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Two decades of vaccine development against atherosclerosis

Miguel A. Moreno-Gonzalez[a,b,1], **Oscar A. Ortega-Rivera**[a,b,1], **Nicole F. Steinmetz**[a,b,c,d,e,f,*]

[a]Department of NanoEngineering, University of California-San Diego, La Jolla, CA 92039, USA

[b]Center for Nano-ImmunoEngineering, University of California-San Diego, La Jolla, CA 92039, USA

[c]Institute for Materials Discovery and Design, University of California-San Diego, La Jolla, CA 92039, USA

[d]Department of Bioengineering, University of California-San Diego, La Jolla, CA 92039, USA

[e]Department of Radiology, University of California-San Diego, La Jolla, CA 92039, USA

[f]Moores Cancer Center, University of California-San Diego, La Jolla, CA 92039, USA

Abstract

Atherosclerosis is an immune-mediated chronic inflammatory disease that leads to the development of fatty plaques in the arterial walls, ultimately increasing the risk of thrombosis, stroke, and myocardial infarction. The immune response in this complex disease is both atheroprotective and pro-atherogenic, involving both innate and adaptive immunity. Current treatments include the adjustment of lifestyle factors, cholesterol-lowering drugs such as statins, and immunotherapy, whereas vaccine development has received comparatively little attention. In this review, we discuss the potential of antigen-specific vaccination as a preventative approach based on more than 20 years of research and innovation. Vaccination targets include proteins that are more abundant in atherosclerotic patients, such as oxidized low-density lipoprotein (LDL), apolipoprotein B-100, proprotein convertase subtilisin/kexin type-9 serine protease (PCSK9), cholesteryl ester transfer protein (CETP), and heat shock proteins HSP60 and HSP65. Immunization with such proteins or their peptide epitopes has been shown to induce T-cell activation, produce antigen-specific antibodies, reduce the size of atherosclerotic lesions, and/or reduce serum cholesterol levels. Vaccination against atherosclerosis therefore offers a new strategy to address the burden on healthcare systems caused by cardiovascular disease, the leading cause of death worldwide.

*Corresponding author at: Department of NanoEngineering, University of California-San Diego, La Jolla, CA 92039, USA. nsteinmetz@ucsd.edu (N.F. Steinmetz).

[1]Co-first author.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Steinmetz is a co-founder of, has equity in, and has a financial interest with Mosaic ImmunoEngineering Inc. Dr. Steinmetz serves as Director, Board Member, and Acting Chief Scientific Officer, and paid consultant to Mosaic. The other authors declare no potential conflicts of interest.

Keywords

Atherosclerosis; Vaccines; Nanotechnology

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, with ~18 million CVD-related fatalities in 2019 representing 31% of all deaths. [1] The major underlying cause of cardiovascular events is atherosclerosis, a chronic inflammatory disease with an autoimmune component, which involves the disruption of normal cholesterol homeostasis. [2,3] In healthy individuals, cholesterol is delivered to cells by low-density lipoprotein (LDL) and is removed for excretion by high-density lipoprotein (HDL). During atherosclerosis, LDL particles accumulate in the arterial intima to form fibro-fatty plaques, which promote local inflammation. This leads to the recruitment of macrophages and T-cells that exacerbate the condition by releasing pro-inflammatory cytokines. Ultimately, the inflammation and hemodynamic anomalies cause local failures of vascular integrity that can trigger thrombosis leading to myocardial infarction and ischemic stroke [2,4,5].

In the clinic, the first-line approach for the prevention and treatment of atherosclerosis is the adjustment of lifestyle factors that contribute to high serum LDL levels, including poor diet, smoking, and lack of exercise. Therapeutic intervention typically involves a group of drugs known as statins, which reduce levels of LDL-cholesterol (LDL-C) in the blood by inhibiting the hepatic enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, a rate-limiting step in the cholesterol biosynthesis pathway. [3] The long-term use of statins, however, can increase the expression of proprotein convertase subtilisin/kexin type-9 serine protease (PCSK9), a hepatic enzyme that inhibits the LDL receptor (LDL-R), thus preventing cholesterol metabolism. Two monoclonal antibody (mAb) therapies against PCSK9 (alirocumab and evolocumab) were therefore approved in 2015 for patients in which statins are ineffective or poorly tolerated. The FDA also recently approved siRNA-based gene therapy against PCSK9 to reduce levels of LDL-C. However, many atherosclerosis patients remain at risk due to chronic inflammation. [6,7].

The ability to therapeutically target components of the cholesterol homeostatic network and/or pro-inflammatory cascade has led to proposals for atherosclerosis vaccines based on two different principles: reducing serum cholesterol levels and blocking the pro-inflammatory response to LDL-C accumulation. In this review, we consider the molecular basis of atherosclerosis and the associated inflammation and then focus on key targets for vaccine development. These include LDL (primarily responsible for the formation of atherosclerotic plaques) and its major component apolipoprotein B (ApoB); cholesteryl ester transfer protein (CETP), which transfers cholesteryl ester between HDL and LDL; PCSK9, which regulates the LDL-R; and heat shock proteins (HSP60 and HSP65), which form part of the stress-response pathway in the arterial walls and are involved in the autoimmune response. We carried out a systematic analysis of patents, academic publications, and clinical trials to provide a comprehensive analysis of the state of the art in atherosclerosis vaccines and to evaluate their feasibility as a strategy to address this chronic disease.

The immunology of atherosclerosis

The formation of atherosclerotic plaques in humans and animal models triggers both innate and adaptive immune responses (Fig. 1). The initiating event is the retention of infiltrating LDL particles on proteoglycans in the subendothelial space. [8] The trapped lipoproteins are modified by proteases and lipases, promoting their ability to bind proteoglycans and thus causing their aggregation. [9] They are also modified by myeloperoxidase, lipoxygenase, and reactive oxygen and nitrogen species to form oxidized LDL (oxLDL), which elicits an inflammatory response from the innate immune system. [10].

Endothelial cells respond to the accumulation of modified lipoproteins by expressing adhesion molecules such as vascular cell adhesion protein 1 (VCAM−1). [11] Circulating monocytes and other leukocytes are recruited to these sites (Fig. 1). Infiltrating monocytes differentiate into macrophages in response to macrophage colony−stimulating factor (M-CSF) and granulocyte–macrophage colony stimulating factor (GM-CSF), which are produced by endothelial cells and several other cell types. Macrophages thus become a major cell population in atherosclerotic plaques. [12] The engulfment of lipoproteins leads to transformation of macrophages into foam cells. Macrophages express scavenger receptors, among which scavenger receptor class A and CD36 are the most important for the uptake of modified LDL. Foam cells lose their ability to migrate and eventually die within the arterial intima, thus creating a core area in the plaque that consists of apoptotic and necrotic cells, cholesterol crystals, and other extracellular material. [13,14].

Atherosclerotic plaques also accumulate T cells and cells that express major histocompatibility complex class II (MHC-II), forming the basis of the adaptive immune response in atherosclerosis. HLA−DR is expressed by several cell types in the plaque and presents antigens to $CD4^+$ T cells. Dendritic cells in atherosclerotic plaques and in the adjacent adventitia take up plaque−derived antigens and migrate to lymph nodes, where they display these antigens to naive T cells. [15,16] Autoantigens derived from LDL particles are important for atherosclerosis and the frequent presence of anti−oxLDL antibodies indicates that B cells react to oxLDL. These antibodies tend to be more prevalent in patients with coronary artery disease than in healthy controls. [17] Anti-oxLDL antibodies can be either IgM or IgG, indicating that an isotype class−switch occurs, which requires assistance from T cells. Indeed, T cells from patients with atherosclerosis recognize LDL components displayed by antigen-presenting cells (APCs). [2,18].

The main adaptive effector cells in atherosclerotic plaques are $CD4^+$ helper T (Th) cells, some of which react to ApoB-100 peptides presented by APCs on MHC-II. [19] Both oxLDL and native LDL contain ApoB-derived T−cell epitopes. However, APCs express scavenger receptors that recognize oxLDL, so oxidation increases the uptake of LDL for antigen presentation. Th1 cells are the most abundant Th cells in the plaques, and they secrete interferon γ (IFNγ), which promotes monocyte infiltration, macrophage activation, and foam−cell formation, as well as inducing Th1 cell differentiation in synergy with IL−12, which is also produced in the plaque. [2] Th2 cells are much less abundant than Th1 cells [12]. The Th2–related cytokine IL−4 appears to promote atherosclerosis, whereas IL−5, IL−13 and IL−33 limit disease development. [2,5] Pro−atherogenic conditions and oxLDL

can also induce a small population of Th17 cells in plaques, although there are conflicting reports concerning the role of their signature cytokine (IL−17 A), which is also produced by other cells. [2,5] Small populations of regulatory T (T_{reg}) cells expressing forkhead box protein P3 (FOXP3) are also found in atherosclerotic plaques, where they control auto-reactive T−cell clones and disease progression and thus play an important protective role during atherogenesis. [20] Whereas $CD4^+$ T cells are present in plaques during all disease stages, $CD8^+$ T cells are more abundant during the very early stages of lesion development but seem to have a lesser impact than $CD4^+$ T cells in hyperlipidemic mice. The proportion of $CD8^+$ T cells gradually decreases as the lesion matures. $CD8^+$ T_{reg} cells may limit atherosclerosis by controlling pro−atherosclerotic pathways. [2,5].

B cells are also found in atherosclerotic plaques. [5] B1 cells represent a first-line innate defense against common pathogens and secrete germline-encoded IgM antibodies in a T-cell–independent manner. In cardiovascular disease, the titers of IgMs recognizing LDL or ApoB epitopes are inversely correlated with atherosclerosis, complications, and outcome. [21] IgMs that recognize oxLDL inhibit its uptake by macrophages and prevent myeloid cell inflammation, suggesting an atheroprotective role for B1 cells. [22] In contrast, B2 cells are activated by Tfh to differentiate into plasma cells that secrete IgG antibodies. IgG titers against native and oxidized LDL or ApoB are positively correlated with atherosclerotic disease in mice and humans, suggesting B2 cells are pro-atherogenic. Accordingly, the inhibition of B2 cells could be atheroprotective, whereas specifically interfering with plasma cell functions is largely pro-atherogenic. [5] However, the GLACIER clinical phase II study using a monoclonal IgG1 antibody against a human ApoB peptide failed to show a reduction in atherosclerotic inflammation, so the role of IgG remains controversial. [23] It is worth mentioning that this clinical trial only tested one mAb, so more trials must be conducted to make general conclusions on the atheroprotective potential of oxidized LDL or ApoB IgG.

Analysis of atherosclerosis treatment: from passive immunotherapy to vaccines

Vaccination is a form of active immunotherapy intended to elicit the production of antibodies by the patient. Before considering the use of vaccination as a strategy to prevent and treat atherosclerosis, it is therefore important to consider passive immunotherapies that have been developed for this disease. This approach involves the therapeutic administration of recombinant antibody products that block the function of target proteins by inhibiting their activity and/or leading to their degradation.

The most advanced antibody therapies against atherosclerosis are those that target PCSK9. Two products have been approved (alirocumab and evolocumab), both of which target and neutralize PCSK9 in the blood, preventing it from binding to the LDL receptor. The FOURIER clinical trial (ClinicalTrials.gov ID: NCT01764633) showed that evolocumab can reduce circulating LDL-C levels by 59% in patients with atherosclerotic cardiovascular disease who are concurrently treated with statins. This was associated with a 15% reduction in the risk of composite of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization. [24] The ODYSSEY

OUTCOMES clinical trial (ClinicalTrials.gov ID: NCT01663402) showed that alirocumab can reduce circulating LDL-C levels by 62.7%, 61.0% and 54.7% after 4, 12 and 48 months of treatment, respectively. [25] There have been 60 clinical trials of evolocumab and alirocumab that have tested various demographics such as age, race, ethnicity and genetics, demonstrating positive results for mortality, safety and efficacy. [26–85] However, the drawbacks of passive immunotherapy include the cost and the need for repeated dosing and lifelong treatment, which make the drugs inaccessible for most patients. The cost in the USA is $4,500-$8,000 per year if used to treat higher-risk patients with LDL cholesterol ≥ 100 mg/dL despite intensive statin therapy, which is prohibitive in developing countries. [86].

