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REVIEW

Immunomodulation of Vascular Diseases: Atherosclerosis and Autoimmunity

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Abstract The autoimmune disease atherosclerosis contributes to several vascular complications. Besides vascular cells, inflammatory cells occur prominently in atherosclerotic lesions; lymphocytes play a detrimental role in the initiation and progression of this common vascular disease. Recent discoveries have led to the identification of several important lymphocyte types within the atherosclerotic lesions. However, peripheral lymphocytes and those in the lymphoid organs both figure critically in the regulation of atherosclerotic lesion growth. Although the concept of atherosclerosis as an autoimmune disease is well known, the ways in which autoantigens and autoantibodies contribute to atherogenesis in human or even in animal models remains largely unknown. For example, autoantigen immunisation can either promote or attenuate atherogenesis in animals, depending on the antigen types and the routes and carriers of immunisation. This article summarises recent findings regarding lesion inflammatory cell types, autoantigens and autoantibody isotypes that can affect the initiation and progression of atherosclerosis from both human and animal studies.
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Atherosclerosis remains the most common cause of vascular complications, including stroke, myocardial infarction (MI) and aortic aneurysms. Data suggest that atherosclerosis also constitutes an autoimmune disease, in which autoantigens and autoantibodies affect this vasculature remodelling process. Processing and presentation of autoantigens and subsequent autoantibody production can occur in lymphoid organs as well as in non-lymphoid tissues such as aortas. In human atherosclerotic lesions, many cells participate in this process, including lymphocytes, macrophages, dendritic cells (DCs), mast cells and even vascular smooth muscle cells (SMCs) or endothelial cells (ECs) (Fig. 1). Many of the inflammatory cells are recruited from the lymphoid organs or the circulation and participate in antigen or autoantigen presentation and T-cell activation. This study discusses our current understanding of how autoantigens and autoantibodies contribute to atherosclerosis in human and animal models.

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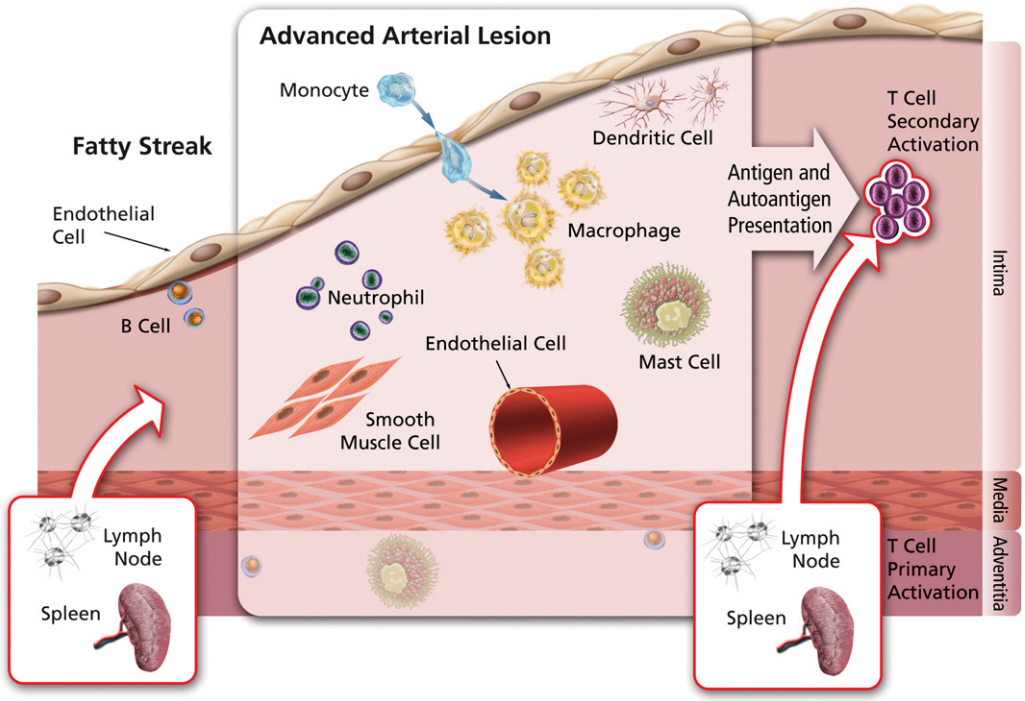

Figure 1 Atherosclerotic lesion professional and non-professional antigen presenting cells and (auto)antigen-mediated T cell activation.

