammatory environment affects the demethylated region in the *Foxp3* locus, downregulating *Foxp3* expression and destabilizing T_{reg} cells⁵⁹. These mechanisms could account for the increased plasticity of T_{reg} cells⁶⁰ and their progressive decline during disease progression, as well as the enrichment for T_{H1}- and T_{H17}-related phenotypes among ApoB-specific T cells of people with atherosclerosis compared with their abundance in those free of ACVD⁴⁷ (Fig. 1).

The role of the various CD4⁺ T cell subsets has been extensively reviewed². Foxp3⁺ T_{reg} cells and IL-10-producing type 1 regulatory T cells are protective, whereas T_{H1} cells promote disease development. The roles of T_{H2} and T_{H17} cells appear to be contextual and most likely depend on the relative abundance of pro- and anti-atherogenic cytokines produced by these T cells^{2,61}. It is important to note that a clear distinction between the various T_H cell archetypes may not be relevant in vivo, and it is highly likely that most plaque T cells display a continuum of helper phenotypes. Indeed, recent single-cell studies in advanced human plaques described an enrichment for chronically activated T_{EM} cells displaying a resident phenotype that are enriched for interferon pathways, but T_H cell-specific transcription factors could not be linked to specific clusters^{52,63}. There is genetic and experimental evidence for a significant role of IL-1, IL-6 and IL-18 pathways in ACVD. Intriguingly, IL-1 and IL-6 signaling pathways were more likely to be activated in CD4⁺ T cells of asymptomatic carotid plaques than in those of symptomatic carotid plaques in humans⁶². The therapeutic implications of this finding remain unclear.

Many other T cell subtypes have been suggested to modulate atherosclerosis, but these subsets, and in particular their antigens,

remain poorly characterized. The preferential distribution of $\gamma\delta$ CD4⁺ T cells, natural killer T cells⁶⁴ and mucosal-associated invariant T cells in the gut and liver suggests that these subsets are more likely to have a role in atherosclerosis in the context of metabolic syndrome, through their immune and metabolic activities⁶⁵. The role of CD8⁺ T cells will require further attention. These cells are enriched in advanced human lesions and display several phenotypes^{62,63}, including central memory, effector memory and cytotoxic phenotypes, with exhausted CD8⁺ T cells being enriched in symptomatic carotid plaques relative to their abundance in asymptomatic carotid plaques⁶². CD8⁺ T cells are suggested to promote atherosclerosis through target-cell lysis or induction of monoapoiesis⁶⁶. Their antigen specificity is still largely unknown, although some CD8⁺ T cells recognize ApoB100-derived peptides⁶⁷. A Qa-1-restricted regulatory CD8⁺ T cell subset has been shown to limit atherosclerosis in mice through the control of the follicular helper T cell (T_{FH} cell) GC B cell response (discussed below)⁶⁸. Its relevance to human atherosclerosis remains unexplored.

Adaptive B cell immunity in atherosclerosis. Follicular B2 cells represent the major part of B cells in secondary lymphoid organs and the circulating blood. B2 cells are involved mainly in T cell-dependent responses, as they differentiate into GC B cells undergoing class-switching and affinity maturation aided by T_{FH} cells. Analyses of atherosclerotic arteries support a relative expansion of B2-like over B1-like cells, and the accumulation of activated plasmablasts^{11,39}. In advanced disease, some VSMCs and other local stromal cells express high levels of CXCL13 and CCL21 and adopt features of lymphoid tissue organizer-like cells. B cells in the adventitia become organized in artery tertiary lymphoid organs (ATLOs)⁶⁹ that represent a site of recruitment to the artery wall and contain different

subsets of B cells that participate in GC responses⁷⁰ (Fig. 1). Despite the documented presence of B cells in the vascular wall, their direct local contribution to atherogenesis is largely unknown. ATLOs are atheroprotective in mice⁶⁹. However, it is unclear whether this is due to protective local B cell functions or to the predominance of induced immunosuppressive T_{reg} cells in ATLOs⁶⁹. Moreover, effector functions associated with antibody production do not require local B cells, as specific antibodies produced in the bone marrow or the spleen can reach plaques via the circulation^{71,72}.

The activation of B2 cells is responsible for the production of class-switched antibodies against modified lipoproteins and other self components. The help of T_{FH} cells, found mainly in B cell follicles, is required for the generation of memory B cells and long-lived plasma cells that secrete affinity-matured class-switched antibodies. T_{FH} cell deficiency appears to limit atherogenesis⁵⁸. However, this requires further exploration, because T_{FH} cells located outside the GC may be required for both switched and unswitched extrafollicular responses, with potentially different effects on atherosclerosis. Of note, activation of marginal zone (MZ) B cells impairs T_{FH} cells via upregulation of PD-L1, thereby limiting proatherogenic T cell-dependent responses⁷³.

