Atherosclerosis is the major cause of acute myocardial infarction (AMI) and stroke, thus accounting for approximately 40% of mortality in developed countries. It is a chronic inflammatory disease affecting large- and mid-sized arteries resulting in development of focal thickenings (plaques) of the arterial intima, preferentially involving branching points and arterial sites exposed to low and/or oscillatory shear stress. Atherosclerotic plaques are characterized by accumulation of lipoprotein-derived cholesterol, inflammatory cells, smooth muscle cells, connective tissue, calcium and neovascularization [1]. Plaques with extensive inflammation, cell necrosis, connective tissue degradation and enhanced neovascularization, also called high-risk plaques or vulnerable plaques, are more likely to trigger acute vaso-occlusive events compared with fibrotic and heavily calcified plaques [2]. Stabilization of such high-risk plaques is the major therapeutic aim in patients with established cardiovascular disease (CVD). Current therapies focusing on risk factor reduction, including lipid-lowering and antihypertensive treatments, have been shown to reduce the risk of ischemic cardiovascular events by up to 40–50% in randomized clinical trials. However, it is difficult to achieve additional effects by risk-factor modification alone, leaving a significant majority of treated subjects without effective protection, emphasizing the need for the development of novel therapies directly targeting the atherosclerotic disease process in the arterial wall. This review discusses the possibilities of developing such therapies based on modulation of immune responses against plaque antigens through using vaccines.

**Keywords:** atherosclerosis • autoimmunity • immunomodulation • low-density lipoprotein • regulatory T cells • vaccine

**Lipids & plaque inflammation**

Inflammation localized to the arterial intima is a key factor responsible for the development and progression of atherosclerosis. There is strong evidence from both human autopsy studies and experimental models of atherosclerosis that this inflammation is initiated by an accumulation of low-density lipoprotein (LDL) in the arterial wall. Oxidation of atherogenic lipoprotein particles trapped in the extracellular matrix of
the arterial wall is associated with the generation of a number of reactive aldehydes, lipid peroxides and phospholipids that activate endothelial expression of adhesion molecules [3]. This leads to the recruitment of monocytes and T cells into the arterial intima where the monocytes differentiate into scavenger receptor-expressing macrophages that ingest oxidized LDL. Accumulations of cholesterol-filled macrophages (so-called foam cells) constitute the first stage of atherosclerosis development, the fatty streak. In situations of continued arterial lipid deposition, the inflammatory process becomes chronic, activating a fibrotic thickening of the arterial intima (Figure 1).

**Toll-like receptors**

The inflammatory process that drives the formation of these early lesions has been shown to depend on activation of innate immune receptors of the Toll-like receptor (TLR) family. TLRs recognize common patterns on microorganisms and play a critical role in the defense against infections [4]. Mice lacking TLRs or TLR signal protein MyD88 are partly resistant to atherosclerosis development [5–8]. These observations indicate that hypercholesterolemia results in the formation of structures that interact with TLRs and that these interactions account for much of early lesion formation. The structures that interact with TLRs in hypercholesterolemia remain to be fully identified but are likely to include oxidized LDL or factors released during LDL oxidation.

**Bioreactive phospholipids**

Lysophosphatidylcholine (lysoPC) and its metabolite lysophosphatic acid (LPA) are other factors that have been proposed to mediate the inflammatory effect of oxidized LDL. LysoPC is formed when oxidized phospholipids in LDL are hydrolysed by Lp-PLA2. Both lysoPC and LPA activate inflammatory responses in cultured vascular cells, and recent studies have shown strong associations between the human atherosclerotic plaque contents of lysoPC, LPA and proinflammatory cytokines [9]. The important role of these phospholipids is further supported by the findings that

![Figure 1. Development of atherosclerotic plaques.](image)

**Figure 1. Development of atherosclerotic plaques.** Circulating LDL particles accumulate in the arterial intima where they become oxidized. This causes an activation of the endothelium, resulting in infiltration of monocytes and T cells. Monocytes differentiate into resident macrophages that take up oxLDL to become foam cells. Some foam cells become apoptotic and die. Lipids and debris from dead macrophages form a necrotic core. Activated macrophages release cytokines and growth factors that stimulate medial smooth muscle cells to migrate into the intima where they help to stabilize the plaque and form a fibrous cap that covers the necrotic core. Macrophages also release matrix-degrading proteases that destabilize the plaque. The stability of the lesion is determined by the balance between extracellular matrix syntheses by smooth muscle cells and the degradation of the matrix by macrophage proteases. The activity of T cells in the lesions is governed by antigen-presenting DCs. When a T cell is presented its antigen by a DC, it will differentiate into a Th1, Th2, Th17 or a suppressive regulatory T cell depending on the cytokines and costimulatory factors expressed by the DC.