Inflammatory Cells in Atherosclerotic Lesions

The best-studied inflammatory cells in atherosclerotic lesions include T cells, B cells, DCs and macrophages. Few studies focus on other 'minor' inflammatory cells such as mast cells and neutrophils. Besides T cells, all other inflammatory cells can present antigens (antigen presenting cells, APCs) to assist T-cell activation, essential for the early progression of atherosclerosis. In human or animal atherosclerotic lesions, T cells include mainly $CD4^+$ and $CD8^+$ cells, although $CD4^+$ cells dominate in number. It has been suggested that T-cell activation occurs initially in lymphoid organs such as spleen and lymph nodes and then migrates to the aorta for secondary activation by APCs that present the same antigens (Fig. 1). In turn, activated T cells can stimulate other inflammatory cells such as macrophages to secrete inflammatory cytokines, proteases and tissue factors.[1]

$CD4^+$ T cells contain two subsets: Th1 and Th2. In human atherosclerotic lesions, Th1 cells expressing the cytokines interferon-γ (IFN-γ) and interleukin 2 (IL2) prevail over IL4-, IL5- and IL10-producing Th2 cells. T cells isolated from human lesions produce a high amount of IFN-γ but a low amount of IL4.[2] B cells, which occur in much fewer numbers than T cells, appear in the fatty streak or the more external layer of the aortic wall (Fig. 1).[1] Both T and B cells play an essential role in the pathogenesis. A reduction of T and B cells can lead to decreased plaque development.[1] With $CD4^+$ T-cell transfer from immunocompetent to immunodeficient apolipoprotein E knockout *Apoe*$^{-/-}$ mice, disease increases dramatically, whereas atherosclerosis-prone but immunodeficient animals show reduced development of early lesions with the fatty streaks.[3] By contrast, low-density lipoprotein receptor-deficient *Ldlr*$^{-/-}$ mice lacking B cells had 30—40% increase in atherosclerosis, resulting in decreased serum anti-ox-LDL autoantibodies — suggesting that the B cells that make autoantibodies are anti-atherogenic. This explains why mice that go through splenectomy have enhanced atherosclerosis, a phenomenon that splenic B-cell reconstitution can reverse.[4]

In contrast to B cells, DCs occur in the sub-endothelial space with other immunocompetent cells. There, they capture autoantigens then migrate to lymphoid stations, where they present autoantigens to T cells, provoking their responses.[5] Thus, T cells isolated from human atheroma can respond to specific autoantigens while DCs and macrophages can present such autoantigens, giving rise to clonal expansion of their specific T cells and autoantibodies.

Compared with macrophages or lymphocytes, mast cells constitute minor inflammatory cells in human atherosclerotic lesions, although their low numbers do not indicate insignificance. Mast cell inactivation reduces atherosclerotic lesion development in *Apoe*$^{-/-}$mice.[6] Using mast cell-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, we demonstrated that these minor inflammatory cells are indeed critical to diet-induced atherosclerosis in *Ldlr*$^{-/-}$ mice. *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice in the background of *Ldlr*$^{-/-}$ are protected from atherogenesis. Using mast cell reconstitution techniques, we found that mast cells release pro-inflammatory cytokines IL6 and IFN-γ to stimulate vascular cell release of matrix-degrading proteases.[7] Therefore, different inflammatory cells serve different roles in atherogenesis.

Autoantigens

Autoantigens refer to those generated by structure modification on specific moieties of endogenous molecules.

Although these antigens occur in human atherosclerotic lesions, they are not aorta-specific. In atherosclerotic lesions, oxidised LDL (ox-LDL), heat-shock proteins (HSPs) and β2-glycoprotein I (β2-GPI) are the most common autoantigens.

In early atherosclerotic lesions, LDLs are trapped in the sub-endothelial space and subsequently oxidised.[8] Two aldehydes with strong immunogenic properties develop: malondialdehyde (MDA) and 4-hydroxynonenal aldehyde (Fig. 2A and B) with anionic valence. This oxidation process associates with major structural modifications of LDL, including fragments of apo B-100 and generation of various aldehyde and phospholipid adducts to apo B-derived peptides. DCs and macrophages uptake ox-LDL and use major histocompatibility complex (MHC) class II molecules to present ox-LDL for recognition by specific $CD4^+$ T cells and then lead to ox-LDL-specific T-cell expansion.[9] Indeed, about 10% of lesional T cells are ox-LDL-specific,[9] which also appear in circulation. Transfer of ox-LDL-reactive T cells to T-cell-deficient *Apoe*$^{-/-}$*scid/scid* mice occurs more efficiently at lesion acceleration than transfer of T cells without antigen specificity.[10] Besides stimulating T cells via APCs, ox-LDL can promote inflammation by attracting monocytes and more T cells to the lesions. Ox-LDL can also be cytotoxic to the ECs (causing a prothrombotic endothelial surface dysfunction) and stimulate the release of various soluble inflammatory and adhesion molecules. Macrophage scavenger receptors (SRs) remove ox-LDL, leading to ox-LDL macrophage intracellular accumulation and foam cell formation. Clinical studies showed the presence of IgM against MDA-modified apo B-100 peptide and association with plasma ox-LDL levels in humans.[11]