B2 cells have antibody-mediated atherogenic functions. Follicular B cells are proatherogenic; their preferential depletion with CD20-targeted antibodies or by genetic targeting of the BAFFR pathway reduces atherosclerosis^{74–76}. Because follicular B cells participate in GC reactions, the likely explanation for their proatherogenic properties is that they contribute to the generation of pathogenic high-affinity IgG antibodies. Hyperlipidemia in mice promotes a GC response with increased serum levels of IgG2b and IgG2c, as well as OSE-specific IgG antibodies and several classical autoantibodies⁷⁷. Of note, *ApoE*^{-/-} mice that are unable to make IgG antibodies due to a deficiency of the activation-induced deaminase, which is critical for class-switch recombination, displayed increased total and malondialdehyde (MDA)-specific IgM levels and developed less atherosclerosis⁷⁸. Genetic ablation of GC-derived antibodies greatly reduced lesion size in *ApoE*^{-/-} mice^{71,79}. Moreover, administration of an IgG preparation from plasma of atherosclerotic *ApoE*^{-/-} mice aggravated disease⁷¹. These data indicate that GC-derived antibodies have the capacity to promote disease. However, few pathogenic IgG antibody responses have been described, such as anti-HSP60/65 IgG antibodies that bind HSP60 in stressed ECs and promote atherogenesis by antibody-dependent cell-mediated cytotoxicity²⁰, and IgG autoantibodies to the 78-kDa glucose-regulated protein, which has been described as another endothelium-derived autoantigen⁸⁰, that trigger EC activation. Thus, there is still a great need to identify the antigens of GC-derived pathogenic antibodies (Box 4).

Antibody-mediated atheroprotective functions of B2 cells. Beyond the atheroprotective properties of B1 cell- and MZ B cell-derived OSE-specific IgM antibodies^{10,81} (Box 1), GC-derived IgG antibodies may also have protective functions⁵⁰, such as promoting the proliferation of smooth muscle cells and plaque stability⁷⁹. Passive immunization of *Ldlr*^{-/-} mice with a recently characterized autoantigen, ALDH4A1, resulted in reduced lipid levels and decreased atherosclerosis⁸². T cell-dependent IgG antibodies with reactivity to ApoB were also shown to protect T cell receptor (TCR)-transgenic mice from atherosclerosis by promoting LDL clearance and reducing serum cholesterol levels⁸³. Moreover, despite *in vitro* studies demonstrating proinflammatory effects of anti-oxLDL IgG in macrophages⁸⁴ and several epidemiological studies demonstrating an association of anti-oxLDL IgG titers with ACVD⁸⁵, direct experimental evidence of a proatherogenic role *in vivo* is missing. In contrast, numerous immunization studies in mice and rabbits, which initially aimed at establishing a proatherogenic role for anti-oxLDL immune responses, demonstrated a robust induction of T cell-dependent

Box 4 | Some considerations related to B cell autoimmunity during atherosclerosis

Experimental data from mouse models of atherosclerosis have clearly established the initiation of a GC reaction (GCR) in response to hypercholesterolemia^{71,77,79}, in association with the development of T cell-dependent antibody responses. It is interesting that most of the B cell antigens that have been characterized so far represent intracellular antigens that are released or expressed in response to stress (for example, HSP60 and GRP78) or carry lipid-peroxidation-derived post-translational modifications (for example, OSEs). Thus, atherosclerosis-related B cell responses may instead target stress-induced antigens or neo-self antigens, and one may speculate that autoantigens recognized in the context of atherosclerosis may in fact be modified — for example, by OSE. The mitochondrial origin of one recently identified candidate antigen, ALDH4A1, would certainly facilitate its modification⁸². Intriguingly, hypercholesterolemia in mouse models is also associated with the generation of autoantibodies to classical antigens, such as anti-nuclear antibodies against Ro-52, Ro-60 or Smd1, suggesting a potential loss of tolerance⁷⁷. Moreover, using an autoantigen array, Hutchinson et al. recently screened serum IgG1 of *ApoE*-knockout mice and identified several potential new candidates, including RSPA, PVRL3, GPBP1 and FAM131c (family with sequence similarity 131 member C)⁷⁸. Indeed, hypercholesterolemia aggravates immune activation in the presence of an autoimmune background¹⁶¹. Although autoimmunity is not typically linked to excessive hypercholesterolemia in humans, these data suggest the possibility that if tolerance is broken in the context of atherosclerosis and dyslipidemia, this in turn could facilitate the activation of autoreactive GCs and B cells against other self antigens, consistent with the epitope spreading seen in autoimmune diseases. Whether OSE DAMPs also promote autoreactive B cells, and how they would do so, remains to be shown. Interestingly, oxidized phospholipids have been suggested to modulate adaptive immunity by promoting a hyperactive DC state¹⁶². All these aspects need to be considered in attempts to identify relevant B cell antigens in atherosclerosis.

IgG antibodies and reduced atherosclerosis¹⁰. For example, immunization with models of OSE generally induced a T_H2-biased response, for which IgG1 is a surrogate marker^{32,86}. Although other mechanisms may be at play, there is also direct evidence for an atheroprotective effect of OSE-specific IgG antibodies. Indeed, infusion of monoclonal human IgG1 against the OSEs MDA-LDL and phosphocholine (PC) protect mice from atherosclerosis^{87,88}. Owing to the human origin of the infused IgG antibodies, the contribution of the IgG subclass cannot be deduced, but it is probably important for the protective capacity of these IgG antibodies. Blockade of the many proinflammatory properties of oxLDL may be part of the protective effect, as mice expressing a single-chain version of the anti-PC IgM, E06, developed reduced inflammation and decreased atherosclerosis⁸⁹.