Active immunization (vaccination) is a promising alternative for the treatment of atherosclerosis, following the remarkable effects of vaccination campaigns against various infectious diseases, [87] as well as more recent advances against cancer and chronic diseases, including CVD. [88] However, given that the therapeutic targets are self-antigens, there are a few risks and challenges that must be addressed. The first obstacle when vaccinating against self-antigens is to overcome immune tolerance. This can be achieved through potent carriers and adjuvants capable of mounting a strong immune response against weakly immunogenic targets. Several nanotechnology-based adjuvants, such as nanoliposomes and virus-like particles (VLPs), have proven effective (this will be elaborated in more detail in the next sections). Based on their size regimen, these nanoparticle formulations are effective at interacting with immune cells, and they have highly immunostimulatory properties. [89,90] For immunotherapies targeting self, the second challenge is avoiding target-specific immunotoxicity. Mounting humoral responses against a self-target is preferred and safer, because cytotoxic $CD8^+$ T cell responses against a self-antigen may lead to autoimmune diseases. [91] However, there are preclinical studies of vaccines that decrease atherosclerotic lesions by increasing $CD8^+$ T cells. [92,93] Overall, if efficacious and safe vaccination can be achieved, this would be superior over other therapeutic approaches such as mAb, siRNA, and gene editing. mAb therapies are effective, but they are expensive, they require repeated dosing, and they lead to poor patience compliance compared to vaccination. In the case of siRNA, there is one FDA approved therapy, Inclisiran, but again requires lifelong treatment. [58] Finally, gene editing is a new and promising alternative, but the approaches are at early stages. [94] Verve Therapeutics recently started the first phase 1b clinical trial to evaluate the safety of PCSK9 gene editing medicine, and they expect results in 2023 (ClinicalTrials.gov Identifier: NCT05398029).

We therefore carried out a detailed quantitative analysis of vaccination approaches for atherosclerosis, aiming to identify all the targets that have been considered in preclinical studies and clinical trials. We searched the peer-reviewed literature in PubMed Central using the keywords “atherosclerosis vaccine” and only included results from the year 2000 onwards since the conception of the idea to develop a vaccine started during the late 90′s. [17,18] We then evaluated the specific targets that have been considered as antigens, using keywords such as oxidized LDL (oxLDL), apolipoprotein B-100 (ApoB-100), heat shock proteins (HSPs), cholesteryl ester transfer protein (CETP), and proprotein convertase subtilisin/kexin type 9 (PCSK9). The relevant results were refined to a list of 113 original research articles and reviews. We also screened the ClinicalTrials.gov database for vaccines

and antibody therapies against atherosclerosis, revealing 7 vaccine products targeting PCSK9, CETP and angiotensin II, as well as 60 clinical trials for monoclonal antibody therapies against PCSK9 and 1 against LDL.

We also conducted a patent search using the website Lens.com and the keywords “atherosclerosis” and “vaccine” in the title, abstract and claims. This resulted in a list of 973 patents (granted patents and applications) which we refined to 248 by restricting the date of registration (starting from 2000) and manual screening for common target antigens. The list included patents covering monoclonal antibodies against PCSK9 (67), CD20 (1) and the phosphorylcholine head group (70).

Ultimately, this combined screen of the academic literature, clinical trials, and patents over the last 21 years (Fig. 2) enabled us to define three broad categories of targets: (1) lipid-related antigens, (2) non-lipid-related antigens, and (3) pathogen-related antigens, because infections can also cause CVD (Table 1). The most prominent lipid-related antigens were LDL, ApoB, CETP and PCSK9, whereas the most prominent non-lipid antigens were HSP60 and HSP65. The intended effect of vaccination was either to reduce serum cholesterol levels or block the inflammatory response to the formation of atherosclerotic plaques.

Next, we compiled Table 2, which lists the 77 preclinical studies on atherosclerosis vaccines targeting LDL, ApoB, CETP, PCSK9, HSP60/HSP65 and angiotensin. The table also shows the year of publication, specific antigen, carrier used (if any), adjuvant used (if any), animal model, and summary of major results that can be used as a point of comparison such as percent reduction in atherosclerotic lesion area (sometimes reported as plaque size, lesion size or lesion area), antibody titers, T cell and T_{reg} activation, protein target inhibition, effects on cholesterol (total cholesterol, LDL-C, and HDL-C) and LDL-R, effects on macrophages and dendritic cells, and effects on certain cytokines, such as IFNγ, tumor necrosis factor α (TNFα), IL-10, IL-4 and transforming growth factor β (TGFβ). Although we could find only one paper describing angiotensin-based atherosclerosis vaccines, most of the clinical trials on atherosclerosis have targeted this protein. The other preclinical studies involving angiotensin mainly focused on hypertension, which is a major risk factor for atherosclerosis, so we decided to include them in this study. The specific amino acid sequence of each peptide vaccine is also included, based on UniProt data. If the organism for each peptide was not specified, we assumed that the authors referred to human proteins. If the protein was described as murine, we assumed the species was the mouse rather than any other rodent.

Major target proteins for vaccine development

Low-density lipoprotein

The predominant reason for the onset of atherosclerosis is the accumulation of LDL on the arterial walls. LDL consists of cholesterol, triglycerides, lipophilic antioxidants, phospholipids and ApoB-100, the major lipoprotein component.[97,166] ApoB-100 in the blood is usually described as “bad cholesterol” because it can causes the buildup of cholesterol in the arteries. Native LDL does not elicit an immune response, but oxLDL

is a major factor in the development of atherosclerosis. [166] One pathway for the oxidation of LDL is through exposure to the enzyme myeloperoxidase (MPO), hydrogen peroxide (H_2O_2) and reactive nitrogen species (NO_2^-). [167].

The pro-atherogenic protein oxLDL is detected by APCs such as macrophages and dendritic cells, which process the protein and present epitopes to T cells. Macrophages engulf oxLDL as a defense mechanism, and the epitopes are presented on the cell surface via MHC-I and MHC-II molecules. [98] $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells then recognize and bind these MHC molecules, initiating the production of oxLDL-specific T cells. As discussed above, these are primarily Th1 cells that produce pro-inflammatory cytokines such as IFNγ, IL-2, IL-12 and TNFα. The uptake of oxLDL by macrophages also promotes the formation of cholesterol-rich foam cells that accumulate on the arterial wall, creating fatty streaks and exacerbating the inflammation. [100].

The oxLDL epitopes also give rise to oxLDL-specific autoantibodies in humans and animal models. [99,96,95] Most such autoantibodies are the Th1-specific IgG2a isotype. [98] A Th1 response is a pro-inflammatory reaction and confers an undesirable prognosis, with higher IgG2a titers correlating with more severe manifestations. [95,166] Based on the recruitment of T cells, the activation of macrophages, and the production of antibodies, it is evident that oxLDL can initiate both cellular and humoral immune responses. [95,100].

Several studies have explored the effects of vaccination with oxLDL epitopes. [99,100,102,95,97] Homologous malondialdehyde (MDA)-LDL antigens were tested for the treatment of apolipoprotein E knockout mice ($ApoE^{-/-}$), revealing no change in the IgM titer but a substantial increase in the titer of T-cell dependent IgG. [95] Treatment with MDA-LDL reduced the mean lesion size by 46%. Similarly, oxLDL-pulsed dendritic cells in LDL-R knockout mice ($Ldlr^{-/-}$) induced high titers of T-cell–dependent IgG against ox-LDL, resulting in an 87% reduction in plaque size in the carotid arteries and a 75% reduction in the abundance of IFNγ. [100] The fact that the upregulation of IgG confers an atheroprotective effect, while high levels of circulating IgG are indicators of a bad prognosis shows how beautiful and complex this disease is. Perhaps this could explain why it has been challenging to develop treatments that can succeed in the clinic.

Another treatment option that has been explored in preclinical models is the induction of mucosal tolerance to oxLDL. [98,101] This is based on the hypothesis that tolerance to the autoantigen should ameliorate the inflammatory effects, as reported for other Th1-mediated autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes. [98] One possible tolerance mechanism mediated by autoantigens is the upregulation of immunosuppressive T_{reg} cells, thus maintaining self-tolerance. It is noted that there are contradictions in targeting oxLDL and distinct approaches are being pursued: While immunization against oxLDL aims to upregulate the IgG humoral immune response, tolerance induction aims to upregulate the T_{reg} cellular immune response to prevent autoimmune response associated with the disease. These are distinct mechanism approaches targeting the same molecule. Oral tolerance to oxLDL was shown to reduce mean lesion areas by up to 71.2% in carotid arteries by the upregulation of $CD4^+CD25^+Foxp3^+$ T_{reg} cells

and TGFβ, [98] whereas nasal tolerance induced a modest 47.6% reduction in lesion size by promoting the accumulation of $CD4^+LAP^+$ and $CD4^+CD25^+Foxp3^+$ T_{reg} cells. [101].

Apolipoprotein B-100

ApoB-100 is a major component of the LDL protein and it binds oxLDL products such as MDA via its histidine and lysine residues. [103] Once MDA binds to ApoB-100, the ApoB-100 protein and its peptide sequences are targeted by the immune system. Plasma antibodies from CVD patients recognize immunogenic ApoB-100 epitopes, confirming this protein is an important target for atherosclerosis vaccines. [103,105,168].

The 302-amino-acid ApoB-100 protein contains several highly immunogenic peptides. [166,104,93,169,103,105,106,170,168,171] Scanning the entire human ApoB-100 protein in 20-amino-acid windows revealed 102 peptides that elicit a humoral immune response. [166,169] The MDA-modified human ApoB-100 peptides p143 ($ApoB_{2131-2150}$, IALDDAKINFNEKLSQLQTY) and p210 ($ApoB_{3136-3155}$, KTTKQSFDLSVKAQYKKNKH) led to a 60% reduction in atherosclerotic lesion area when administered with an alum adjuvant. [103] Similarly, MDA-modified p45 (human $ApoB_{688-707}$, IEIGLEGKGFEPTLEALFGK) led to a 48% reduction in atherosclerotic lesion area, whereas the lesser-known p74 ($ApoB_{1123-1142}$, VISIPRLQAEARSEILAHWS) led to a 31% reduction in aortic lesion area. [105] More importantly, both peptides converted a pro-inflammatory Th1 response to an anti-inflammatory Th2 response, as evident from the isotype switch from IgG2a to IgG1. The same group also tested the p45 (human $ApoB_{661-680}$,IEIGLEGKGFEPTLEALFGK) and p210 peptides, which reduced atherosclerotic lesion area by 66% and 59%, respectively despite no increase in the titer of peptide-specific IgG, which was initially considered an important correlate of efficacy. [106] This suggested that the protective effect of the vaccines was independent of the antibodies, and may reflect a cellular rather than a humoral immune response. It is important to note that despite both studies being done by the same researchers, they identified P45 with two different sequences, but with the same amino acids.