Autoantigen HSP shows high-sequence homology from bacteria to humans. For example, mycobacterial and chlamydial HSP65 show considerable mimicry with human HSP60.[12] Most known risk factors or stressors evoke HSP expression on ECs, SMCs and macrophages to maintain cell viability. In turn, HSP induces production of inflammatory cytokines and proteases from macrophages and mediates monocyte adhesion to the endothelium. In subjects with

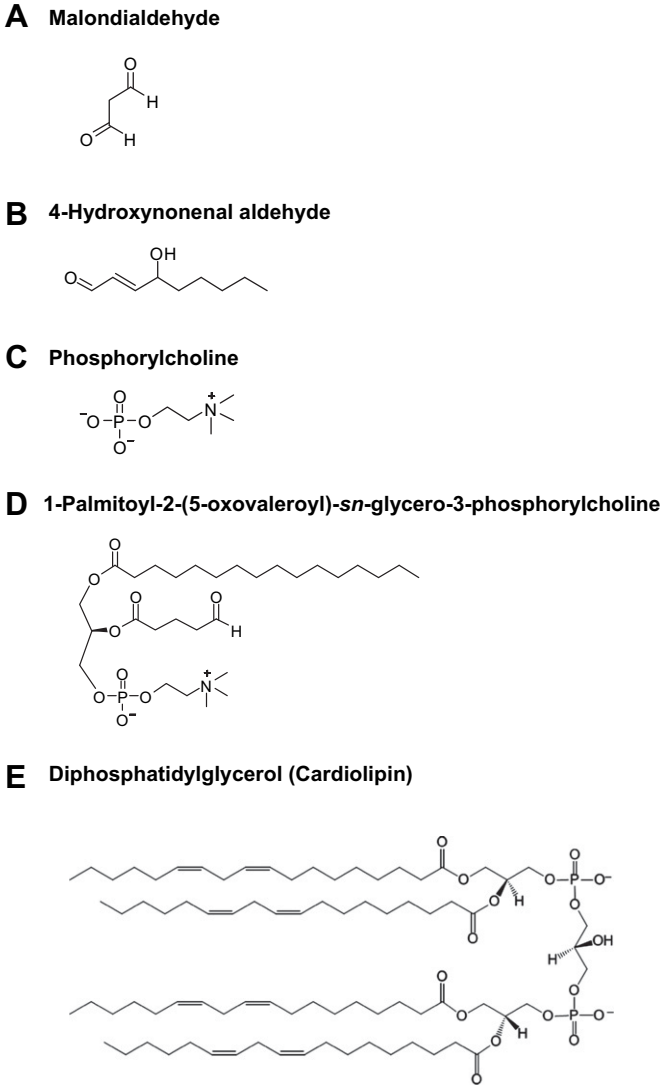

Figure 2 Chemical structures of common autoantigen modification moieties or antigenic epitopes (**A**—**D**). **E**. Autoantigen cardiolinpin.

established cardiovascular disease (CVD), antibodies against HSP60/65 increase and predict further development of the disease.[13] These antibodies specifically react with cells in the plaques and mediate lysis of stressed ECs and macrophages. Chlamydial HSP65 isolated from human serum are cytotoxic to heat-stressed human ECs, and antibodies to microbial HSP65 recognise specific epitopes on human HSP60 present in the arterial wall of healthy young people.[12] Intralesional cells have significantly increased T-cell reactions against human HSP60 compared with peripheral T cells, and T cells isolated from human atherosclerotic lesions showed significant reaction against human HSP60 as well as chlamydial and mycobacterial HSP.[14] On challenge with human HSP60, plaque T cells express Th1 functions, including cytotoxicity and monocyte tissue factor production.[14]