Fc receptor-mediated effects of antibodies. To fully understand the contribution of class-switched antibody responses in atherosclerosis, not only antigen specificity but also the different effector functions of antibody subclasses need to be considered⁸¹. Key effector functions that also need to be explored in context of the bound antigens are neutralization and clearance of antigens (for example, of oxLDL and self antigens), antibody-dependent cell-mediated cytotoxicity (for example, of HSP60-expressing ECs), complement activation and inflammation, as well as cellular activation. For the latter, the role of Fcγ-receptors has been studied in a series of settings,

and in general indicated an overall pathogenic role for activating Fcγ receptors and protective effects of the inhibitory FcγRIIB^{10,90}. However, these effects are complicated by sexual dimorphism, disease-stage-specific effects, cell-type-specific differences and potential non-canonical roles of these receptors^{10,90}. Future studies need to further dissect the involvement of Fcγ receptors in mediating IgG effector functions independent of immunomodulatory roles and in an antigen-dependent manner. A clear proatherogenic role has been found for IgE antibodies, which trigger mast-cell and macrophage activation through FcεR in the artery wall^{91,92}. Whether these effects involve the recognition of specific antigens remains to be shown.

Other effector functions of B cells. B cells also contribute to atherosclerosis through cytokine secretion. For example, expression of TNF by B cells may be in part responsible for the proatherogenic effects of B2 cells⁹³, and innate response activator B cells are GM-CSF-secreting B cells that promote atherogenesis through GM-CSF-mediated DC activation⁹⁴. The role of B cell-derived IL-10 is still unclear and requires further studies¹⁰. For all these subsets, it remains uncertain whether their effects are mediated as simple bystander activities or are associated with certain B cell receptor identities.

Finally, recent studies have indicated that the peripheral nervous system is involved in an artery–brain cross-talk that affects local neuro–immune–vascular interactions in the adventitia, with important consequences for atherosclerosis progression in mice¹⁹. The implication of this circuit for human atherosclerosis is currently unknown.

Adaptive immunity in response to cardiac ischemic injury

Resident DCs and T cells in normal hearts. The normal heart harbors several populations of resident macrophages and several populations of DCs, including monocyte-derived DCs, cDC1 and cDC2 (ref. ⁹⁵). The precise spatial distribution of DC subsets is poorly characterized. However, these DCs, namely IRF8-dependent cDC1, drive the generation of autoreactive T_{reg} cells that are specific for cardiac self antigens, particularly α-myosin heavy chain⁹⁶. This is an important homeostatic mechanism that ensures peripheral non-deletional tolerance to a self antigen that escapes central negative selection⁹⁷ (Fig. 2a). T cells in normal hearts are enriched for selective TRBV-J rearrangements and TRBV gene segments compared with those in peripheral blood, indicating that they may recognize and tolerate against tissue-specific antigens⁹⁸. Single-cell TCR sequencing will facilitate the characterization of these T cells and their antigen specificities. It will also be important to dissect the different steps involved in maintaining peripheral tolerance to cardiac-specific antigens. A well-developed and functional cardiac lymphatic network could facilitate this immune-surveillance task. Biomechanically induced IL-33 may promote and/or maintain immunosuppressive and reparative ST2⁺ cardiac T_{reg} cells⁹⁹. Furthermore, the heart produces hepatocyte growth factor, which is known to induce immune-regulatory DCs and instructs T cell cardiotropism by binding to its receptor c-Met in draining LNs, inducing the release of β-chemokines and promoting T cell recruitment through CCR5 (ref. ¹⁰⁰). This mechanism may be required for cardiac T_{reg} cell accumulation at steady state, and is consistent with the role of CCR5 in promoting T_{reg} cell recruitment to the heart after myocardial infarction (MI), suppressing inflammation and reducing adverse remodeling¹⁰¹ (Fig. 2a).

Adaptive T cell immunity in response to MI. Ischemic injury releases cardiac self antigens in an inflammatory milieu that promotes maturation and activation of APCs⁹⁶ (Fig. 2b). The infiltrating monocytes facilitate cardiac self-antigen trafficking to draining LNs, promoting the development of pathogenic, autoreactive T cells¹⁰². With the exception of pDCs, all other DC subtypes