To test this hypothesis, other groups investigated the MHC-II-restricted murine ApoB epitopes $ApoB_{3501-3516}$ (SQEYSGSVANEANVYL) and $ApoB_{978-993}$ (TGAYSNASSTESASYY), [110] p101 ($ApoB_{705-720}$,), p102 ($ApoB_{441-456}$, TLYALSHAVNSYFDVD) and p103 ($ApoB_{3953-3968}$, LYYKEDKTSLSASAAS), [112] p18 ($ApoB_{3030-3044}$, SLFFSAQPFEITAST), [114] and p6 ($ApoB_{978-993}$, TGAYSNASSTESASY) [113] rather than B-cell epitopes, achieving lesion size reductions of 35–52% accompanied by significant increase in IL-10 production.

This newfound knowledge inspired other researchers to look for different pathways that could explain the atheroprotective effects of ApoB-100 epitope vaccines. The depletion of T_{reg} cells is known to worsen atherosclerotic plaques, whereas the addition of these cells ameliorates the condition. [107] One promising alternative was to investigate the potential upregulation of T_{reg} cells by ApoB-100 vaccines as already shown in oxLDL tolerance experiments. Researchers at Lund University immunized mice with the so-called aBp210 vaccine based on p210, resulting in a 37% reduction in atherosclerotic lesion area in the aorta accompanied by a fivefold expansion of splenic $CD4^+CD25^+Foxp3^+$ T_{reg} cells. [107]

To confirm that the atheroprotective effect was mediated by the expansion of T_{reg} cells, they administered anti-CD25 antibodies that block the effect of circulating T_{reg} cells and found that this neutralized the effects of the aBp210 vaccine. Other researchers working with p210 obtained similar results accompanied by an expansion of $Foxp3^+$ T_{reg} cells, a decrease in the levels of pro-inflammatory cytokines such as IFN-γ and IL-17, and an increase in the level of anti-inflammatory cytokine IL-10. [109,20,111].

To determine whether the atheroprotective effect could also be mediated by $CD8^+$ cytotoxic T cells, the $CD4^+$ vs $CD8^+$ immune response was evaluated following the administration of p210. [92] This achieved a significant 57% reduction in aortic lesion area, but the $CD4^+$ T cell levels did not increase following immunization. Surprisingly, the $CD8^+$ T-cell population expanded whereas dendritic cells were depleted at the immunization sites, suggesting that the adaptive immune response was interfering with the innate response. $CD8^+CD25^+$ T cells were responsible for the effectiveness of the p210 vaccine, and that the adoptive transfer of $CD8^+$ T cells from the p210-immunized mice to recipient mice could also protect against atherosclerosis.

Cholesteryl ester transfer protein

High levels of HDL-C correlate with a lower risk of CVD. [172] Perhaps the most promising target to increase HDL-C levels is CETP, a hydrophobic glycoprotein that circulates in the plasma and is mainly secreted by the liver. [166,173] This protein binds to HDL and promotes the removal of cholesterol from tissues and its transport to the liver for reverse cholesterol transfer, which leads to cholesterol excretion. CETP maintains equilibrium lipid concentrations by mediating the transport of cholesteryl esters, triglycerides and phospholipids between lipoprotein particles, but it also promotes atherogenesis by transferring cholesteryl ester from the antiatherogenic HDL to the pro-atherogenic LDL and very-low-density lipoprotein (VLDL). CETP therefore has both pro-atherogenic and anti-atherogenic functions (Fig. 3).

The pro-atherogenic properties of CETP were originally investigated in transgenic mice. Expression of the human *CETP* gene in mice reduced HDL levels and led to an increase in the abundance of VLDL and LDL. [173] Furthermore, expression of the human *CETP* gene in $ApoE^{-/-}$ and $Ldlr^{-/-}$ mice resulted in the development and progression of atherosclerosis. [166] Based on these results, the pro-atherogenic activity of CETP has been inhibited using anti-CETP antibodies, which were shown to lower the plasma concentration of CETP and in turn increase the amount of HDL-C in circulation. [116,117,123,119,115,120] CETP-specific antibodies have been induced by administering a DNA vaccine consisting of the plasmid pCR-X8-HBc-CETP, which encodes the B-cell epitope of the CETP C-terminal fragment (CETPC) displayed by hepatitis B virus core (HBc) particles. [119] Intramuscular immunization of female New Zealand white rabbits resulted in the production of anti-CETP antibodies, leading to an 80.6% reduction in aortic lesion area, lower total cholesterol and LDL-C levels, and higher HDL-C levels compared to control rabbits on a high cholesterol diet. The same pCR-X8-HBc-CETP plasmid combined with the linear polysaccharide chitosan to form chitosan/pCETP nanoparticles was used for the intranasal immunization

of male New Zealand white rabbits, leading to a 59.2% reduction in aortic lesion size accompanied high titers of anti-CETP IgG and lower levels of LDL-C. [120].

Researchers at the Universidad Nacional Autonoma de Mexico recently administered their therapeutic intranasal vaccine (HB-ATV-8) to pigs, which more closely resemble human anatomy, physiology and biochemistry than rodents, thus providing a better prediction of performance in clinical trials. [126] The HB-ATV-8 vaccine is based on micellar nanoparticles consisting of the C-terminal residues H486–S496 of CETP, which are necessary for lipid binding and transfer. As expected, the immunized animals produced anti-CETP IgG and the aortal atherosclerotic lesions were less severe. Indeed, 50% of the vaccinated pigs lacked any atherosclerotic lesions and only type I lesions (with isolated macrophage foam cells) were found in the other 50%. These are excellent results compared to the pigs that consumed a high fat diet but received no vaccination, which developed type II and type III lesions characterized by intracellular lipid accumulation and small extracellular lipid pools, respectively.

Researchers have also developed vaccines that combine B-cell epitopes of CETP with tetanus toxoid (TT) residues that act as helper T cell epitopes. [124,125,127,118,122,115,121] The first CETP/TT vaccine combined $TT_{830\text{-}843}$ (QYIKANSKFIGITE) with human $CETP_{461\text{-}476}$ (INPEIITRDGFLLLQM, the residues needed for lipid transfer). [115] The intramuscular immunization of New Zealand white rabbits led to a 39.6% reduction in aortic lesion area, higher HDL-C levels, and lower LDL-C levels. Similarly, the recombinant chimeric enzyme vaccine AnsB-TTP-CETPC combined the enzyme asparaginase (AnsB), $TT_{831\text{-}854}$ (YIKANSKFIGITELKKLESKINKV) and human $CETP_{448\text{-}476}$ (ALMNSKGVSLFDIINPEIITRDGFLLLQM). [118] This vaccine, administered subcutaneously, induced anti-CETP antibodies that inhibited CETP activity, increasing HDL-C levels and reducing LDL-C levels in the immunized rabbits, and reducing the size of aortic atherosclerotic plaques by 42.3% compared to the ovalbumin (OVA) neutral control and by 47.6% compared to the rHSP65 negative control. Subcutaneous CETP/TT vaccines have also been shown to reduce atherosclerotic lesion area and the levels of IL-4, but surprisingly increased the level of IFNγ. [125,127] Other CETP/TT vaccines have been combined with human intestinal trefoil factor (TFF3) to withstand acidity and proteases in the gastrointestinal tract. [122] Oral administration reduced CETP activity and atherosclerotic lesion area, increased the abundance of anti-atherogenic IL-10 and TGFβ, but in contrast to earlier reports also depleted pro-atherogenic TNFα and IFNγ. Only one CETP vaccine has been tested in a clinical trial (ClinicalTrials.gov ID: NCT01284582). [174] This phase I clinical trial tested the safety, immunogenicity and dose response of ATH03, a vaccine against CETP, in healthy male subjects with HDL-C blood concentrations equal or below 80 mg/dl from May 2011 to July 2012, but no results have been made publicly available.

Proprotein convertase subtilisin/kexin type 9

LDL-C is removed from circulation by its receptor (LDL-R) located on the surface of hepatocytes, which is followed by endocytosis and lysosomal fusion. [128] Within the lysosome, LDL-C is removed and the receptor is recycled back to the cell surface. The

liver also produces the protease PCSK9, which regulates the hepatic availability of LDL-R. [128,175] PCSK9 binds to the extracellular epidermal growth factor-like repeat A (EGFA) domain of LDL-R and prevents recycling, resulting in the lysosomal degradation of the receptor. [89] PCSK9 thus reduces the availability of LDL-R, which in turn causes the levels of LDL-C in the circulation to increase (Fig. 4). PCSK9 gain-of-function mutations are linked to hypercholesterolemia, which is characterized by high levels of LDL-C and an increased risk of atherosclerosis, whereas loss-of-function (LOF) mutations are linked with hypocholesterolemia, which leads to low levels of LDL-C and a lower risk of CVD. [128,175] Therapeutic strategies targeting PCSK9 levels in the blood are therefore an effective approach against atherosclerosis. Fig. 5.

As discussed above, two monoclonal antibodies have already been approved for the targeted inhibition of PCSK9. Alternative approaches include the use of antisense oligonucleotides that interfere with the transcription of the PCSK9 gene [176] or the translation of PCSK9 mRNA. [177] An antisense oligonucleotide targeting the mouse PCSK *9* gene resulted in a 92% reduction in hepatic *PCSK9* mRNA levels in hyperlipidemic mice, boosting the levels of LDL-R substantially. [176] This caused a 52% reduction in total cholesterol, a 32% reduction in LDL, and a 54% reduction in HDL. Furthermore, siRNA molecules in lipidoid nanoparticles have been used to silence the PCSK9 gene by targeting the mRNA for degradation, resulting in lower levels of PCSK9, ApoB and LDL-C in mouse, rat and nonhuman primate models. [178] Built on this, the FDA recently approved siRNA-based gene therapy (Novartis Leqvio® (inclisiran) https://www.novartis.com) against PCSK9 to reduce levels of LDL-C. Yet another approach is to use CRISPR gene editing technologies to delete the PCSK9 gene altogether. Musunuru et al. administered CRISPR base editors using lipid nanoparticles (LNPs) in cynomolgus monkeys, and they found that a single dose resulted in a sustained 8 month decrease of PCSK9 and LDL-C of about 90% and 60%, respectively. [94].

The success of PCSK9-specific therapies has generated interest in the development of vaccines based on PCSK9 peptides. [130,179,129,133,131,137,89,134,136,132,135] One promising approach is the use of VLPs based on bacteriophage as a combined carrier and adjuvant for PCSK9 peptides. [130] Specifically, Qβ-PCSK9 vaccines were produced by conjugating human PCSK9 peptides ($PCSK9_{68-76}$, $PCSK9_{153-163}$ or $PCSK9_{207-223}$) to bacteriophage Qβ particles in vitro, and MS2-PCSK9 vaccines were produced by the genetic fusion of PCSK9 peptide sequences ($PCSK9_{153-163}$, $PCSK9_{188-200}$, $PCSK9_{208-222}$ and $PCSK9_{368-381}$) to the MS2 coat protein gene. Both VLP vaccines elicited high titers of IgG antibodies, blocking the binding of PCSK9 to LDL-R, and reducing total cholesterol and LDL-C levels. Mice receiving vaccine PCSK9Qβ−003, consisting of the human PCSK9 B-cell epitope peptide ($PCSK9_{150-157}$) conjugated to Qβ particles, produced high titers of IgG specific for the PCSK9-003 peptide and were characterized by lower total cholesterol levels, 30% lower free PCSK9 levels, higher levels of LDL-R, lower levels of LDL-C, and a reduction in aortic lesion area. [132,135].