High levels of autoantigen β2-GPI alongside $CD4^+$ and $CD8^+$ T cells appear in atherosclerotic lesions. β2-GPI binds strongly to negatively charged molecules such as phospholipids and to activated platelets and apoptotic cell membranes, which mediate clearance of apoptotic cells from the circulation. In atherosclerotic lesions, β2-GPI colocalises with ox-LDL and often forms covalent complexes that occur in autoimmune diseases. In patients with systemic lupus erythematosus (SLE) or anti-phospholipid syndrome (APS), serum complexes and anti-β2-GPI-ox-LDL complex autoantibodies are elevated.[15] Similarly, such complexes and antibodies present in the bloodstream of patients with vascular complications, such as MI and unstable angina, strongly associate with arterial thrombosis. These complexes inhibit ox-LDL uptake by macrophages through SRs, but are more rapidly internalised by macrophages as IgG immune complexes in the presence of anti-β2-GPI autoantibodies. These macrophages will then present β2-GPI antigenic peptides (on MHC class II) to specific T cells. Therefore, circulating IgG immune complexes containing ox-LDL and β2-GPI can be pro-atherogenic. Indeed, adoptive transfer of β2-GPI-reactive lymphocytes enhances early atherosclerosis in $Ldlr^{-/-}$ mice.[16]

Autoimmune Diseases and Atherosclerosis

Atherosclerosis shares many similarities with other chronic autoimmune diseases such as SLE, rheumatoid arthritis (RA), APS, vasculitis and type I diabetes. They all have evidence of activation of macrophages, lymphocytes and ECs; alteration in the Th1/Th2 ratio; and elevation of inflammatory cytokines. Vascular inflammation in autoimmune diseases may cause LDL oxidation and interaction of ox-LDL with various plasma proteins such as β2-GPI. These events may favour autoantibody production and accelerate arterial thrombosis.

Patients with SLE, a chronic autoimmune inflammatory disease, are 5—6 times more likely to have coronary events than the normal population. In young women, the risk can increase to 50-fold.[17] A large case-control study of non-hospitalised SLE patients with no signs of renal failure showed that the presence of plaques was much more common among SLE patients than among controls. A recent case-control study indicated that coronary artery calcification was also more frequent in patients with lupus than in controls.[18] Anti-ox-LDL antibody titres associate with disease activity in SLE. Increased atherosclerosis was also found in lupus mice. Lupus-susceptible $Ldlr^{-/-}$ mice develop atherosclerosis under moderate dyslipidaemia (chow diet), and overt accumulation of atherogenic lipoproteins can enhance SLE disease.[19]

RA, another prototypic autoimmune disease, also associates with accelerated vascular risk, resulting in early mortality and excess morbidity. RA patients can have up to double the risk of a cardiovascular event irrespective of the traditional CVD risk factors. Many of the same cells that comprise the inflammatory infiltrates in an RA joint lining are likewise found in the atherosclerotic plaques. Both aberrant cellular and humoral immune responses are integral to the pathogenesis of the two conditions. The immune dysregulation that characterises RA seems to have a key role in the accelerated form of atherosclerosis present in this autoimmune disease.[20] Examination of carotid intimal thickening (IMT) shows greater values of IMT in RA patients than in controls. A recent study also showed an association between ox-LDL autoantibodies and CVD in RA.[21]

One in three APS patients develops arterial thrombosis (e.g., MI, stroke and angina) during the disease evolution.[22] β2-GPI appears to constitute the major antigenic target for anti-phospholipid antibodies and plays a central role in the development of clinical complications of APS.

Vasculitis is a chronic inflammatory disease of the large (aorta, coronary, pulmonary and cervical arteries), medium-sized (subclavian, mesenteric, iliac and temporal arteries) and small (skin, intestine, kidney, respiratory tract, etc.) vessels. Like atherosclerotic lesions, vasculitic lesions also contain T cells, monocytes, DCs and mast cells. These pro-inflammatory cells are critical to the pathogenesis. For examples, depletion of DCs abrogates large-vessel vasculitis whereas stimulation of these cells in normal temporal arteries induces vasculitis.[23] Similar to atherosclerotic patients, those with large-vessel vasculitis show high lesion $CD4^+CD28^-$ Th1 cell contents while defect in regulator T cells (T_{reg}) and their serum matrix metalloproteinase (MMP) levels often increase, but anti-inflammatory IL10 decreased compared to control subjects.[24] Like other autoimmune diseases, vasculitis often associates with increased IgG autoantibodies against ox-LDL, HSP and β2GP-1.[25—27] Besides those inflammatory signatures, vasculitic patients demonstrate endothelial cell injury, high IMT, arterial stiffness or thoracic dilatation. Therefore, patients with vasculitis have high risk of developing atherosclerosis. Indeed, both populations share similar risk factors, such as diabetes, hypertension and high serum levels of CRP and IgG autoantibodies against ox-LDL, HSP and β2-GPI (Table 1).