DC: Dendritic cell; LDL: Low-density lipoprotein; oxLDL: Oxidized low-density lipoprotein.
treatment with an inhibitor of Lp-PLA2 reduces the development of coronary atherosclerosis in experimental animals [10] and halts the progression of plaque necrotic core expansion in humans [11]. Oxidized LDL may also promote intimal inflammation by toxic injury of arterial cells.

**Modulation of atherosclerosis by adaptive immune responses**

Atherosclerosis has long been accepted as an inflammatory disease, but has only recently been recognized that this inflammation is orchestrated by a complex array of adaptive immune responses (Figure 2). The first indications of an involvement of adaptive immunity in the disease process came from studies performed in the 1980s, demonstrating expression of MHC class II antigen molecules and presence of activated T cells in human atherosclerotic lesions [12]. Subsequent experimental studies in hypercholesterolemic mice with severe immune deficiencies such as severe combined immunodeficiency, Rag-1 and Rag-2 mice yielded conflicting results and suggested a limited role of adaptive immunity in the disease process [13]. In severe combined immunodeficiency/ApoE-/- mice, the development of atherosclerosis was reduced by 70%, and this protection disappeared following transfer of CD4+ T cells from immune-competent mice [14]. However, in Rag-1/ApoE-/- mice, atherosclerosis was reduced only when the mice were given a chow diet but not when they were given a high-fat diet [15]. In Rag-2/ApoE-/- mice, there was no effect on atherosclerosis independent of the diet given [16]. These conflicting results were initially difficult to interpret. However, subsequent studies have revealed that the role of the immune system is much more complex than initially anticipated and that both protective and pathogenic immune responses are involved.

**CD4+ effector T cells**

CD4+ T cells are a heterogeneous group of cells that include both pro- and anti-inflammatory cells (Figure 2). Release of the cytokines IL-12 and IL-18 from antigen-presenting cells promotes naive CD4+ T cells to express the Th1-specific transcription factor Tbet, leading to Th1 cell differentiation. Tbet deficiency reduces atherosclerosis in Ldr+ mice, indicating that Th1 cells are pro-atherogenic [17]. Th1 cells produce proinflammatory cytokines such as IFN-γ and TNF-α. Hypercholesterolemic mice lacking IFN-γ or TNF-α are characterized by reduced atherosclerosis, providing further support of a pro-atherogenic role of Th1 cells [18,19]. Th2 cells represent a lineage of CD4+ T cells that primarily interact with B cells. The role of Th2 cells in atherosclerosis appears to be more complex than that of Th1 cells and both pro- and anti-atherogenic actions have been reported. Th2 cell differentiation is initiated by the Th2 cell signature cytokine IL-4. The role of IL-4 and Th2 cells in experimental atherosclerosis remains to be fully elucidated, and hypercholesterolemic mice deficient in IL-4 have been reported to have reduced, as well as unaltered, atherosclerosis [20,21]. Activated Th2 cells typically also produce the cytokine IL-5 [22–24]. IL-5 has a protective effect on atherosclerosis in hypercholesterolemic mice, a phenomena that has been attributed to the ability of IL-5 to stimulate the synthesis of so-called natural antibodies from B cells of the B1 type [25,26]. These antibodies are IgM that bind to phospholipid epitopes in oxidized LDL and prevent scavenger receptor-mediated uptake of oxidized LDL in macrophages [27].

**Atherogenic immune responses**

![Atherogenic immune responses diagram](image)

**Atheroprotective immune responses**

![Atheroprotective immune responses diagram](image)

**Figure 2. Atherogenic and atheroprotective immune responses.** The inflammatory activity in atherosclerotic lesions is determined by the balance between proinflammatory (atherogenic) and suppressive (atheroprotective) immune responses. Activated DCs presenting peptide antigens can induce formation of Th1 cells in the presence of IL-12 and IL-18, Th2 in the presence of IL-4, and Th17 in the presence of IL-6 and TGF-β. Antigen presentation by nonactivated, tolerogenic DCs induces formation of regulatory T cells while DCs presenting lipid antigens activate NKT cells. Th2 cells interact with B1 cells through secretion of IL-5 and B2 cells through secretion of IL-4.