PCSK9 and TT have also been combined with a nanoliposome carrier. In the first such example, the PCSK9 peptide (SIPWNLERITPVR) was combined with TT (AQYIKANSKFIGITEL), resulting in the elicitation of anti-PCSK9 antibodies that inhibited

PCSK9 activity. [89] The specific human PCSK9 sequence was searched in the uniport database, and we found $PCSK9_{153\text{-}165}$ corresponds to SIPWNLERITPPR, but the authors have intentionally altered the sequence in accordance to the AFFiRiS group. This minor change to the sequence helps overcome B cell tolerance as the organism can mount an immune response against the “foreign” rather than “self” PCSK9 peptide. The tetanus toxin sequence could not be found in the uniport database. In a mouse atherosclerosis model, this vaccine reduced lesion sizes by 39.13%, free PCSK9 levels by 58.5%, PCSK9–LDL-R binding by 69.86%, total cholesterol by 44.7%, and LDL-C by 57%. [134,136] Testing in rhesus macaques stimulated the production of anti-PCSK9 IgG that inhibited PCSK9–LDL-R biding by 33% with no adverse effects. [137] There has been one clinical trial of PCSK9 vaccines AT04A and AT06A from July 2015 to August 2017, which was completed successfully (ClinicalTrials.gov ID: NCT02508896). [180].

Heat shock proteins

Under normal physiological conditions, HSPs facilitate the folding and transport of proteins, [181] and thereby support cell proliferation, immune responses, and protect cells against apoptosis. [182] Humans have evolved cellular and humoral immunity against bacterial HSP65, the homolog of the 60-kDa human heat shock protein 60 (HSP60). [181] However, when cells are exposed to stress factors caused by smoking, hypertension or chronic infection, including the buildup of oxLDL on arterial walls, HSP60 expression is upregulated and may trigger an autoimmune response which is a major factor in the initiation and progression of atherosclerosis. [138,141] Mitochondria in endothelial cells release excess HSP60, which is secreted from the cell. The extracellular HSP60 (exHSP60) then binds to Toll-like receptor 4 (TLR4), inducing the production of the pro-inflammatory cytokines TNFα and IL-6. [182] As the disease progresses, anti-HSP60 antibodies induce complement-mediated or antibody-dependent cellular cytotoxicity (ADCC), resulting in the lysis of endothelial cells. [183].

Researchers have explored the use of vaccines to reinstate HSP60/HSP65 tolerance. However, the subcutaneous administration of HSP60/HSP65 was pro-atherogenic, resulting in larger atherosclerotic plaques. [147] In contrast, oral and nasal vaccines induced the desired tolerance. [145,184,139,142,144,143,140,148,146] In the most effective example, the size of atherosclerotic plaques was reduced by 80.7% and 83.3% following the oral administration of full-length HSP60 protein and the $HSP60_{253\text{-}268}$ peptide (SIQSIVPALEIANAHR), respectively, in *Ldlr*$^{-/-}$ mice. [140] This induced a significant increase in the abundance of $CD4^+CD25^+Foxp3^+$ T_{reg} cells, TGFβ and IL-10. Similar results were achieved by nasal immunization with recombinant *Helicobacter pylori* HSP60, showing that the reduction of atherosclerotic plaques size can be attributed to the upregulation of $CD4^+CD25^+GARP^+$ T_{reg} cells that suppress Th1 and Th17 responses, [146] as well as $CD4^+CD25^-LAP^+$ T_{reg} cells. [144] HSP60 tolerization may also involve the upregulation of myeloid derived suppressor cells (MDSCs). [147].

The mucosal administration of mycobacterial HSP65 delivered by *Lactococcus lactis* achieved a 39.83% reduction of atherosclerotic lesion area, which was attributed to the downregulation of IFNγ and the upregulation of IL-10. [142] Oral tolerance has also been

achieved in *ApoE*$^{-/-}$ mice using recombinant mycobacterial HSP65. [148] As expected, the treated mice had significantly smaller aortic lesions, a larger number of T_{reg} cells in the spleen and draining lymph nodes, and a lower titer of anti-mbHSP65 antibodies compared to untreated controls.

Combinations of vaccines

ApoB-100 has been combined with other antigens such as HSP60, [185,162–164] *Chlamydia pneumonia* (Cpn), [162,164] IL-10, [108] CETP and PCSK9. [90] Most of these antigens have been discussed already with the exception of Cpn antigens, which are known to interact with MHC and may therefore lead to cytotoxic T lymphocyte (CTL) activation. [162] Cpn and HSP60 peptides have been incorporated into different ApoB-100 vaccine formulations with promising outcomes. A vaccine comprising human $ApoB_{688\text{-}707}$ (IEIGLEGKGFEPTLEALFGK), human $HSP60_{153\text{-}163}$ (AELKKQSKPVT), human $HSP60_{303\text{-}312}$ (PGFGDNRKNQ), and a Cpn peptide was tested using the repetitive immunization multiple sites strategy (RIMMS) which achieved a reduction in atherosclerotic lesion area of 63.8%. [162] The same group also tested this vaccine in an oral dosing study and found a more modest reduction of atherosclerotic lesions of 46.5%. [164] The same researchers also developed a vaccine using $ApoB_{661\text{-}680}$ (FDPNNYLPKESMLKTTLTAF) and human $HSP60_{153\text{-}163}$, which achieved a 41.3% reduction in early atherosclerotic lesion area, [185] and a 39.9% reduction in early plaque size development. [163] A combination of dendritic cells pulsed with ApoB-100 and the immunosuppressive cytokine IL-10 achieved a 70% reduction in aortic lesion area on the descending thoracic aorta. [108] Finally, we recently described an implant-based vaccine comprising the peptides human $ApoB_{3163\text{-}3182}$ (KTTKQSFDLSVKAQYKKNKH), human $CETP_{461\text{-}476}$ (FGFPEHLLVDFLQSLS), and human $PCSK9_{207\text{-}223}$(NVPEEDGTRFHRQASKC) conjugated to bacteriophage Qβ VLPs. [90] These trivalent slow-release implants elicited high titers of antigen-specific IgG, inhibiting all three protein targets and reducing total cholesterol levels.

CETP and HSP65 have also been combined to counteract the lipid homeostasis and inflammation pathways simultaneously. An Hsp65-CETP-PADRE-TT-CETP (HCPTC) fusion protein was administered intranasally to male New Zealand white rabbits, leading to a significant reduction in aortic atherosclerotic plaques size, the production of anti-CETP and anti-HSP65 IgG and protective IgA antibodies, lower levels of LDL-C and IFNγ, and an increase in the abundance of IL-10. [165].

Other protein targets

In addition to the major proteins discussed above, other targets have also been explored for vaccination, albeit to a lesser extent, including interleukins, CD99, apoC-III, ANGPTL3, S100A9, and angiotensin. [152,160,186,187,154,188,150,189–192,149,193,194,153,195, 158,157,196,197,155,161,198,156,199–201,159,151] Interleukins play a major role in atherogenesis and act as key indicators of vaccine efficacy, but some vaccines have been designed to target these molecules directly, especially pro-inflammatory interleukins such as IL-1α, [199] IL-12, [194] and IL-15. [190] The IL-1α-C-Qβ vaccine consisted of the full IL-1α protein chemically conjugated to Qβ VLPs, and was shown to elicit high titers of IL-1α-specific antibodies, reducing plaques size in the descending aorta by 50% and in

the aortic root by 37%. [199] There was also a significant 42% and 32% decrease in the abundance of the inflammatory markers VCAM-1 and ICAM-1, respectively. Vaccination against IL-12 also achieved a 68.5% reduction in atherosclerotic lesion area, [194] whereas vaccination against IL-15 resulted in a 75% reduction in plaque size. [190].

CD99 is a membrane protein found on the surface of leukocytes that promotes the recruitment of macrophages and T cells. [200] The oral administration of a DNA vaccine consisting of *Salmonella typhimurium* transformed with mouse complementary DNA encoding the CD99 extracellular region inhibited CD99 expression in T cells and macrophages, increasing the number of $CD8^+$ T cells that induced apoptosis when CD99 is overexpressed, reducing the size of carotid artery lesions by 69%. [200].

ApoC-III and angiopoietin-like protein 3 (ANGPTL3) can be grouped as the only two protein targets with focus on the reduction of triglyceride (TG) levels, a major risk factor for atherosclerosis. TG is transported by pro-atherogenic lipoproteins such as LDL and VLDL, but traditional statin therapies that lower LDL levels are ineffective at controlling TG levels; hence there is a need for more comprehensive therapeutic approaches. [191] ApoC-III is a protein expressed on hepatocytes whose overexpression leads to increased plasma TG levels and cardiovascular disease (CVD) risk. [202] In addition, ApoC-III increases the binding of LDL with the artery wall proteoglycans and activates adhesion molecules on monocytes which leads to more cholesterol retention in the arterial walls. [202] Chackerian et al. developed a VLP-based vaccine using ApoC-III and they saw a 51% reduction in TG levels. [196] Although a permanent reduction in ApoC-III levels by the vaccination with VLPs successfully reduced plasma TG levels in mice, the effects of an ApoC-III targeting vaccine on atherosclerosis and cardiovascular disease remain unknown. ANGPTL3 is a protein secreted by hepatocytes that inhibits lipoprotein lipase (LPL) which results in increased plasma TG. [192] ANGPTL3 loss-of-function mutations result in decreased LDL-C, TG, and CVD risk, making it a promising vaccination target. Unsurprisingly, vaccination against mouse $ANGPTL3_{32\text{-}41}$ (EPKSRFAMLD) resulted in high antibody titers, a reduction in serum triglycerides and LDL-C, and most importantly a reduction in atherosclerotic lesion sizes in an $Apoe^{shl}$ mouse model. [192] Similarly, $ANGPTL3_{32\text{-}47}$ (EPKSRFAMLDDVKILA) conjugated to Qβ VLPs led to high IgG titers accompanied by a 34% decrease in TG and an increase in LPL. [191] However, effectiveness of these vaccine candidates in preclinical models of atherosclerosis or cardiovascular disease models has not yet been reported.

S100A9 was recently used in our lab as a novel target for the treatment of atherosclerosis. [198] S100A9 is an inflammatory marker secreted by neutrophils of the innate immune system, and it is highly involved in vascular inflammation and atherothrombosis. The vaccine was made by fusing mouse $S100A9_{101\text{-}110}$ (RGHGHSHGKG) to the C-terminus of the Qβ coat protein (CP) with a GSG linker and it was expressed using *E. coli*. Each nanoparticle had ~90 S100A9 peptides conjugated to the surface which led to high IgG titers. Once the immunogenicity of the soluble vaccine was confirmed, we developed slow release PLGA implants using hot-melt extrusion. The implants elicited high IgG titers with a slight Th1 bias which led to a 32% reduction in atherosclerotic lesion size.

Although angiotensin has not yet been thoroughly investigated as a target for the treatment of atherosclerosis, it is a main target for the treatment of hypertension, another major risk factor for atherosclerosis. This hormone has been the focus of many clinical trials, so we decided to include it in this project. The renin-angiotensin-aldosterone system (RAAS) is a major regulator of systemic blood pressure and blood volume. Briefly, renin is an enzyme secreted by the kidneys which results in the production of the angiotensin I (Ang I) hormone. Ang I is then cleaved to Ang II by the angiotensin-converting enzyme (ACE). [152] Ang II is an active hormone that causes vasoconstriction, high blood pressure, and aldosterone secretion when it binds to the angiotensin receptor subtype 1 (AT_1R). [152,156] The increase in blood pressure leads to the growth of vascular smooth muscle cells and oxidative stress which can contribute to the narrowing of the arterial wall. [203] Ang II is also involved in the recruitment of pro-inflammatory cytokines by activating AT_1R, which leads to the formation of atherosclerotic plaques. [159] Thus, Ang II has emerged as a novel target for atherosclerosis vaccination. In 2016, an AT_1R vaccine consisting of the ATR-001 peptide (CAFHYESQ) conjugated to Qβ VLPs was administered subcutaneously to *ApoE*$^{-/-}$ mice, reducing the plaque area of the aortic sinus by 28% and substantially attenuating VCAM-1 and MCP-1 expression. There was also a decrease in the expression of AT_1R and immunostaining revealed less macrophage infiltration in the aortic sinus. As this is the only preclinical study we could find, more research must be conducted to validate Ang II as a viable target for the treatment of atherosclerosis.