Autoantigen Immunisation and Atherosclerosis Prevention

Autoantigen immunisation-associated atherosclerosis is probably one of the most difficult phenomena to understand. The same autoantigens may yield controversial atherosclerosis phenotypes if different immunisation protocols were used (Table 2). Atherosclerosis-prone mice receiving mucosal (oral or intranasal) immunisation of the autoantigens ox-LDL, HSP or β2-GPI without adjuvant demonstrated reduced atherosclerosis. Mouse lesions had

Table 1 Vasculitis and pathophysiology.

Group	Example	Lesion location	Preferred population	Vascular complication	Risk factor
Large-vessel	Giant cell arteritis	Aorta and major branches	Caucasian, >50 y/o	Accelerated atherosclerosis high aortic aneurysms but low IMT	Diabetes, hypertension, high c-reactive protein (CRP) and autoantibody titers
	Takayasu arteritis	Aorta, pulmonary, coronary, and cervical arteries	Young women 15–45 y/o Asian Latin Americans	Accelerated atherosclerosis vasa vasorum EC injury thrombus formation thoracic dilatation, rupture	Viral infection and genetic factor
Medium-sized vessel	Kawasaki disease	Subclavian, mesenteric, iliac, temporal arteries	Infants and <5 y/o Asian Latin Americans	Coronary artery dilatation cardiac failure high IMT and arterial stiffness	Diabetes, hypertension, high CRP, autoantibody titers
	Polyarteritis Nodosa	Temporal arteries and medium-to-small muscular arteries in kidney, gastrointestinal tract, skin, nerve, joint, and muscle	Children and adults affected with hepatitis B or with atherosclerosis HIV or streptococcal virus	Unknown relationship	Viral infection
Small vessel	Grouped according to whether they contain ANCA (anti-neutrophil cytoplasmic autoantibody)	Small arteries in skin, intestine, kidney, respiratory tract, or any other organs	Children and adults affected with hepatitis C	CVD as a major cause of mortality in $ANCA^+$ patients, arterial stiffness, and impaired function, high CRP level and high autoantibody titers	Viral infection, diabetes, hypertension, impaired renal

fewer numbers of macrophages and $CD4^+$ cells and IFN-γ staining. Mechanistically, autoantigen mucosal immunisation induces antigen-specific T_{reg} and adaptive immune cells to produce anti-inflammatory IL4, IL10 and transforming growth factor beta (TGF-β) to inhibit plaque inflammation and thus the progression of atherosclerosis and reduction in the reactivity of lymph node lymphocytes against autoantigens.[28] Strong evidence indicates that T_{reg} participate in both early and advanced stages of atherosclerosis, particularly in preventing and/or reversing plaque instability. T_{reg} can be activated by rapamycin, anti-CD3 monoclonal antibodies or indirectly via DC activation. Naive $CD4^+$ T cells express CD25 and forkhead boxP3 (FoxP3) after incubation with rapamycin-treated and HSP60-loaded DC, and display antigen specificity. Adoptive transfer of these HSP60-specific $CD4^+CD25^{high}$ T cells inhibited plaque formation in *Apoe*$^{-/-}$ mice.[29] T_{reg} cell activation requires CD28-CD80/CD86 co-stimulation. Deficiency of these co-stimulatory molecules or depletion of CD25 using CD25-neutralising antibodies leads to increased atherosclerosis.[30]