DC: Dendritic cell; LDL: Low-density lipoprotein; oLDL: Oxidized low-density lipoprotein.
Another CD4+ T-cell type that has recently been implicated in atherosclerosis is the Th17 cell. It is a lineage of T-helper cells distinct from both Th1 and Th2 cells and characterized by expression of the cytokine IL-17 and the transcription factor ROR-γt [27]. The formation of Th17 cells is dependent on IL-6 and TGF-β. They have been shown to play an important role in clearance of extracellular pathogens by recruiting neutrophils and by producing antibacterial peptides, but have also been implicated in several autoimmune diseases. Recent studies have implicated Th17 cells in atherosclerosis, but their exact role in the disease process remains to be fully understood since both pro-atherogenic [28–31] and protective effects [32] have been reported. Th17 cells targeting collagen V have been shown to aggravate atherosclerosis [33] and Xie et al. have suggested that progression of atherosclerosis can occur as a result of an imbalance between Th17 and Tregs [34]. The plasma level of IL-17 correlates with that of IFN-γ in coronary artery disease patients [35]. The same cytokine expression pattern has been identified in coronary artery-infiltrating T cells, suggesting that these cytokines may act synergistically to promote atherosclerosis [35].

Natural killer T (NKT) cells share properties of both CD4+ T cells and NK cells. Many of these cells recognize the CD1d molecule, an antigen-presenting molecule that resembles MHC class I but that instead of peptides binds self and foreign lipids and glycolipids. It is interesting to note that that lysPC has been identified as one of the most common antigens presented on CD1d and that this results in activation of CD1d-restricted, lysPC-specific NKT cells [36]. A functional role of the CD1d–NKT pathway in atherosclerosis is supported by observations of reduced plaque development in hypercholesterolemic mice lacking CD1d. In accordance, activation of NKT cells by a synthetic agonist has been shown to result in increased plaque development [37–39]. NKT cells have also been found to be involved in vascular repair processes [40].

Regulatory T cells
Tregs are immune-inhibitory cells characterized by expression of CD25, the transcription factor FoxP3 and anti-inflammatory cytokines such as IL-10 and TGF-β. They suppress pathogenic immune responses to self-antigens but may also suppress immune responses against foreign antigens such as allergens and dietary antigens [41]. Tregs are found in very low numbers in atherosclerotic plaques as compared with other chronically inflamed tissues [42,43]. This suggests that local tolerance is impaired in the plaques, which could contribute to increased arterial inflammation. Depletion of Tregs through deletion of CD80/86, CD28 or ICOS, as well as anti-CD25 antibody treatment, significantly increases plaque formation [44,45]. Similarly, inhibition of Tregs through deletion of the T-cell receptor for TGF-β markedly enhances the progression of the disease [46], while administration of a clone of ovalbumin-specific Tregs together with its cognate antigen ovalbumin inhibited plaque development in ApoE−/− mice [47]. Inhibition of atherosclerosis in ApoE−/− mice has also been observed in response to transfer of Tregs [48]. Collectively, these studies provide convincing evidence for a protective role of Tregs in experimental atherosclerosis. The authors have recently reported that low baseline levels of Tregs, defined as CD4+FoxP3+ T cells, was associated with an increased risk for development of AMI during a 15-year follow-up of 700 subjects taking part in the cardiovascular substudy of the Malmö Diet and Cancer study [49]. The hazard ratio for suffering a coronary event in the lowest tertile of CD4+FoxP3+ T cells was 1.9 compared with the highest tertile, and this increase in risk was independent of other cardiovascular risk factors. Decreased levels of circulating Tregs have also been reported in patients with acute coronary syndrome [50–53].