Other clinical trials

The only other vaccine trial found in the clinical trials database consisted of an oral vaccine that was composed of pig adipose tissue (ClinicalTrials.gov ID: NCT03042741). This atherosclerosis vaccine, named V6, has been through two small-scale phase II open label clinical trials and it has shown significant improvement in lipid profile in patients with overweight or obesity. However, the ID for this phase II trials or any previous trial are not available in the ClinicalTrials.gov website and are mentioned in the description of phase III trial and the results for this two small-scale phase II can be accessed only as academic publications. [204,205] This phase III trial started in February 2017 and is still in the recruiting stage. For practical purposes we considered this clinical trial as an ApoB/LDL vaccine during data analysis.

Conclusion

Our comprehensive analysis of the scientific literature, clinical trials and patents surrounding atherosclerosis vaccines over the last two decades has confirmed that the vaccines show promise but that more clinical trials and testing in animal models that closely replicate human lipid metabolism are necessary. We found that the vaccines target two major processes, namely the lowering of cholesterol levels in serum and the blocking of inflammatory responses by inhibiting the pro-inflammatory cascade. We also discovered four major targets (ApoB/LDL, CETP, PCSK9 and HSP60/HSP65), which have been explored extensively along all stages of vaccine innovation (patents, academic publications, and clinical trials). We conclude that the development of atherosclerosis vaccines has the

potential to alleviate the burden of cardiovascular diseases in a cost-effective manner, especially in developing countries, but we are far from a solution.

While the preclinical development pipeline focused on atherosclerosis vaccine candidates is moving rapidly, success in clinical trials is yet to be seen. With improved understanding of the underlying biology and advances in technology, i.e. nanotechnology delivery and adjuvant systems, the field is poised to make a clinical impact. Nanoparticles as carriers and/or adjuvants provide potent alternatives to the traditional adjuvants and delivery technology. Nanoparticles such as nanoliposomes and virus-like particles (VLPs) have already been researched in the setting of atherosclerosis vaccine development, and there is continued effort to move these candidate treatments toward clinical development and testing. Being a plug-and-play technology, vaccine design utilizing nanotechnology can draw from other application areas and implement designs to achieve potency while maintaining safety. Nanoparticles can target and stimulate innate immune cells and promote immunostimulation through promotion of cytokine/chemokine secretion. Targeted and carefully engineered formulations carrying adjuvants, co-stimulatory molecules, as well as antigens or epitopes can be designed to achieve potency while mitigating off-target toxic effects. The COVID-19 pandemic has highlighted that nanotechnology can enable rapid development of vaccines, and with the emergence of novel nanotechnologies, we are confident that platforms will become available that can help address the unique needs for a successful atherosclerosis vaccine, e.g., the need to target self in a chronic disease setting.

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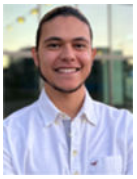
Biographies



Miguel Moreno is a PhD student in NanoEngineering at the University of California, San Diego. Miguel obtained his B.S. in Nanoengineering at UCSD in 2020, and he plans to finish his PhD in 2025. His research focuses on vaccines and immunotherapies based on viral nanoparticles for treatment and prevention of atherosclerosis and cancer. He was awarded an NCI diversity supplement to support his research.

Dr. Ortega-Rivera earned his PhD in Mexico working with oncolytic viruses for cancer immunotherapy. He spent four years as post-doc in the Steinmetz Lad at the University of California, San Diego, where he developed multiple vaccines for cardiovascular diseases, cancer immunotherapy, and against infectious diseases (HPV and SARS-CoV-2). The vaccine development included the combination of virus-like particles and polymers for a slow-release approach as well as microneedle patches for self-administration. He has been honored with prestigious fellowships from CONACYT (Mexico) and UC-MEXUS (UC San Diego). His inventions have been licensed by biotech companies such as Mosaic ImmunoEngineering Inc. and Glycopep Therapeutics.

Dr. Steinmetz is a Professor of NanoEngineering at the University of California, San Diego and Founding Director of the Center for Nano-ImmunoEngineering and Co-Director for the Center of Engineering in Cancer. Dr. Steinmetz trained at The Scripps Research Institute and obtained her PhD from the John Innes Centre in the UK. Her early training was at the RWTH-Aachen University in Germany. Dr. Steinmetz is a Fellow of NAI, BMES, FIAAM, RSC, and AIMBE. She has published more than 200 peer-reviewed publications and is the inventor on more than 100 patents and patent applications, with several patents licensed to biotech companies.

Data Availability

Data will be made available on request.

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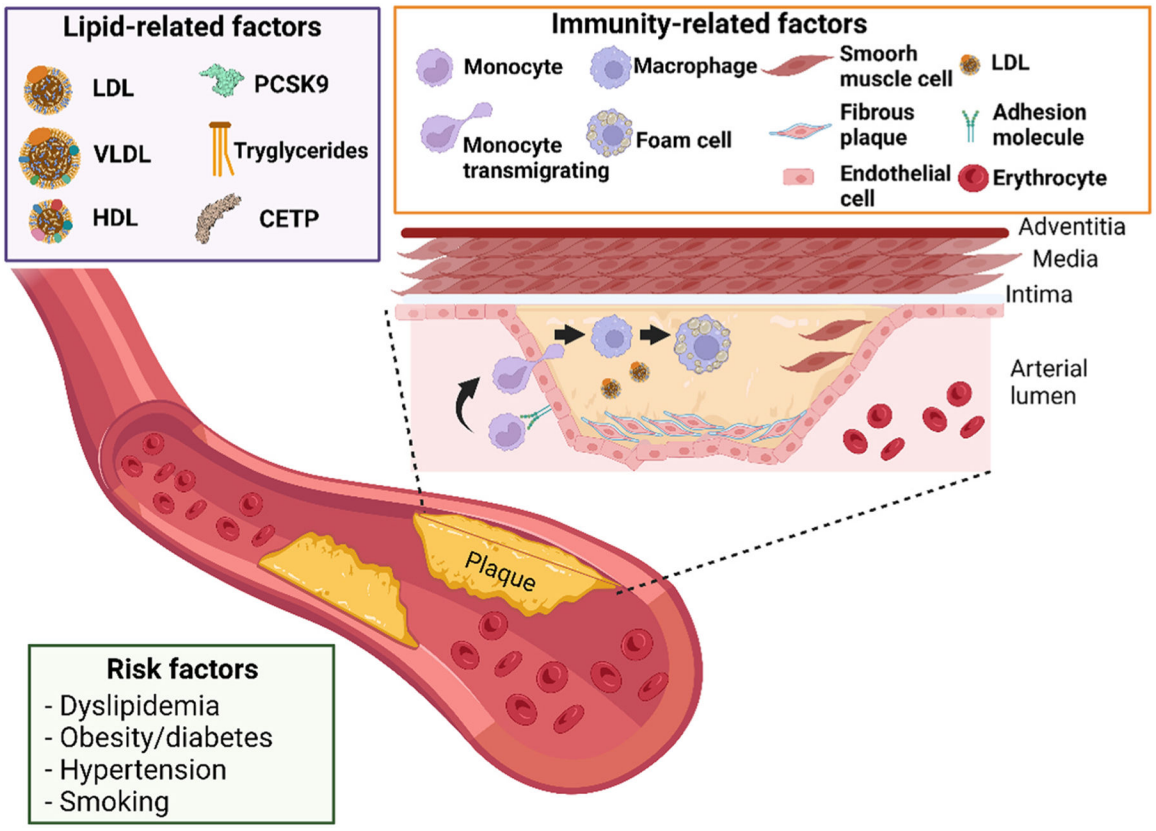
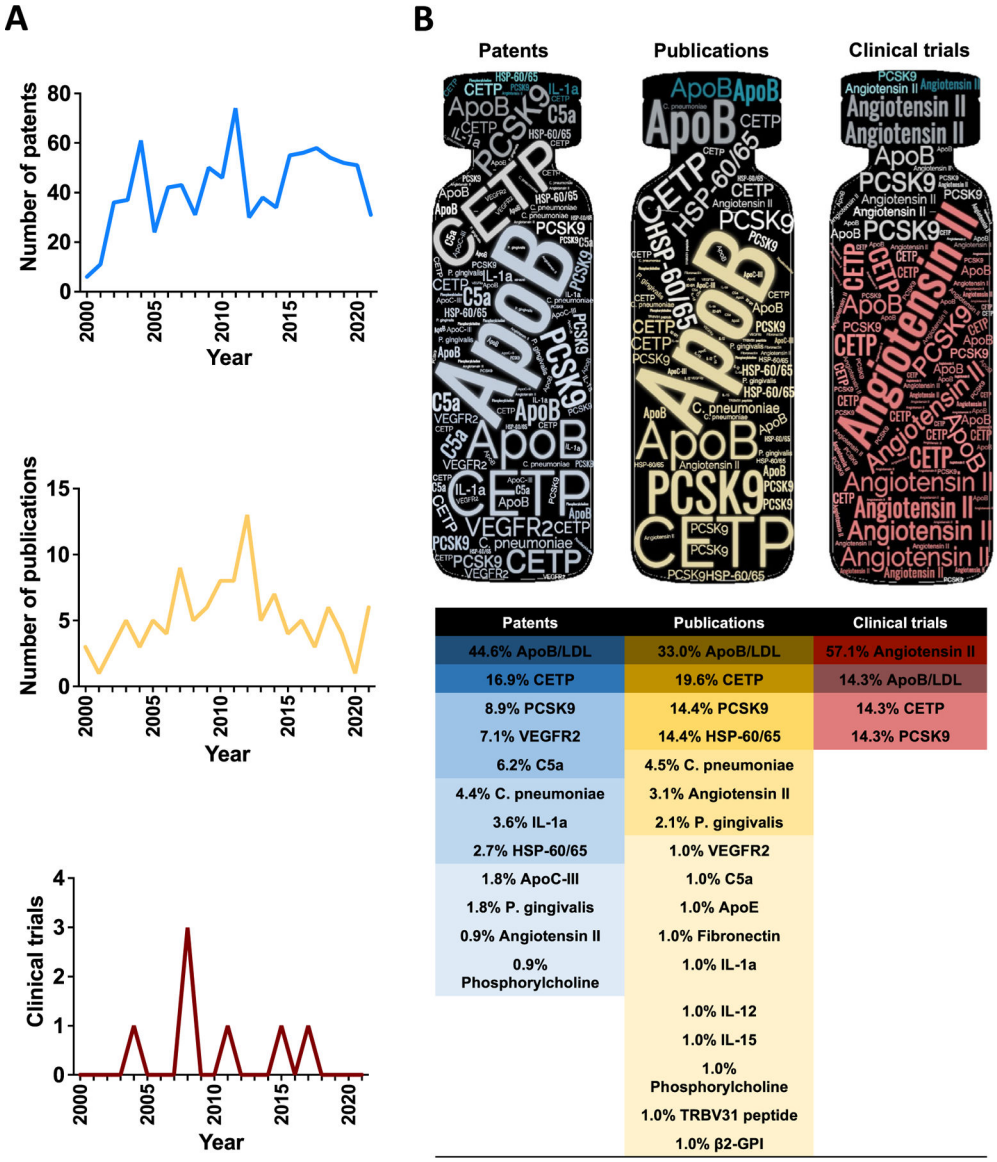


Fig. 1. Lipid-related, environmental, and cell-dependent factors that influence atherosclerotic plaque formation

. LDL retention initiates atherosclerosis. The subendothelial accumulation of lipoproteins leads to the upregulation of adhesion molecules on the endothelial surface and the recruitment of monocytes to the lesion. Monocytes transmigrate into the subendothelial space and differentiate into macrophages in response to macrophage-colony stimulating factor (M-CSF) and granulocyte–macrophage colony stimulating factor (GM-CSF) produced by endothelial cells. Smooth muscle cells can also transdifferentiate into macrophage-like cells. Scavenger-receptor-mediated uptake of lipoproteins by macrophages leads to the formation of foam cells. Printed with permission from BioRender.