were shown to license pathogenic autoreactive T_H cells and/or T_{eff} cells^{96,98,103} (Fig. 2b). The mechanisms responsible for the recruitment, retention and further activation of pathogenic CD4⁺ T cells post-MI remain poorly understood. CX3CR1 expression on human CD4⁺ T cells post-MI was associated with their decline in the circulation, potentially attracted to the injured myocardium by increased production of CX3CL1 (ref. ¹⁰⁴). This is consistent with the mechanisms of tissue recruitment of T_{EM} cells, but additional mechanisms are likely at play (Fig. 2b). CD4⁺ T cells in the ischemic heart show a preferential expansion of TRBV gene segments and TRBV-J rearrangements relative to their abundance in circulating T cells⁹⁸. It is still unclear, however, whether these clones are different from those found in non-ischemic hearts, or whether the same clones that are present before ischemia switch from naive and regulatory to effector memory and T_H1/T_H17 phenotypes. This early CD4⁺ T cell activation post-MI is detrimental overall and contributes to increased infarct size and reduced heart function¹⁰⁵. CD8⁺ T cells are also activated and accumulate in the ischemic heart⁹⁸, producing IFNγ and displaying cytotoxicity toward cardiomyocytes¹⁰⁶ (Fig. 2b). Although their antigen specificity is still unknown, their granzyme B-dependent¹⁰⁶ detrimental effect¹⁰⁷ requires the recognition of self antigens in an MHC class I-restricted manner¹⁰⁶. This is consistent with the detrimental role of CLEC9A-expressing cDC1s, which promote CD8⁺ T cell cross-priming¹⁰⁷. As in CD4⁺ T cells, a role for the CX3CR1–CX3CL1 pathway has been proposed in myocardial homing of CD8⁺ T cells and their detrimental role¹⁰⁴. The same group described accelerated senescence of circulating effector memory TEMRA CD8⁺ T cells¹⁰⁸. However, the implication of this finding to post-MI cardiac remodeling is unclear. The role of other T cell subsets requires further investigation. The TCR repertoire of γδ T cells becomes significantly restricted in people with acute MI and is associated with increased expression of IL-17A¹⁰⁹. CD4[−]CD8[−] (double-negative) T cells also accumulate in large numbers in ischemic hearts¹⁰⁷, but their significance and role remain unexplored.

The appearance of pathogenic, autoreactive T cells post-MI may constitute a threat to immune homeostasis. However, in most circumstances and in the absence of an autoimmune-prone background, overt cardiac autoimmunity does not develop. This may be explained, in part, by the concomitant increase in cardiac self-antigen-specific T_{reg} cells^{3,99,110}. The ischemic heart sustains the recruitment of circulating T_{reg} cells and their local proliferation and expansion⁹⁹, and induces the conversion of recruited conventional T cells into T_{reg} cells⁵. However, the mechanisms responsible for these effects are poorly understood. Enhanced release of IL-33 from stressed necrotic cells may have a role. Other data point to the presence of a local paracrine–autocrine adenosinergic loop enhancing T_{reg} cell immunosuppressive effects^{105,111}. Several additional hypotheses merit exploration. The hypoxic environment could maintain a tolerogenic DC phenotype. Furthermore, the site of T cell priming outside the heart may play a role. Autoreactive T cell priming by DCs in the spleen after MI has been shown to promote T_{reg} cell generation¹¹², whereas DC-dependent priming of T cells in draining LNs, supported by high levels of hepatocyte growth factor (HGF) and CXCL10, may drive a sustained recruitment of pathogenic T cells¹⁰⁰ (Fig. 2b).

Although CD4⁺ T cell depletion is protective in the acute phase of MI¹⁰⁵, total CD4⁺ T cell deficiency in mice impairs long-term cardiac remodeling and the recovery of heart function, suggesting that CD4⁺ T cells have an overall protective role¹¹³. Indeed, a series of experimental studies point to a protective role of T_{reg} cells in experimental MI^{114,115}, and low levels of circulating CD4⁺Foxp3⁺ T_{reg} cells in humans correlate with increased risk of acute coronary events at follow-up¹¹⁶. Cardioprotective effects of T_{reg} cells are diverse and include the regulation of adaptive and innate immune responses, their impact on stromal cells, modulating fibroblast activation and matrix deposition^{99,115}, and their regulation of cardiomyocyte