CD8+ T cells
The role of CD8+ T cells in atherosclerosis development is less well studied than that of CD4+ T cells. Whereas MHC class I-deficient C57Bl/6 mice on high-fat diet develop increased atherosclerosis [54], CD8+ T-cell deficient as well as MHC class I-deficient ApoE−/− mice have similar lesion size as CD8-competent ApoE−/− mice [55,56]. A prerequisite for specific involvement in the disease process is that the CD8+ T cells are present in atherosclerotic lesions. Indeed, CD8+ T cells are found together with CD4+ T cells in lesions of both mice [57] and humans [58]. In advanced human lesions, they even appear to be the predominating T-cell type [58]. The authors recently reported activation of CD8+ T cells in response to diet-induced hypercholesterolemia in ApoE−/− mice and that this precedes that of CD4+ T cells in lymph nodes draining atherosclerotic lesions [59].

B cells
B cells are present in atherosclerotic lesions, although they are less frequent than T cells [60]. The spleen is a major B-cell reservoir, and splenectomy leads to increased atherosclerosis that can be reversed by B-cell replacement [61]. It has also been shown that hypercholesterolemic mice deficient in B cells (through deletion of the gene encoding the µ-chain of the BCR, µMT) have more atherosclerosis [62]. However, the role of B cells in atherosclerosis remains to be fully elucidated since subsequent studies have shown that blocking B cells by treatment with an antibody against CD20 decreases atherosclerosis development in mice [63,64]. Recent studies by Kyaw et al. have suggested a pro-atherogenic role of B2 but not of B1 cells [65].

Autoantigens in atherosclerosis
LDL-associated antigens
Several lines of evidence suggest that immune responses against LDL-associated antigens are of key importance in atherosclerosis. Antibodies against oxidized LDL are found both in the circulation [66] and in atherosclerotic plaques [67]. Oxidized LDL-specific T cells are present in the circulation [68] and accumulate in human atherosclerotic lesions [69]. Interestingly, Hermansson et al. recently identified the presence of apoB100-reactive CD4+ T cells in ApoE−/− mice, and demonstrated that the deletion of these cells resulted in reduced atherosclerosis, suggesting that immune responses against unmodified LDL might also play an important role in the disease process [70].
Heat-shock proteins
Heat-shock proteins (HSPs) represent a class of autoantigens that have been implicated in atherosclerosis. Human HSP60 shows considerable mimicry with mycobacterial HSP65 and the chlamydial HSP60 [71,72], suggesting that immune responses against microbial HSP could crossreact with HSPs expressed by stressed arterial cells. Immunization of LDL receptor-deficient mice [73] and hypercholesterolemic rabbits [74] with HSP65 has been shown to promote the development of atherosclerosis. In clinical studies, increased titers of antibodies against mycobacterial HSP65 has been found in subjects with established coronary [75] and carotid atherosclerosis [76].

Extracellular matrix antigens
It has been recently shown that reactive aldehydes released during LDL oxidation cause MDA modifications not only of apoB but also of surrounding extracellular matrix proteins. The possibility that these modifications become the target of autoimmune responses is supported by the presence of autoantibodies against aldehyde-modified fibronectin, laminin, collagen type I, type III and tenascin-C in human plasma [77]. Interestingly, the presence of high levels of autoantibodies against MDA fibronectin was associated with a lower risk for development of myocardial infarction in a small, prospective, nested case–control study [77] and immunization of ApoE-/- mice with MDA fibronectin has been found to reduce atherosclerosis development, suggesting that this immune response may be protective [78]. By contrast, immunization of ApoE-/- mice with MDA laminin results in more aggressive development of atherosclerosis [79].

Atherosclerosis vaccines
Immunizations with oxidized & aldehyde-modified LDL
Early studies evaluating the functional role of immune responses against oxidized LDL were based on immunization with oxidized or MDA-modified LDL (MDA is one of the dominant antigens in oxidized LDL) in hypercholesterolemic rabbits [80–82]. The studies were designed to test the hypothesis that activating immunity against oxidized LDL would result in a more aggressive development of atherosclerosis. However, these studies unexpectedly revealed that stimulating immune responses against oxidized or MDA-modified LDL was associated with a partial protection against atherosclerosis. This finding was subsequently confirmed by a number of studies performed in ApoE-/- and ldlr-/- mice [83–85]. Interestingly, with the results of the targeted immune-deficiency studies in hypercholesterolemic mice discussed earlier now at hand, it is now clear that the hypothesis originally put forward by the authors identified three 20-amino acid long apoB peptides (Table 1) that when used for immunization together with an albumin carrier and aluminium hydroxide (Alum) as adjuvant reduced the development of atherosclerosis by up to 60–70% in ApoE-/- mice [87–89]. A vaccine containing one of these peptide sequences (p210) is currently in final preclinical development for human safety and efficacy studies.