Complete Freund's adjuvant (CFA)-mediated immunisation of autoantigen MDA-LDL (primary subcutaneous immunisation in CFA followed by subsequent immunisation in incomplete Freund's adjuvant, IFA) also yielded decreased atherosclerotic lesion sizes in Watanabe heritable hyperlipidaemic rabbits and in *Ldlr*$^{-/-}$ mice,[31,32] but through different mechanisms from those of mucosal immunisation (Table 2). CFA-mediated immunisations of MDA-LDL or its fragments of human apo B-100 produce a T-cell-dependent increase in the titres of autoantibodies against MDA-LDL and great reduction of atherosclerosis in *Apoe*$^{-/-}$ mice with increased IgG1 (Th2 specific). Such atheroprotective effects of autoantigen immunisation occur independently of adjuvant, regardless of whether using Freund's adjuvant (FA) or cationised bovine serum albumin (BSA) and aluminium.[31–47] Injection of high-dose ox-LDL in neonatal *Apoe*$^{-/-}$ mice without adjuvant also reduced atherosclerosis and resulted in suppression of the T-cell response against ox-LDL.[39] These findings concur with the observations that patients with MI have lower levels of MDA-LDL autoantibodies, supporting the notion that antibodies to autoantigens are atheroprotective. This conclusion is not only true in ox-LDL-immunised animals, but also in animals receiving pathogenic bacteria, which also act through increased ox-LDL autoantibodies. Binder *et al.* demonstrated that *Streptococcus pneumoniae* immunisation resulted in increased ox-LDL autoantibody T15 titre and concomitant reduction in atherosclerotic lesion size in *Ldlr*$^{-/-}$ mice after a similar immunisation protocol to those of MDA-LDL discussed above.[31,32,40] In addition to increased T15 titre, pneumococci-immunised mice also displayed increased *in vivo* formation of circulating IgM immune complexes with apo B-containing particles. Treatment with anti-phosphorylcholine IgM isolated from a T15 idiotype hybridoma reduced vein graft atherosclerosis in *Apoe*$^{-/-}$ mice, suggesting the importance of phosphorylcholine moiety (Fig. 2C) on autoimmunogens. In supporting this hypothesis, immunisation with phosphorylcholine-coupled keyhole limpet haemocyanin (KLH) associates both with a threefold increase in specific antibodies and a 40% reduction in atherosclerosis at the aortic root, even using

Table 2 Immunization-mediated anti- and pro-atherogenesis in animal models.

Antigen	Immunisation Protocol	Carrier/Adjuvant	Animal (sex)	Induction of atherosclerosis	Reference
Anti-atherogenic immunisation:					
Human ox-LDL	Oral immunisation	none	*Ldlr*$^{-/-}$ Mice (unknown gender)	Atherogenic diet	[33]
Mycobacterium HSP65	Oral or Nasal immunisation	none	*Ldlr*$^{-/-}$ Mice (female)	*M. tuberculosis* immunisation in IFA or atherogenic diet	[34,35]
Human or bovine β2-GPI	Oral immunisation	none	*Ldlr*$^{-/-}$ Mice (female)	Atherogenic diet	[36]
WHHL rabbit MDA-LDL	Subcutaneous immunisation	CFA and IFA	WHHL rabbit (both genders)	Atherogenic diet	[31]
Murine MDA-LDL	Subcutaneous immunisation	CFA and IFA	*Ldlr*$^{-/-}$ Mice (female)	Atherogenic diet	[32]
Human apo B-100 peptide-45, -74, -143, -210, or -240	Subcutaneous immunisation	cationized BSA /aluminium	*Apoe*$^{-/-}$ mice (male)	Atherogenic diet	[37,38]
Human ox-LDL	Intraperitoneal injection	none	neonatal *Apoe*$^{-/-}$ mice (both genders)	Atherogenic diet	[30]
S. pneumoniae	Subcutaneous immunisation	CFA and IFA or none	*Ldlr*$^{-/-}$ mice (both genders)	Atherogenic diet	[40]
Phosphorylcholine-KLH	Intraperitoneal immunisation	Oligonucleotide CpG	*Apoe*$^{-/-}$ (female)	Chow diet	[41]
None	Subcutaneous immunisation	Aluminium hydroxide	*Apoe*$^{-/-}$ (male)	Chow diet	[42]
Pro-atherogenic immunisation:					
Human β2-GPI	Subcutaneous immunisation	CFA	*Ldlr*$^{-/-}$ or *Apoe*$^{-/-}$ mice (female)	Chow or atherogenic diet	[43,44]
Recombinant HSP65 or *M. tuberculosis*	Subcutaneous immunisation	IFA	C57BL/6J mice (female)	Atherogenic diet	[45]
Recombinant HSP65 or *M. tuberculosis*	Subcutaneous immunisation	IFA	*Ldlr*$^{-/-}$ mice (female)	Chow diet	[46]
Mycobacterium HSP65	Intracutaneous injections	CFA and IFA	NZW rabbits (male)	Chow or atherogenic diet	[47]

Abbreviations: WHHL: Watanabe heritable hyperlipidemic; NZW: New Zealand White; KLH: keyhole limpet hemocyanin; CFA: complete Freund's adjuvant, IFA: incomplete Freund's adjuvant; BSA: bovine serum albumin.

oligonucleotide CpG as adjuvant,[41] which does not possess intrinsic atheromodulating properties. Therefore, stimulation of the immune response against oxidised phospholipids represents one possible approach for the development of an immunomodulatory therapy for atherosclerosis.