Patents	Publications	Clinical trials
44.6% ApoB/LDL	33.0% ApoB/LDL	57.1% Angiotensin II
16.9% CETP	19.6% CETP	14.3% ApoB/LDL
8.9% PCSK9	14.4% PCSK9	14.3% CETP
7.1% VEGFR2	14.4% HSP-60/65	14.3% PCSK9
6.2% C5a	4.5% C. pneumoniae	
4.4% C. pneumoniae	3.1% Angiotensin II	
3.6% IL-1a	2.1% P. gingivalis	
2.7% HSP-60/65	1.0% VEGFR2	
1.8% ApoC-III	1.0% C5a	
1.8% P. gingivalis	1.0% ApoE	
0.9% Angiotensin II	1.0% Fibronectin	
0.9% Phosphorylcholine	1.0% IL-1a	
	1.0% IL-12	
	1.0% IL-15	
	1.0% Phosphorylcholine	
	1.0% TRBV31 peptide	
	1.0% β2-GPI	

Fig. 2. The development of vaccines to treat atherosclerosis.

(A) The number of patent registrations (top), academic publications (middle), and registered clinical trials (bottom) between the years 2000 and 2022. (B) Word clouds showing the frequency of different targets (percentage representation) and their rankings in patents, academic publications, and clinical trials (excludes monoclonal antibody therapies).

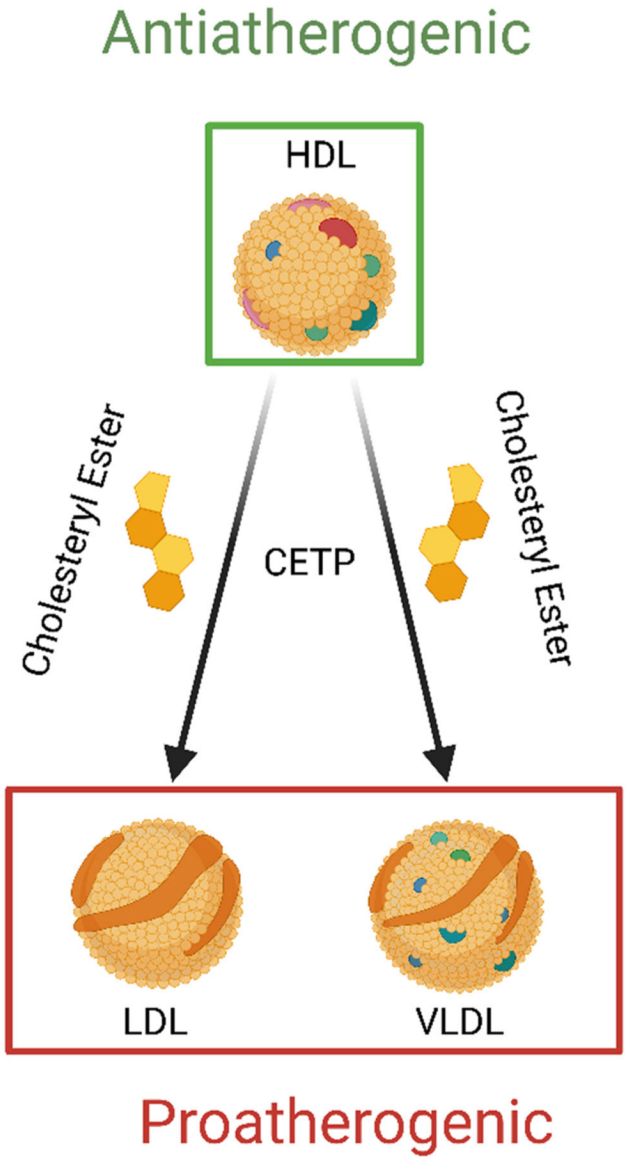


Fig. 3. Proatherogenic properties of CETP.

CETP acts as a proatherogenic protein by promoting the transfer of cholesteryl esters from the antiatherogenic HDL to the proatherogenic LDL and VLDL. As CETP concentration increases, there is a lipid imbalance between the lipoproteins which leads to atherogenesis. CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein. Printed with permission from BioRender.

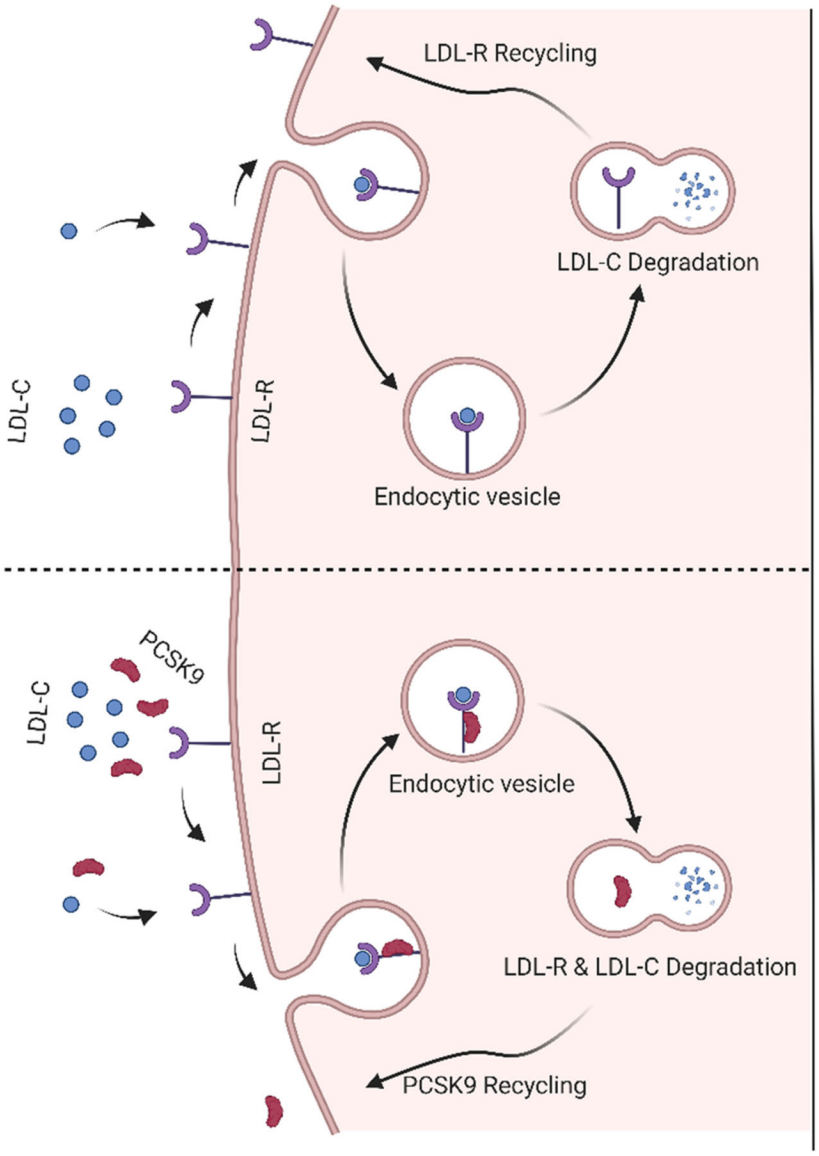


Fig. 4. The PCSK9 mechanism of action.

Under normal physiological conditions (top), LDL-C is removed from blood circulation by LDL-R on the surface of hepatocytes. LDL-C is degraded inside the hepatocyte, and LDL-R is recycled back to the cell surface where it can bind to more LDL-C. In the presence of PCSK9 (bottom), both LDL-C and PCSK9 bind to LDL-R. LDL-C and LDL-R undergo lysosomal degradation, whereas PCSK9 is recycled back to the cell surface where it can continue to deplete the receptors. PCSK9, proprotein convertase subtilisin/kexin type 9; LDL-C, low-density lipoprotein cholesterol; LDL-R, low-density lipoprotein receptor. Printed with permission from BioRender.

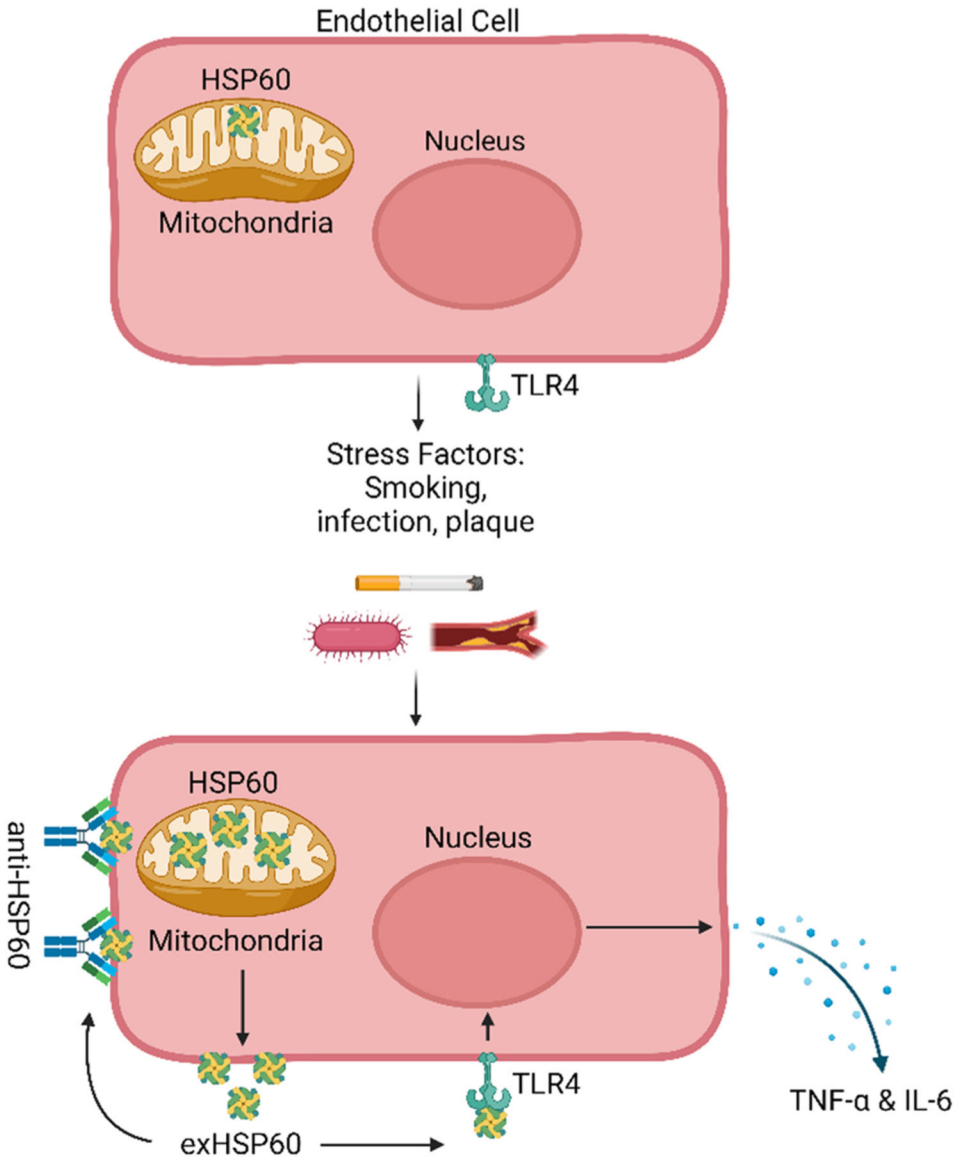


Fig. 5. Role of HSP60 in atherosclerosis autoimmunity.