Mode of action of apoB peptide vaccines
The possible mechanisms involved in the protective effects of apoB peptide vaccines are outlined in Figure 2. Immunization with the MDA-modified apoB peptide p45 (amino acids 661–680) has been shown to be associated with the generation of MDA-p45 specific IgG [88]. To investigate the role of these antibodies, recombinant human IgG with the same specificity were generated. Treatment of ApoE-/- mice with these antibodies was found to inhibit the development of atherosclerosis, reduce inflammation of remaining plaques and to facilitate plaque regression when applied in combination with lipid lowering, suggesting that the protective effects of apoB vaccines may be explained at least in part by the generation of peptide-specific IgG [90,91]. The possible atheroprotective effect of these antibodies in humans is presently being investigated in the GLACIER trial.

Interestingly, subsequent studies revealed that apoB peptide vaccines can inhibit atherosclerosis without activating an antibody response, suggesting that other mechanisms also could be of importance [92]. The observation by Chyu et al. that the atheroprotective effect of apoB peptide immunization is absent...
in splenectomized mice but may be conveyed to nonimmunized mice via adoptive transfer of splenocytes from apoB peptide immunized mice indicated the involvement of cellular immunity [89]. Subsequent studies have identified several possible cellular mediators of the protective effect of apoB peptide immunization. Wigren et al. showed that immunization with apoB peptides is associated with a relative expansion of Tregs and that concomitant treatment with CD25-blocking antibodies inhibited both the Treg expansion and the atheroprotective effect of the vaccine [98]. These observations are indicative of an involvement of Tregs in mediating the effect of apoB peptide vaccine but does not exclude a role for other CD25-expressing cells. In another cell transfer study, Chyu et al. found that transfer of CD8+ Tcells, but not CD4+ T cells, isolated from the spleens of the apoB peptide immunized mice recapitulated the protective effect of immunization [94]. The latter study suggests that the protective effect of apoB peptide vaccines is mediated by CD8+ T cells rather than Tregs. However, since Tregs have been shown to lose their suppressive properties following adoptive transfer [95], it is possible that the lack of effect of CD4+ T-cell transfer in the latter study is explained by this phenomenon.

Alternative strategies for development of LDL tolerogenic vaccines

The identification of the protective role of Tregs in atherosclerosis has stimulated the development of tolerogenic vaccines activating LDL-specific Tregs. From a theoretical perspective, this approach has the advantage of being able to direct the suppressive action of Tregs to locations of LDL accumulation rather than inducing a systemic immune suppression that could compromise infection and tumor defenses. Mucosal delivery of antigens is a well-established approach to induce tolerance, and studies by van Puijvelde et al. have shown that oral administration of oxidized LDL results in generation of oxidized LDL-specific Tregs and inhibition of atherosclerosis [96]. Klingenberg et al. developed an intranasal vaccine containing a recombinant fusion protein encompassing the native apoB sequence 3136–3155 (p210) and the B-unit of cholera toxin (CTB) [97]. CTB promotes uptake of antigens via the nasal and oral mucosa and CTB antigen conjugates have been used to induce tolerance in a variety of autoimmune diseases. Immunization with p210–CTB for 12 weeks caused a reduction in aortic lesion size in ApoE-/- mice. This effect was accompanied by induction of Tregs that markedly suppressed effector T cells rechallenged with apoB100 and increased numbers of IL-10 and CD4+ T cells.

An alternative strategy used by Herbin et al. to activate apoB100 tolerance applied subcutaneously implanted mini-osmotic pumps to continuously administer low doses of apoB100 peptides in absence of adjuvant [98]. This treatment was shown to reduce lesion development in young ApoE-/- mice and to completely halt atherosclerosis progression in older ApoE-/- mice. The protective effect was associated with activation of apoB100-specific Treg response and inhibited by depletion of Tregs.