Importantly, not all autoantigen immunisations yield atheroprotective phenotypes. Immunisation of *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mice with β2-GPI induced an increase of atherosclerosis.[43,44] Similarly, parental immunisation of *Ldlr*$^{-/-}$ mice or normocholesterolaemic rabbits with mycobacterial HSP65 in IFA results in the development of atherosclerosis, and T cells isolated from these plaques respond specifically to HSP65 *in vitro* (Table 2).[46,47] Transfer of HSP65-reactive cells also accelerates disease in hypercholesterolaemic animal models. Of importance, when CFA (containing mineral oil and heat-killed mycobacterial HSP65) is injected into non-hypercholesterolaemic animals, atherosclerotic lesions develop in the aorta, principally in sites exposed to an elevated haemodynamic stress.[48] Such adjuvant-initiated atherosclerosis did not occur in IFA-immunised animals, likely due to the lack of mycobacterial HSP65 contaminations in IFA. Therefore, selection of the adjuvant in autoantigen immunisation can prove critical to atherosclerosis outcomes.

Autoantibodies and Atherosclerosis

Immunisation of atherosclerosis-prone animals with ox-LDL, regardless of adjuvant selection, demonstrated the importance of autoantibodies in atherosclerosis animal models.[31–39] The most extensively characterised anti-ox-LDL autoantibody is an IgM called EO6, which reacts against an oxidised phospholipid in modified LDL that has been identified as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine (Fig. 2D). Palinski *et al.*[49] first cloned this ox-LDL monoclonal antibody that recognises the phosphorylcholine epitope in oxidised phospholipids and those on apoptotic cell surfaces from B-cell hybridoma from *Apoe*$^{-/-}$ mice. This autoantibody is indistinguishable from the natural antibody T15, which is secreted by a specialised subclass of B lymphocytes termed B-1 cells in the peritoneal cavity and spleen. Functionally, these autoantibodies block the selective binding to ox-LDL and apoptotic cells by SRs in a dose-dependent manner and thereby prevent macrophage lipid accumulation. In addition to IgM, IgG also inhibits ox-LDL uptake by macrophages. In MDA-LDL-immunised *Apoe*$^{-/-}$ mice, the IgG titres against MDA-LDL correlate inversely with plaque size and serum cholesterol levels.[50] These antibodies neutralise the many pro-atherogenic effects of ox-LDL and minimally oxidised LDL and inhibit apoptotic blebs to induce monocyte adhesion by ECs. In humans, antibodies present in the plasma target a series of MDA-modified peptide sequences in apo B-100. Clinical studies indicated that IgM against MDA-LDL and ox-LDL associated inversely with carotid or femoral IMT, and IgG against ox-LDL also correlated inversely with carotid IMT. In a nested case-control study, these IgM predicted the risk of developing acute MI and causing cardiac death.[11]

By contrast, anti-bacterial HSP65 antibody levels correlate positively with the presence of sonographically visible atherosclerotic lesions in the carotid arteries and established coronary atherosclerosis, and associate with elevated levels of coronary calcification independent of established coronary artery disease (CAD) risk factors. A subsequent follow-up study confirmed that such correlation held true in a 5-year period of observations, especially in those with progressive carotid atherosclerosis.[13] Human serum HSP65 antibodies react with recombinant form of human HSP60 and those present in ECs, macrophages and SMCs in atherosclerotic lesions. Purified anti-HSP65 antibodies are indeed cytotoxic to ECs. Similarly, antibodies targeting human HSP60 also associate with severity of CAD. Increased levels of soluble HSP60 and antibodies to HSP60 predict increased risk of CAD. A large population-based study showed the presence of serum soluble HSP60 in patients with carotid atherosclerosis, a correlation that held independent of age, sex and other risk factors.[51]