HSP60 is present in the mitochondria of healthy endothelial cells. As these cells are presented with stress factors such as smoking, chronic infections, and plaque buildup, HSP60 gets over-expressed and released from the cell. The exHSP60 gets recognized by the immune system as foreign and anti-HSP60 lead to ADCC. The exHSP60 is also recognized by TLR4 which leads to the production of pro-inflammatory cytokines TNFα and IL-6. HSP60, heat shock protein 60; exHSP60, extracellular heat shock protein 60, ADCC, antibody-dependent cellular cytotoxicity; TLR4, toll-like receptor 4; TNFα, tumor necrosis factor alpha; IL-6, interleukin 6. Printed with permission from BioRender.

Table 1

Major antigens considered during the development of atherosclerosis vaccines. Red shading indicates antigens tested in clinical trials. The rest have only been tested in preclinical models.

Lipid-raleted antigen	Non-lipid-related antigen	Pathogen-related antigen
Low density lipoprotein(LDL)	Heat shock protein (HSP60/HSP65)	*Chlamydia pneumoniae*
ApoB-100-derived peptides	Angiotensin II	*Porphyromonas gingivalis*
Cholesteryl ester transfer protein (CETP)	Interleukin 1α/12/15	
Proprotein convertase subtilisin/kesin 9 (PCSK9)-derived peptides	CD99	
ApoE-derived peptides	Fibronectin	
ApoC-III-derived peptides	Vascular endothelial growth factor receptor 2 (VEGFR2)	
Phosphocholine head group	Complement 5a protein (C5a)	
	T-cell receptor β variable 31-derived peptide (TRBV31)	

Table 2

Preclinical studies of atherosclerosis vaccines 2000–2022. UniProt ApoB (human): P04114. UniProt HSP60 (human): P10809. UniProt ApoB (mouse): E9Q414. UniProt CETP (human): P11597. UniProt tetanus toxin: P04958. UniProt PCSK9 (human): Q8NBP7. UniProt PCSK9 (mouse): Q80W65.

Lipid-Related Antigens							
Protein Target	**Year**	**Antigens**	**Carrier**	**Adjuvant**	**Model**	**Results**	**Reference**
Low density lipoprotein (LDL)	2001	Homologous atherosclerotic plaque homogenate/ homologous MDA-LDL	N/A	Complete and incomplete Freund's adjuvant	*ApoE*$^{-/-}$ mice	46% lesion size ↓, T-cell–dependent IgG ↑	[95]
	2004	Murine native LDL	N/A	Murine IL-12	*ApoE*$^{-/-}$ mice	46% plaque size ↓, oxLDL IgG ↑,	[96]
	2005	Homologous MDA-LDL	N/A	Complete and incomplete Freund's adjuvant	*ApoE*$^{-/-}$ and CD4/*ApoE*$^{-/-}$ mice	41% lesion size ↓, MDA-LDL IgM and IgG ↑	[97]
	2006	oxLDL	N/A	Dimethyl dioctadecyl ammonium bromide	*Ldlr*$^{-/-}$ mice	71.2% plaque size ↓, $CD4^+CD25^+Foxp3^+$ T_{reg} ↑, TGFβ ↑	[98]
	2007	MDA-LDL or Cu-LDL	N/A	Freund's adjuvant	New Zealand white rabbits	Fatty streak lesions ↓, LDL antibodies ↑, LDL-cholesterol ↓	[99]
	2010	oxLDL-pulsed DCs	N/A	N/A	*Ldlr*$^{-/-}$ mice	87% plaque size ↓, T cell-dependent Cuox-LDL IgG↑	[100]
	2012	Human copper-oxLDL	N/A	N/A	*ApoE*$^{-/-}$ mice	47.6% lesion size ↓, $CD4^+LAP^+$ and $CD4^+$ $CD25^+Foxp3^+$ T_{reg}↑, TGBβ↑	[101]
	2022	MDA-LDL	N/A	Complete Freund's adjuvant	*Ldlr*$^{-/-}$*mice*	atherosclerotic lesion area ↓, memory B cells ↑, germinal center B cells ↑, IgM/IgG1/IgG2b/IgG2c ↑	[102]
Apolipoprotein B 100 (ApoB-100)	2003	MDA modified human ApoB-100 peptide 143 (aa 2131–2150: IALDDAKINFNEKLSQLQTY) and peptide 210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH)	Cationized bovine serum albumin (cBSA)	Alum (aluminum hydroxide)	*ApoE*$^{-/-}$ mice	60% atherosclerotic lesion area ↓, MDA peptides IgG ↑	[103]
	2005	Human ApoB-100 peptide-1 (aa: EEEMLENVSLVCPKDATRFK) and human ApoB-100 peptide-2 (aa: ATRFKHLRKYTYNYEAESSS)	N/A	Alum	*ApoE*$^{-/-}$ mice	42% aortic atherosclerotic lesion area ↓, peptide-specific IgG and IgM ↑	[104]
	2005	MDA-modified human ApoB-100 peptide 45 (aa 688–707: IEIGLEGKGFEPTLEALFGK) and peptide 74 (aa 1123–1142: VISIPRLQAEARSEILAHWS)	cBSA	Alum	*ApoE*$^{-/-}$ mice	48% and 31% atherosclerotic lesion area ↓, peptide-specific IgG ↑, Th2 shift	[105]
	2008	Native human ApoB-100 peptide 45 (aa 661–680: IEIGLEGKGFEPTLEALFGK)	cBSA	Alum	*Ldlr*$^{-/-}$ / human apoB-100 transgenic mice	66% atherosclerotic lesion area ↓, native LDL and copper-ox LDL IgM ↑	[106]

Lipid-Related Antigens							
Protein Target	**Year**	**Antigens**	**Carrier**	**Adjuvant**	**Model**	**Results**	**Reference**
		and peptide 210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH)					
	2010	Human ApoB peptide 210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH)	N/A	CTB (B subunit of cholera toxin)	*ApoE*$^{-/-}$ mice	35% aortic lesion area ↓, peptide specific IgG↑, Foxp3 mRNA ↑	[20]
	2011	Human ApoB peptide 210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH)	cBSA	Alum	*ApoE*$^{-/-}$ mice	37% atherosclerotic lesion area ↓ $CD4^+CD25^+$ T cell and $CD4^+CD25^+$Foxp3, Treg↑	[107]
	2011	Human ApoB-100 and IL-10	Dendritic cells	N/A	huB100^(tg) × *Ldlr*$^{-/-}$ mice	70% lesion area ↓, ApoB100 IgG ↑, $CD4^+$ ↓, IFNγ ↓	[108]
	2012	Mixture of human ApoB-100 peptide 210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH), peptide 240 (aa 3586–3605: FPDLGQEVALNANTKNQKIR), and MDA-P210	N/A	N/A	*ApoE*$^{-/-}$ mice	40% lesion area ↓, 30% $CD4^+CD25^+Foxp3^+$ T_{reg} ↑, 45% T-cell ↓	[109]
	2012	Human ApoB100 peptide 210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH)	cBSA	Alum	*ApoE*$^{-/-}$ mice	57% atherosclerotic lesion area ↓, peptide-specific IgG and IgM ↑, $CD8^+CD62L^+$ and $CD8^+CD25^+$IL-10^+ T cells ↑	[92]
	2013	Murine ApoB-100 (aa 3501–3516: SQEYSGSVANEANVYL) and murine ApoB-100 (aa 978–993: TGAYSNASSTESASYY)	N/A	Complete and incomplete Freund's adjuvant	*ApoE*$^{-/-}$ mice	40% lesion size ↓, peptide-specific IgG ↑	[110]
	2013	Human ApoB-100-derived peptides P2 (amino acid sequence not specified), P45 (aa 661–680: IEIGLEGKGFEPTLEALFGK) and P210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH)	BSA and dendritic cells	N/A	*ApoE*$^{-/-}$ mice	$CD4^+CD25^+FoxP3^+$ T_{reg} ↑, peptide-specific IgG1 ↑, peptide-specific IgG1 ↑, IL-10 ↑	[111]
	2017	Murine ApoB peptides P101 (aa 705–720: FGKQGFFPDSVNKALY, P102 (aa 441–456: TLYALSHAVNSYFDVD, and P103 (aa 3953–3968: LYYKEDKTSLSASAAS)	N/A	Complete and incomplete Freund's adjuvant	*ApoE*$^{-/-}$ mice	40% atherosclerotic lesion area ↓, peptide-specific IgG1 and IgG2c ↑, $FoxP3^+CD4^+$ T cells ↑	[112]
	2018	Murine ApoB peptide P6 (aa 978–993: TGAYSNASSTESASY)	N/A	Addavax	*ApoE*$^{-/-}$ mice	57% lesion area ↓	[113]
	2018	Murine ApoB peptide P18 (aa 3030–3044: SLFFSAQPFEITAST)	N/A	Complete and incomplete Freund's adjuvant	*ApoE*$^{-/-}$ mice	~35% lesion size ↓, $CD25^+FoxP3^+CD4^+$ T_{reg} ↑, peptide-specific IgG1 and IgG2c ↑	[114]
	2022	Human ApoB100 peptide 210 (aa 3136–3155: KTTKQSFDLSVKAQYKKNKH)	Peptide amphiphile micelles (PAMs)	N/A	*A2Kb-Tg ApoE−/− mice*	atherosclerotic lesion area ↓, CD4 + effector memory T cells ↓, CD8 + central memory T cells ↑, CD4 +CD25 +FoxP3 + T_{reg} ↑, LDL-C ↓	[93]
CETP	2000	Human CETP (aa 461–476: FGFPEHLLVDFLQSLS) and tetanus toxin (aa 830–843: QYIKANSKFIGITE)	N/A	Complete and incomplete Freund's adjuvant	New Zealand white rabbits	39.6% aortic lesion area ↓, CETP antibodies↑, CETP activity ↓, HDL ↑, LDL ↓	[115]