As discussed earlier, the existence of apoB-reactive T cells was recently discovered. The initial identification of these cells came from studies in which in human apoB100 transgenic mice were immunized with oxidized LDL and T-cell hybridomas subsequently generated by fusing cells from draining lymph nodes with thymoma cells [70]. Unexpectedly, it was found that several of the generated hybridomas were specific for apoB100, whereas none reacted to oxidized LDL. The apoB-reactive T-cell hybridomas were all characterized by expression of the T-cell receptor variable β-chain TRB31. The authors subsequently demonstrated that immunization with a TRB31-derived peptide blocked T-cell recognition of apoB100 and inhibited the development of atherosclerosis, providing a novel vaccine approach for suppression of autoimmune responses against LDL.

An additional strategy that has been used to induce tolerance against LDL antigens is through dendritic cell (DC) therapy. DCs can be isolated and kept in cell culture under conditions that stimulate expression of costimulatory cell surface receptors and cytokines that will lead to the generation of either pro-inflammatory Th1 cells or suppressive Tregs. DCs that have been pulsed with MDA-LDL in presence of the TLR4 activator LPS and subsequently transferred into ApoE-/- mice have been shown to aggravate atherosclerosis development [99]. By contrast, transfer of DCs pulsed with apoB in the presence of IL-10 has been found to inhibit disease development [100].

Immune responses against phospholipid antigens in LDL

Immune responses against LDL also target nonpeptide structures such as phospholipids. One antigen that has been found to be of particular importance is phosphatidylcholine (PC), which is present on the surface of oxidized LDL. PC is recognized by both scavenger receptors and a type of germline-encoded IgM antibodies referred to as natural antibodies [101]. Removal of damaged lipoproteins through scavenger receptors and natural IgM probably plays an important role in reducing inflammation and injury to the surrounding tissue. Treatments with the PC-specific T15-type of natural antibodies have been shown to inhibit the development of vein graft atherosclerosis in ApoE-/- mice [102]. Immunization of ApoE-/- mice against PC using a Streptococcus pneumoniae vaccine induced a PC–antibody response and inhibited the development of atherosclerosis [103]. Similar observations were made in ApoE-/- mice immunized with PC linked to a carrier protein [104]. Accordingly, it may be possible to develop atherosclerosis vaccines based on the PC epitope or to enhance the efficacy of atherosclerosis vaccines based on other antigens through PC modification of these antigens.

Traditional immunization with HSP using subcutaneous administration of the antigen is associated with an enhanced development of atherosclerosis as discussed earlier. Several investigators have studied if the response can be shifted into antheroprotective tolerogenic response through mucosal administration of the antigen. Harats et al. reported that oral administration of HSP65 inhibits atherosclerosis in LDL receptor deficient mice [105]. Similar observations were made by Maron et al. using both oral and nasal administration of the antigen [106]. Subsequent studies by van Puijvelde et al. showed that the protective effect of mucosal immunization with HSP65 could be explained by generation of antigen-specific Tregs releasing IL-10 and TGF-β [107]. Interestingly, Lu et al. have reported that subcutaneous
immunization with a combination of peptides derived from apoB and HSP60 is atheroprotective [108].

There have also been attempts to develop vaccines for atherosclerosis targeting immune responses against aldehyde-modified extracellular matrix proteins. The results of these studies have been inconsistent, with increased development of atherosclerosis in response to immunization with MDA laminin [79], while immunization with MDA fibronectin was associated with partial protection against atherosclerosis development [78]. However, the interpretation of the latter finding was complicated by the fact that immunization with native fibronectin also reduced atherosclerosis and that immunization with native as well as MDA fibronectin markedly reduced the levels of both fibronectin and cholesterol in plasma.

**Role of adjuvants & carriers**

It is well known that both adjuvants and the carrier molecules required for small peptide antigens critically influence the effectiveness and functional characteristics of vaccines. However, unexpectedly it has also been found that adjuvants can have direct effects on atherosclerosis. Khallou-Laschet et al. were the first to report atheroprotective effects of several adjuvants including Alum [109]. The authors could subsequently demonstrate that treatment of *ApoE*−/− mice with Alum results in activation of Tregs, and that this was mediated by facilitating uptake of oxidized LDL antigens by tolerogenic antigen-presenting cells [110].