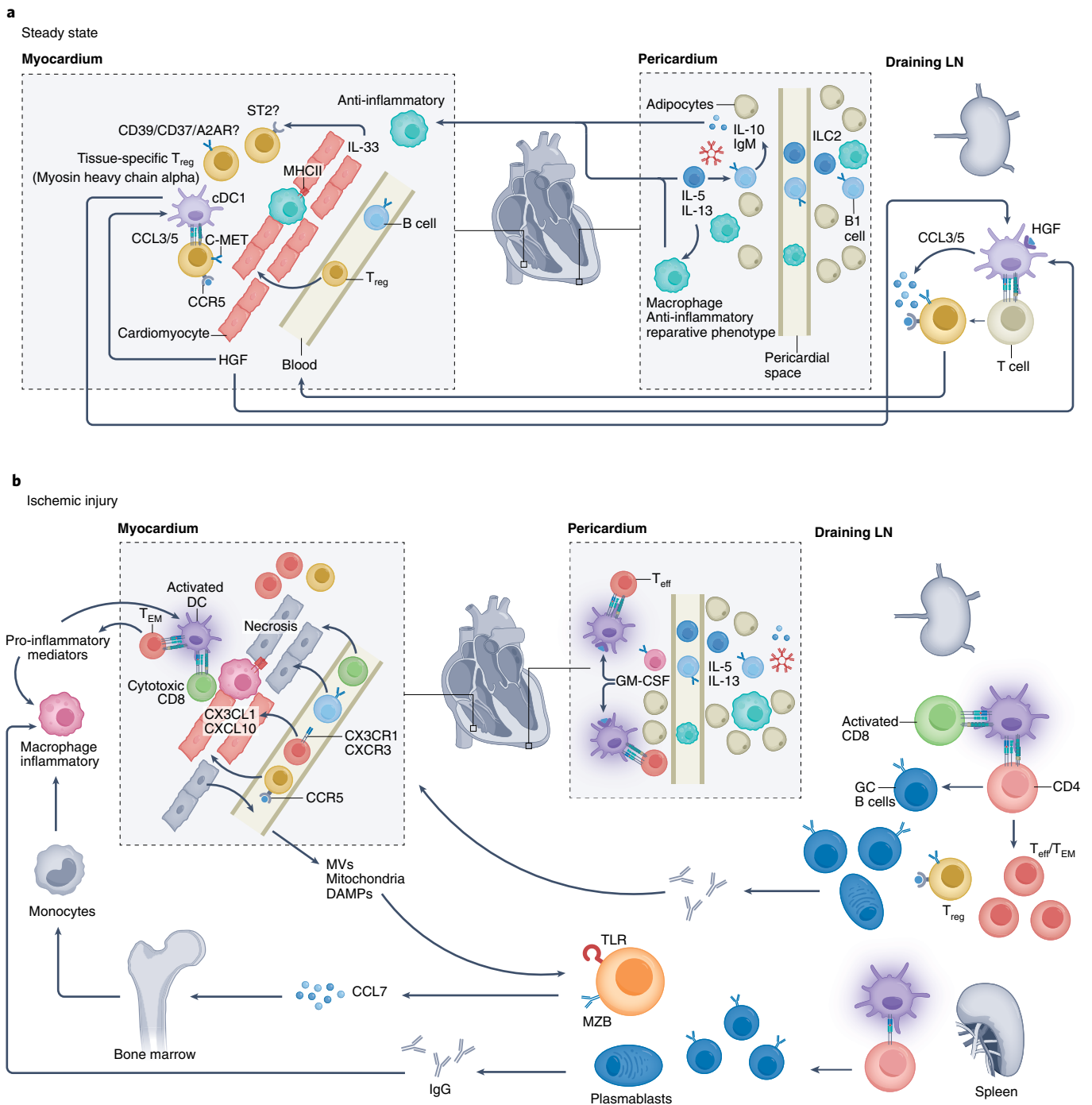


Fig. 2 | Adaptive immune responses in the healthy and ischemic heart. The heart and its adjacent pericardial and adipose tissue harbor several types of immune cells. **a**, At steady state, conventional DCs (cDCs), particularly cDC1, home to draining LNs and instruct the generation of tissue-specific (for example, myosin heavy chain- α) T_{reg} cells¹⁴⁶, which establish peripheral tolerance. Heart-derived HGF binds to DCs and induces an immune-regulatory phenotype. HGF also promotes chemokine production (for example, of CCL5) by c-MET-expressing T cells, which drives CCR5-dependent recruitment and cardiac T_{reg} cell accumulation. T_{reg} cell immunosuppressive function is further promoted through a local (cardiac) paracrine/autocrine adenosinergic loop, involving CD39/CD73 and adenosine A2A receptor (A2AR), or by engaging ST2 signaling with heart-derived IL-33. Pericardial innate lymphoid cells type 2 (ILC2)-derived IL-5 and IL-13 activate innate-like B cells to produce natural IgM antibodies and anti-inflammatory IL-10, which contribute to an anti-inflammatory and reparative macrophage phenotype. **b**, During ischemic injury, DCs acquire an inflammatory phenotype that provides instructions for the development of T_H cells, T_{eff} cells and T_{EM} cells, as well as CD8⁺ cytotoxic T cells. Enhanced production of GM-CSF by pericardial innate-like B cells further promotes DC activation and the generation of T_H cells and T_{eff} cells. These T cells upregulate CX3CR1 and CXCR3, and their recruitment into the ischemic heart is facilitated by heart-derived CX3CL1 and CXCL10. GC reactions develop in the spleen and draining LNs, leading to the production of (heart-specific) autoantibodies, with potential detrimental consequences. Splenic MZ B cells are activated by the release of DAMPs, including in the form of mitochondria-containing microvesicles, which activate Toll-like receptors (TLRs), leading to CCL7 production. The latter promotes inflammatory monocyte mobilization from the bone marrow, enhancing the accumulation of inflammatory macrophages within the ischemic myocardium. In most cases, the ischemic heart is able to maintain the presence of a sufficient number of T_{reg} cells to prevent the occurrence of overt full-blown cardiac autoimmunity.

Box 5 | General therapeutic considerations in people with atherosclerotic disease

Treatment of modifiable classical risk factors will remain an essential component of therapeutic management. This is particularly the case for lipid-lowering therapies, which will continue to evolve with the aim to ensure early, substantial and sustainable reduction of circulating lipid levels. However, not all lipid-lowering therapies are equal in terms of their potential impact on the immuno-inflammatory response, and consideration must be given to whether specific lipid-lowering therapies could be more appropriate than others in particular settings. For example, SLE is characterized by an increased type I interferon response, which reduces the intracellular cholesterol-synthesis pathway¹⁶³. This is a vicious circle, given that reduced cholesterol synthesis drives more type I interferon production¹⁶³. Will statin therapy be the most effective strategy to reduce plasma LDL-C levels in SLE, or might individuals with SLE derive greater benefit from another LDL-C-lowering (PCSK9-targeted) strategy? We believe this question merits appropriate investigation.