Bacterial infection-associated atherosclerosis has been linked to HSP60/65. Anti-HSP60 antibody correlates strongly with human IgA to *Chlamydia pneumoniae* and with IgG to *Helicobacter pylori*. Infection with *C. pneumoniae* showed pro-atherogenic effect. Research suggests that bacterial HSP60 product exerts a direct pro-atherogenic effect by stimulating TNFα and MMP production[52] and IgA anti-HSP60 and anti-*C. pneumoniae* increases the risk of coronary events seven times.[53] Serum IgG antibody against particular sequences of the *H. pylori*-derived HSP60 appeared predominantly in CVD patients. *H. pylori* infection induces atherosclerosis in mice by causing autoimmunity to endogenous HSP60 due to molecular mimicry and enhancing HSP-specific Th1 immune responses. Antibiotics or anti-HSP60 antibodies reduce atherosclerosis in these mice.[54]

Similar to antibodies to HSP60/65, those against autoantigens β2-GPI or cardiolipin (Fig. 2E) also positively correlate with cardiovascular events. Case-control studies demonstrated the association of anti-cardiolipin antibodies (aCLs) with stroke and acute MI,[55] and IgG/IgM/IgA aCL and IgA for anti-β2-GPI associate with increased risk of ischaemic stroke, arterial thrombosis, atherosclerotic immune process, acute MI and peripheral vascular diseases. Although the exact mechanisms remain unknown, anti-β2-GPI was thought to interact with β2-GPI on EC membrane and induce pro-inflammatory and procoagulant endothelial phenotype that is pro-atherognic.[56]

Autoantigen-Associated Clinical Trials

As discussed in Table 2, several promising observations were made from animal studies using autoantigen mucosal immunisation or parental immunisation with oxidisation-modified antigens. These data suggest the possibility of using the same approaches in human atherosclerosis prevention. Although direct application of the same immunisation protocol in treating human atherosclerosis is waiting to be developed, autoantigen HSP mucosal immunisation or vaccination did prove effective in patients with other autoimmune diseases (e.g., RA and type I diabetes) or in different cancer patients (Table 3). As we discussed above (Table 2), animals receiving ox-LDL immunisation demonstrated anti-atherogenic phenotype mainly by increasing anti-ox-LDL antibodies, suggesting the possibility of using anti-ox-LDL as a therapy for human atherosclerosis. Indeed, a phase II

Table 3 Few selected atherosclerosis autoantigen-related clinical trials.

Antigen and protocol	Status	Reference or institution information
HSP oral immunisation, once a day, 6 month	Safe, developed tolerogenic T cell response, showed reduced TNFα, increased IL10, and improved RA	[57]
dnaJP1 (HSP peptide), oral, once a day	Phase II trial for RA, achieved >40% ACR20 response and 80% reduction of *in vitro* T cell TNFα production and increase in tolerogenic cytokines IL10 and FoxP3; ready for phase III trial	Adeona Pharmaceuticals, Inc. Ann Arbor, MI
DiaPep277 (HSP peptide), subcutaneous injection	Phase II trial for type I diabetes, preserved endogenous insulin production	[58]
HSP peptide p27 and p35	Phase I clinical trial for type I diabetes	Hadassah University Hospital, Jerusalem, Israel
HSP96 vaccine	Phase III on melanoma, showed 29% survival extension	[59]
HSP70 vaccine	Phase I trial on genital herpes	[59]
HSP65 vaccine	Phase II trial on intraepithelial neoplasia, showed >50% reduction	[59]
HSP65 vaccine	Phase II trial on cervical intraepithelial neoplasia	[59]
Anti-ox-LDL	Phase II clinical trial on cardiovascular diseases	Genentech, Inc. South San Francisco, CA

*ACR20: a composite endpoint developed by the American College of Rheumatology and accepted as an US Food and Drug Administration approvable scoring criteria.

clinical trial using this strategy has been initiated by Genentech, Inc., South San Francisco, California.

It is intriguing that *Apoe*$^{-/-}$ mice receiving aluminium hydroxide subcutaneous immunisation without any antigen had reduced atherosclerosis compared with those that received saline alone, presumably via down-regulated T-cell activation but increased T_{reg} activity.[42] Indeed, aluminium hydroxide is the most common vaccine adjuvant in both humans and animals.[60] It is primarily used in tetanus, diphtheria, pertussis and poliomyelitis due to its confirmed safety. Therefore, it is possible that aluminium hydroxide vaccination without any antigen may be used in treating human atherosclerosis.

Together, different autoantigens, autoantibodies and bacterial infections can either promote or protect against atherogenesis — depending on the type of antigens, antibodies, bacterial strains and even immunisation protocols with mechanisms more complicated than we currently know.

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