**Clinical evidence for a role of immunity in CVD**

There is still limited clinical data on the role of immunity in CVD. HLA genotypes have been associated with risk for development of several types of inflammatory diseases with autoimmune characteristics, with increased prevalence of HLA-DR3 and -DR4 in Type 1 diabetes, HLA-DR2 and -DR3 in systemic lupus erythematosus and HLA-DR1 in rheumatoid arthritis [111]. Similar associations also exist in CVD but are less pronounced. In a population-based cohort of 1188 AMI patients and 1191 matched healthy controls, the authors found that the HLA-DRB1*1001 allele was associated with increased risk for AMI (odds ratio: 1.24; 95% CI: 1.00–1.54), while the DRB1*07 and DQA1*02 alleles (odds ratio: 0.78; 95% CI: 0.65–0.95 for both) conferred protection. A polymorphism in *MHC2TA*, the MHC class II transactivator gene, resulting in decreased expression of MHC class II on activated leukocytes, has also been linked to increased susceptibility to AMI [112].

A large number of studies have been carried out to determine the association between autoantibodies against oxidized LDL and CVD. High levels of circulating autoantibodies have been reported to be associated with both less and more severe atherosclerosis [66]. The reason for these inconsistencies remains to be fully clarified but may involve difficulties in standardizing the LDL antigens used in the antibody assays. One approach to overcome the standardization problem has been to determine autoantibodies against specific native or MDA-modified apoB100 peptide sequences. These studies have primarily used the p45 and p210 apoB100 peptides (Table 1) used in the development of immune-modulatory therapies discussed earlier. High levels of autoantibodies against these apoB100 peptides have been associated with a less severe atherosclerosis in the coronary and carotid arteries as well as with a lower risk for AMI [113–116].

**Expert commentary**

Although the currently available therapies for prevention of acute cardiovascular events focusing on risk-factor intervention have proven very effective, they still leave the majority of treated subjects without effective protection, emphasizing the need for development of novel therapies directly targeting the atherosclerotic disease process in the plaque. Such therapies should preferentially act through specific inhibition of plaque inflammation, and downregulation of proinflammatory autoimmune responses against plaque antigens represents a promising approach to achieve this. Several tolerogenic plaque-antigen vaccines have been shown to be effective in animal models of atherosclerosis. However, the challenge in translating these results into clinically effective therapies should not be underestimated. Most of the available knowledge of the role of immunity in atherosclerosis is based on studies performed in mice, and our understanding of the importance of these disease mechanisms in humans remains limited. There is an urgent need for studies identifying and validating immune biomarkers for cardiovascular risk in humans as well as to develop biomarkers that can be used to monitor the effect of atherosclerosis vaccines in clinical trials. Another important limitation of the animal studies is that they with few exceptions have demonstrated the effect of vaccines on early development of atherosclerosis rather than on the more clinically relevant advanced plaques. It is not unlikely that strategies for immune-modulatory therapy targeting advanced established plaques need to be different from those aiming to prevent early stages of disease. In this context, it is encouraging to note that Herbin et al. were able to completely halt the progression of advanced atherosclerosis by subcutaneous infusion of apoB peptides [98]. Despite these limitations, it is clear that downregulation of plaque inflammation through treatment with tolerogenic plaque-antigen-specific vaccines represents one of the most promising approaches for development of novel cardiovascular therapies that can act on top of current risk-factor lowering medications.

**Five-year view**

Recent research has provided a detailed characterization of the role of the immune system in the development of experimental atherosclerosis in mice. Within the next 5 years, the importance of these immune responses for the human disease process will be clarified and a number of immune-modulatory therapies for treatment and prevention of CVD will have entered early clinical safety and proof-of-concept trials.

**Financial & competing interests disclosure**

J Nilsson and PK Shah are signed as coinventors on patents for the use of apoB peptides in atherosclerosis vaccines. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.
Key issues

- Loss of tolerance against self-antigens in atherosclerotic lesions aggravates local inflammation and promotes plaque growth and destabilization.
- Low-density lipoprotein is the most important auto-antigen in atherosclerosis.
- Antibodies and regulatory T cells help to remove oxidized low-density lipoprotein from plaques and maintain immunological tolerance.
- Generation of such antibodies and regulatory T cells by vaccines represents a novel approach for treatment of cardiovascular disease.
- The role of the immune system in the development of atherosclerosis in humans remains poorly described. This represents an important obstacle in translating emerging findings from experimental studies into clinical application.

References

Vaccines against atherosclerosis

Review


Vaccines against atherosclerosis

Review