Others have suggested that sustained reduction of circulating lipid levels could make anti-inflammatory therapy redundant. We believe this is unlikely to be the case. A large proportion of individuals recruited in contemporary trials still show a residual inflammatory risk (on-treatment high-sensitivity C-reactive protein (hsCRP) level above 2 mg/L)¹⁶⁴, and are at substantial lifetime risk of cardiovascular events. Experimental models have established inflammation as an obligatory pathway for atherosclerosis development. Therefore, appropriate targeting

of this biological process is expected to yield exceptional benefits.

We would also like to stress the fact that hsCRP, although currently useful, is certainly not the optimal marker to stratify individuals for anti-inflammatory therapy. Circulating levels of hsCRP are exclusively dependent on the IL-6 pathway. Atherosclerosis is unlikely to be exclusively driven by IL-6, and individuals with low hsCRP (<2 mg/L) may still mount IL-6-independent inflammation and be susceptible to atherosclerosis and cardiovascular complications. Moreover, increased hsCRP may preferentially reflect a state of metabolic inflammation in the liver, rather than vascular inflammation. Thus, the optimal circulating marker of plaque inflammation is still to be found. This is particularly true for markers of adaptive immune responses, which will require the development and measurement of antigen-specific responses (for example, antigen-specific tetramers, autoantibodies).

A final general consideration relates to the increased risk of serious infections that could result from the long-term use of anti-inflammatory therapies. This was indeed responsible for halting the development of canakinumab as a potential therapy for people with ACVD. We believe the infectious risk is minimal with the use of therapies that target antigen-specific adaptive immunity compared with therapies that target broad systemic inflammatory pathways. Furthermore, T_{reg} cell suppression of autoreactive T cells does not compromise, and may even boost, T cell immunity directed toward infectious non-self antigens.

apoptosis and proliferation¹¹⁷. Despite the relative preservation of T_{reg} cells at the acute phase of MI, a subset of them may become dysfunctional and potentially pathogenic over time¹¹⁸. Those cells appear to have lost their suppressive properties, upregulated the expression of proinflammatory mediators (TNE, TNFR1, IFN γ) and acquired anti-angiogenic properties¹¹⁸. The molecular pathways responsible for these alterations require further investigation.

B cell responses to cardiac ischemic injury. B cells, including B1-like and B2-like cells, represent a large portion of resident leukocytes in the myocardium^{119,120}. They represent circulating cells that slowly transit through the myocardium to support cardiac homeostasis. Additional resident B1-like cells are found in the pericardial adipose tissue as part of FALCs^{121,122} (Fig. 2a). Intravascular myocardial B cells appear to support the expression of MHC class II on resident cardiac macrophages¹²³. However, the (patho)physiological relevance of this finding is currently unclear. Studies in mice and rats have shown that within 7 days following MI, more B cells accumulate in the myocardium and pericardial FALCs^{121,124}. Pericardial B cells produce GM-CSF post-MI and promote CCR7-mediated DC migration and T cell activation in pericardial adipose tissue, with detrimental consequences on post-MI remodeling and recovery of heart function¹²¹ (Fig. 2b). Systemic B cells, and more particularly MZ B cells, also become activated early after MI, and their depletion improves cardiac remodeling and the recovery of heart function^{124,125}. The detrimental effect of mature B cells is attributed to their proinflammatory role, producing CCL7 and promoting monocyte mobilization and recruitment^{124,125} (Fig. 2b). Except for the production of certain natural IgM antibodies against non-myosin heavy chain II that promote myocardial damage¹²⁶, the immediate effects of B cells are unlikely to depend on specific antibodies. However, following the acute phase, myocardial ischemia triggers GC formation in draining mediastinal LNs⁴ (Fig. 2b). Such newly induced autoantibodies, such as those against cardiac myosin, have been associated with post-MI heart failure¹²⁷. Thus, it is possible

that MI leads to the emergence of autoantigens released during myocardial damage, which in turn trigger autoimmune responses and memory B cells that worsen cardiac function and may even accelerate atherosclerosis post-MI⁴. The latter is particularly relevant in the setting of secondary prevention. However, not all B cells are detrimental. IL-10-producing B cells of the pericardial adipose tissue may help in inflammation resolution and heart recovery post-MI¹²² (Fig. 2).

Targeting adaptive immune responses for patient benefit

A few important points should be considered regardless of which therapeutic strategy is pursued to target the immune response in ACVD (Box 5).

Harnessing the adaptive immune system for patient benefit could be pursued through various modalities. The therapeutic objective is to break the vicious circle of pathogenic adaptive immune activation and promote regulatory homeostatic immunity. Here, we will highlight a few immunomodulatory approaches in development (Fig. 3).

Deleting/neutralizing proatherogenic immunity. *Targeting pathogenic B cells.* CD20-mediated B cell depletion reduces atherosclerosis, MI remodeling and post-MI accelerated atherosclerosis in experimental models^{4,74,75,124}. CD20 antibodies preferentially deplete B2 cells and preserve atheroprotective B1 cells. The RITA-MI phase 1/2a trial tested the safety and tolerability of a single intravenous injection of rituximab in people with acute ST-elevation MI (STEMI)¹²⁸. The treatment led to a rapid and dose-dependent reduction in circulating B cells, and echocardiographic data suggested that LV-ejection fraction was improved after 6 months. This impact on cardiac remodeling is now being tested in the phase 2b RITA-MI2. A phase 2 trial of rituximab in people with systolic heart failure is also underway (NCT03332888).

Belimumab is approved for treatment of SLE. It targets BAFF, a cytokine of the BAFF-APRIL system that is essential for B2 cell

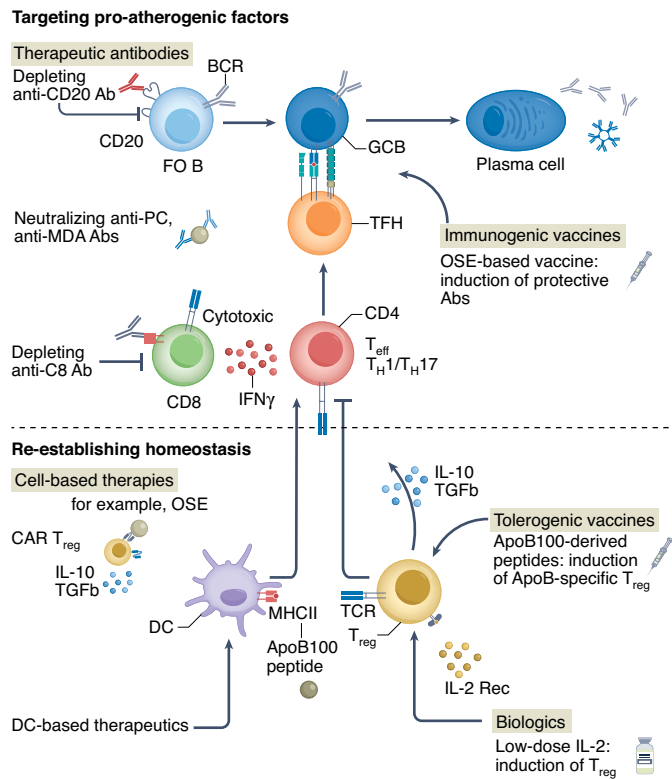


Fig. 3 | Therapeutic strategies to target adaptive immune responses.

The figure, which is not exhaustive, highlights some of the most promising therapeutic strategies to manipulate the adaptive immune system (through neutralizing pathogenic or promoting regulatory immunity) for the benefit of individuals with, or at risk of, ischemic cardiovascular diseases. Current strategies tested in the clinic include the use of a monoclonal antibody (Ab) against CD20 (that is, rituximab) to deplete mature B cells, and the use of recombinant (rec) low-dose IL-2 to promote T_{reg} cells. Promising strategies in development include the development of CD8-depleting monoclonal antibodies, neutralizing antibodies to block proinflammatory OSEs, such as anti-PC and anti-MDA antibodies, CAR- T_{reg} cells targeting OSEs, RNA-based immunogenic vaccines targeting OSEs and RNA-based tolerogenic vaccines using ApoB100-derived peptides. FO B, follicular B cell; GCB, GC B cell.

survival. Although targeting of BAFFR was atheroprotective in mice⁷⁶, BAFF neutralization was proatherogenic owing to non-canonical atheroprotective effects of BAFF signaling in myeloid cells that inhibit TLR9–IRF7-dependent inflammatory responses¹²⁹. Thus, caution is warranted regarding this specific B cell-depletion approach.

Several other B cell-depleting agents are being developed (some, for example, target CD22 and CD19), but they may also deplete protective subsets, and data in experimental atherosclerosis are lacking.

Monoclonal anti-OSE antibodies. Neutralizing different OSEs, such as MDA or PC, with specific humanized monoclonal antibodies has been successful in preclinical ACVD models^{87,88}. The efficacy of anti-MDA antibodies in humans has been tested in the GLACIER trial, which assessed aortic ¹⁸F-FDG-PET uptake in people receiving orticumab (human anti-MDA-ApoB100 IgG1) over a period of 12 weeks¹³⁰. Although this trial did not show an effect, two randomized double-blind placebo-controlled phase 2 trials with different study designs are underway. NCT04776629 is investigating the effect of orticumab on coronary plaque burden in individuals with psoriasis who have increased CVD risk, and NCT03991143 is investigating the effect of a human IgG1 against PC (ATH3G10) on CV

outcomes in participants with STEMI. Future approaches could employ RNA-based technologies to produce sustained levels of protective antibodies.

Immunogenic vaccination strategies. A range of vaccination strategies that raise anti-OSE antibodies are atheroprotective in animal models^{40,86,131,132}. In particular, the molecular mimicry between PC and the capsular polysaccharide of *Streptococcus pneumoniae* has been exploited to trigger high levels of atheroprotective anti-PC IgM antibodies in *Ldlr*^{-/-} mice by immunizing them with pneumococcal extracts¹³¹. This prompted the idea to translate these findings and evaluate whether vaccination with approved polyvalent pneumococcal vaccines also induces anti-PC antibodies and reduces cardiovascular events. However, PC is not a major constituent of pneumococcal vaccine preparations, and small studies using the 13-valent conjugate pneumococcal vaccine Prevnar 13 failed to show a robust induction of anti-oxLDL or anti-PC antibodies, and special vaccination protocols may be required^{133,134}. The effect of the 23-valent vaccine Pneumovax 23 on cardiovascular outcomes (acute coronary syndrome (ACS), ischemic stroke) is currently being tested in the randomized placebo-controlled AUSPICE trial¹³⁵. It will be interesting if this study confirms the cardioprotective signals from a meta-analysis of several observational studies¹³⁶.

Re-establishing immune homeostasis. IL-2-based therapeutics. The concept of low-dose IL-2 therapy in CVD¹³⁷ builds on the essential role of IL-2 in promoting T_{reg} cells and the exquisite sensitivity of these cells to ultra-low doses of IL-2. IL-2 also limits the switch of autoreactive T_{reg} cells to T_{eff} cells that could result from TCR overactivation⁵⁹. A first proof-of-concept safety and biological efficacy trial, LILACS, was completed. Low-dose IL-2 significantly expanded T_{reg} cells in people with stable and unstable ischemic heart disease without adverse events of major concern¹³⁸. A dose of 1.5×10^6 IU was selected and is currently being tested in IVORY (NCT04241601), a randomized, double-blind, placebo-controlled trial testing the superiority of low-dose IL-2 in reducing vascular inflammation in individuals with ACS. Low-dose IL-2 therapy may have protective effects beyond T_{reg} cells. In LILCAS, low-dose IL-2 increased the activation of ILC2 (ref. ¹³⁹), an immune-cell population with protective roles in atherosclerosis³¹ and cardiac remodeling post-MI¹³⁹.

Other IL-2 biologics. More targeted approaches to enhance IL-2 selectivity are being developed; for example, an antibody–IL-2 conjugate that induces a conformational change resulting in lower affinity for IL-2R α and competitive advantage for T_{reg} cells¹⁴⁰. Other approaches include IL-2 muteins with enhanced T_{reg} cell selectivity¹⁴¹, some of which are being tested in clinical trials (NCT03422627; NCT03451422; NCT03410056). Cell-therapy approaches are also in development using engineered T cells that express defined ligand (normal or mutated IL-2)–receptor (mutated versions of the IL-2R subunits) complexes, and that signal exclusively together¹⁴².

Cell-therapy-based approaches. Chimeric antigen receptor (CAR) expression renders a population of T cells uniformly specific for a defined antigen and has revolutionized T cell therapy. Engineered CAR- T_{reg} cells that express scFv antibodies directed against atherosclerosis-relevant epitopes, such as OSEs, are expected to preferentially home to disease sites and suppress local inflammation. Such CAR- T_{reg} cells may also be engineered to express their own IL-2, further stabilizing them and enhancing their efficacy. Technological breakthroughs allowing for the use of allogeneic cells would overcome major hurdles in translating this technology to the clinic.

Tolerogenic vaccination strategies. The discovery that T_{reg} cells are atheroprotective¹⁴³ opened the possibility for therapeutic strategies

that promote antigen-specific T_{reg} cells¹⁴⁴. Several tolerogenic strategies have been tested, particularly using MHC class II-restricted ApoB peptides, and these were shown to reduce atherogenesis, likely in a T_{reg} cell-dependent manner^{45,145}. Unfortunately, translation of this knowledge to the clinic has been very slow. This is due to several factors, including but not limited to insufficient knowledge and characterization of the adaptive immune response in human atherosclerosis and the need for correct HLA class II matching for peptide-based vaccines, as well as uncertainty about the vaccination route and the type of adjuvant needed to induce protective autoantibodies (while preventing the generation of pathogenic T cells and autoantibodies), or required to elicit a tolerogenic T_{reg} cell response. Increasing knowledge of the adaptive immune response of human atherosclerosis, boosted by the advent of single-cell multiomics technologies, along with the revolution in vaccine design and delivery, as shown by the exceptional efficacy of combining RNA and lipid nanoparticle technologies to combat COVID-19, will help overcome these hurdles and will accelerate the development of various immunogenic and tolerogenic vaccines to prevent and treat ACVD.

Perspectives and conclusion

Despite unequivocal evidence for a fundamental role of the immune system in ACVD, newly developed anti-inflammatory treatments have been shown to be incremental or ineffective⁵. We believe that two major areas with fundamental gaps in knowledge deserve particular attention.

Despite substantial evidence from GWASs for a major role of inflammation in human atherosclerosis, there is a huge gap in characterizing the causal, mechanistic pathways. We operate at a distance from biology, and we desperately lack the comprehensive cell-type and disease-specific phenotyping information that will enable us to produce useful knowledge.

The second gap is due to our reliance on an outdated morphological classification of atherosclerosis. We need to move toward the medicine of the future and establish a molecular classification of atherosclerosis. This will require comprehensive insight at single-cell resolution by studying healthy and diseased arteries, at all major arterial sites and across space and time. Deep characterization of antigen-specific T cells and B cells, along with their antigens and antibodies, must be conducted in a systematic way, at different anatomical sites (arteries, SLOs, ATLOs and blood), in large numbers of individuals and at various time points of the disease process. This effort will be facilitated by the continuous development of advanced single-cell technologies and computational analysis, coupled with powerful antigen-screening libraries and advanced proteomics, allowing for the prioritization of atherosclerosis-specific immune receptors and their relevant antigens and antibodies. These can then be harnessed for the development of transformative diagnostic and therapeutic strategies. The exceptional recent advances in RNA-based and cell-based therapies are expected to further facilitate and accelerate the translation of this knowledge into benefit for patients.

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Competing interests

The authors declare no competing interests.

Additional information

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