Lipid-Related Antigens							
Protein Target	**Year**	**Antigens**	**Carrier**	**Adjuvant**	**Model**	**Results**	**Reference**
	2004	Human CETP (aa 451–476: RDGFLLLQMDFGFPEHLLVDFLQSLS)	HSP65	N/A	C57BL/6 J mice	anti-CETP antibodies ↑	[116]
	2005	Human CETP (aa 448–476: IITRDGFLLLQMDFGFPEHLLVDFLQSLS)	HSP65	Alum	New Zealand white rabbits	30.8% aortic lesion area↓, anti-CETP antibodies↑, HDL-C↑, LDL-C↓	[117]
	2005	Asparaginase (AnsB), tetanus toxin (aa 831–854: YIKANSKFIGITELKKLESKINKV), and human CETP (aa 448–476: IITRDGFLLLQMDFGFPEHLLVDFLQSLS)	N/A	Alum	New Zealand white rabbits	47.6% atherosclerotic plaque area↓, anti-CETP antibodies ↑, HDL-C ↑, LDL-C ↓	[118]
	2006	pCR-X8-HBc-CETP	hepatitis B core (HBc)	HBc	New Zealand white rabbits	80.6% aortic lesion area↓, anti-CETP antibodies↑, HDL-C ↑, LDL-C↓	[119]
	2008	pCR-X8-HBc-CETP	Chitosan	HBc	New Zealand white rabbits	59.2% aortic lesion area ↓, anti-CETP IgG ↑, LDL-C ↓	[120]
	2009	Human CETP (aa 461–476: FGFPEHLLVDFLQSLS), tetanus toxin (aa 830–843: QYIKANSKFIGITE) and PADRE T cell epitope (aK-Cha-VAAWTLKAa)	N/A	Alhydrogel and VaxImmune	New Zealand white rabbits and BALB/c mice	anti-CETP antibodies ↑	[121]
	2011	Human CETP (aa 461–476: FGFPEHLLVDFLQSLS) and tetanus toxin (aa QYIKANSKFIGIT)	Human intestinal trefoil factor (TFF3)	N/A	New Zealand white rabbits	atherosclerotic lesion area ↓, anti-CETP antibodies ↑, plasma CETP activity ↓, HDL-C ↑, LDL-C ↓, TNFα and IFNγ ↓, IL-10 and TGFβ ↑	[122]
	2014	Fc-CETP6	N/A	Complete and incomplete Freund's adjuvant	New Zealand white rabbits	atherosclerotic lesion area↓, anti-CETP antibodies ↑, CETP activity ↓, HDL-C ↑, ApoA-I ↑, ox-LDL ↓	[123]
	2016	Human CETP (aa 461–476: FGFPEHLLVDFLQSLS) and tetanus toxoid (aa 830–845: QYIKANSKFIGITELK)	N/A	Complete and incomplete Freund's adjuvant	New Zealand white rabbits	anti-CETP antibodies ↑, CETP activity ↓	[124]
	2016	Human CETP (aa 461–476: FGFPEHLLVDFLQSLS) and tetanus toxoid (aa CQYIKANSKFIGITE)	N/A	MF59	New Zealand white rabbits	atherosclerotic lesion area ↓, IFNγ ↑, IL-4 ↓	[125]
	2018	CETP (aa H486–S496)	Micellar nanoparticle	N/A	Pigs (large white × landrace)	atherosclerotic lesion area ↓, anti-CETP IgG ↑	[126]
	2020	Human CETP (aa 461–476:FGFPEHLLVDFLQSLS)) and tetanus toxoid (830–845: QYIKANSKFIGITELK)	Liposome	CpG oligonucleotide	White rabbits	atherosclerotic lesion area ↓, anti-TT-CETP antibodies ↑, IL-4 ↓, IFNγ ↑	[127]
PCSK9	2012	Human PCSK9 protein	N/A	CpG oligonucleotide	BALB/c mice	anti-hPCSK9 antibodies ↑, total cholesterol and HDL-C and LDL-C ↓, plasma mPCSK9 ↓, LDLR ↑	[128]
	2014	PCSK9 peptides (amino acid sequence not specified)	KLH	Alhydrogel	BALB/c mice	anti-hPCSK9 and anti-mPCSK9 antibodies ↑, total cholesterol ↓, LDLR ↑, LDI ↑, LDI ↓.	[129]

Lipid-Related Antigens							
Protein Target	**Year**	**Antigens**	**Carrier**	**Adjuvant**	**Model**	**Results**	**Reference**
	2015	Human PCSK9 (aa 207–223: NVPEEDGTRFHRQASKC)	Qβ VLPs	Qβ VLPs	BALB/c mice and macaque monkeys	peptide-specific and recombinant human PCSK9 IgG ↑, 55% total cholesterol ↓, 50% free PCSK9 ↓, 15% LDL-C ↓	[130]
	2017	Human and mouse PCSK9 protein (aa 153–692)	N/A	N/A	APOE* 3Leiden.CETP transgenic mice	64% lesion size ↓, PCSK9 antibodies ↑, plasma muPCSK9 ↓, 53% total cholesterol ↓, LDL-C ↓	[131]
	2017	PCSK9 peptide V150–157 (aa: FAQSIPWN)	Aluminum hydroxide gel	Qb VLP	*Ldlr*$^{+/-}$ mice	30% free PCSK9 ↓, cholesterol and LDL-C ↓, LDLR ↑	[132]
	2018	Mouse PCSK9 antigen (aa 682–690: CRSRPSAKA)	KLH	complete or incomplete Freund's adjuvant	*ApoE*$^{-/-}$ mice	Anti-PCSK9 antibody ↑, LDLR ↑, total cholesterol ↓	[133]
	2019	PCSK9 (aa: SIPWNLERITPVR) and tetanus toxin (aa: AQYIKANSKFIGITEL)	Nanoliposome	Alum	BALB/c mice	PCSK9 peptide IgG ↑, 52.2% plasma PCSK9 ↓, 50.2% PCSK9-LDLR binding ↓	[89]
	2019	PCSK9 (aa: SIPWNLERITPVR) and tetanus toxin (aa: AQYIKANSKFIGITEL)	Nanoliposome	Alum	C57BL/6 mice	39.13% lesion size ↓, PCSK9 peptide IgG ↑, 58.5% plasma PCSK9 ↓, 44.8% PCSK9-LDLR binding ↓, 44.7% total cholesterol ↓, 51.7% LDL-C ↓, LDLR ↑	[134]
	2020	PCSK9–003 peptide (aa: FAQSIPWN)	Qb VLP	Qb VLP	*ApoE*$^{-/-}$ mice	aortic lesion area ↓, peptide-specific antibody ↑, total cholesterol and LDL-C ↓, LDLR ↑, macrophage infiltration ↓	[135]
	2021	PCSK9 (aa: SIPWNLERITPVR) and tetanus toxin (aa: AQYIKANSKFIGITEL)	Nanoliposome	Alum	C57BL/6 mice	24.25% lesion size ↓, PCSK9 peptide IgG ↑, 40% plasma PCSK9 ↓, 69.86% PCSK9-LDLR binding ↓, 38.13% total cholesterol ↓, 57%LDL-C ↓, LDLR ↑	[136]
	2021	PCSK9 (aa: SIPWNLERITPVR) and tetanus toxin (aa: AQYIKANSKFIGITEL)	Nanoliposome	Alum	Rhesus macaque monkeys	PCSK9 IgG ↑, 33% PCSK9-LDLR binding ↓	[137]
Non-Lipid Related Antigens							
HSP60/65	2002	*Mycobacterium* HSP-65	N/A	N/A	*Ldlr*$^{-/-}$ mice	lesion size ↓, anti-HSP antibody ↓, macrophages and CD4^{+} T cells and IFNγ ↓, IL-10 ↑	[138]
	2002	*Mycobacterium* HSP-65	N/A	N/A	*Ldlr*$^{-/-}$ mice	lesion size ↓, IL-4 ↑	[139]
	2007	*Mycobacterium* HSP60 and HSP60 peptide (aa 253–268: SIQSIVPALEIANAHR)	N/A	N/A	*Ldlr*$^{-/-}$ mice	83.3% plaque size↓, CD4^{+}CD25^{+}Foxp3^{+} T_{reg}↑ , TGFβ and IL-10↑	[140]

Lipid-Related Antigens							
Protein Target	**Year**	**Antigens**	**Carrier**	**Adjuvant**	**Model**	**Results**	**Reference**
	2009	*Mycobacterium* HSP-65	N/A	CTB	New Zealand white rabbits	atherosclerotic lesion area ↓, IL-10 ↑, total cholesterol and LDL-C and HDL-C ↓,	[141]
	2011	*Mycobacterium* HSP-65	*Lactococcus lactis*	N/A	*Ldlr*$^{-/-}$ mice	39.83% atherosclerotic lesion area ↓, IFNγ ↓, IL-10 ↑	[142]
	2012	DNA encoding Hsp65 (Hsp65 DNA) and *Mycobaterium tuberculosis* Hsp65 protein	N/A	N/A	New Zealand White rabbits	lesion area ↓, anti-Hsp65 IgG and IgA ↑, IFNγ ↓, IL-10 ↑, total cholesterol and LDL-C ↓	[143]
	2012	recombinant H. Pylori HSP60	N/A	N/A	*ApoE*$^{-/-}$ mice	53.7% atheroscleroticplaque size ↓, $CD4^+CD25^-LAP^+$ and $CD4^+CD25^+Foxp3^+$ T_{reg} ↑, 50% IFNγ ↓, TGFβ ↑	[144]
	2015	Recombinant mycobacterial HSP65	N/A	N/A	*ApoE*$^{-/-}$ mice	lesion size ↓, splenic T_{reg} ↑, IL-10 ↑	[145]
	2016	Recombinant *Helicobacter pylori* HSP60	N/A	N/A	*ApoE*$^{-/-}$ mice	30.6% atherosclerotic plaque size ↓, $CD4^+CD25^+GARP^+$ and $CD4^+CD25^+FoxP3^+$ T_{reg} ↑, IFNγ ↓, IL-10 and TGFβ ↑	[146]
	2018	HSP-60 (organism not specified)	N/A	N/A	*ApoE*$^{-/-}$ mice	atherosclerotic plaque size ↓, MDSCs↑, IFNγ ↓, IL-10↑	[147]
	2018	Recombinant mycobacterial HSP65	N/A	incomplete Freund's adjuvant	*ApoE*$^{-/-}$ mice	atherosclerotic lesion area ↓, total cholesterol ↓, T_{reg} ↑	[148]
Angiotensin	2000	Angiotensin I (AngI) and Angiotensin II (AngII) analogues	Tetanus toxoid	Alum	Sprague-Dawley rats	anti-Ang IgG ↑, AngI response ↓	[149]
	2003	Angiotensin I (AngI) analogue	KLH	Alum	Sprague-Dawley rats	anti-AngI IgG and IgM ↑, blood pressure ↓	[150]
	2006	Angiotensin II type 1 receptor peptide ATR12181 (aa: AFHYESR)	Tetanus toxoid	Complete and incomplete Freund's adjuvant	Spontaneously hypertensive Rats	peptide-specific antibody ↑, blood pressure ↓	[151]
	2007	Angiotensin II (aa 1–8: DRVYIHPF)	Qβ VLP	Qβ VLP	Spontaneously hypertensive Rats	anti-AngII IgG ↑, blood pressure ↓, AngII ↑ (antibody-bound AngII)	[152]
	2011	Angiotensin I (aa: DRVYIHPFSL)	N/A	Alum	Spontaneously hypertensive Rats	anti-AngI-R and anti-AngII antibody ↑, blood pressure ↓, AngI and AngII ↓	[153]
	2013	Angiotensin II type 1 receptor peptide ATR-001 (aa: CAFHYESQ)	Qβ VLP	Qβ VLP	Spontaneously hypertensive Rats	peptide-specific antibody ↑, blood pressure ↓, AT_1R ↓	[154]
	2013	Angiotensin II (peptide not specified)	KLH	Complete and incomplete Freund's adjuvant	Spontaneously hypertensive Rats	anti-Ang II antibodies ↑, blood pressure ↓, IFNγ and IL-4 ↑	[155]

Lipid-Related Antigens							
Protein Target	**Year**	**Antigens**	**Carrier**	**Adjuvant**	**Model**	**Results**	**Reference**
	2013	pHAV–4Ang IIs	HAVLP	HAVLP	Spontaneously hypertensive Rats	anti-AngII IgG ↑, blood pressure ↓, Ang II ↓	[156]
	2014	Angiotensin II type 1 receptor peptides ATR12181 (aa: AFHYESR), ATR12185 (aa: ESRNSTL), and ATR 10014 (aa: IQDDCPK)	Tetanus toxoid	Complete and incomplete Freund's adjuvant	Spontaneously hypertensive Rats	peptide-specific antibody ↑, ATR12181 blood pressure ↓	[157]
	2015	pcDNA3.1-HBc-Ang II	HBc	HBc	Spontaneously hypertensive Rats	anti-Ang II antibody ↑, blood pressure ↓, AngII ↓, IFNγ and IL-2 ↑	[158]
	2016	Angiotensin II type 1 receptor peptide (aa: CAFHYESQ)	Qβ VLP	Qβ VLP	*ApoE*$^{-/-}$ mice	28% plaque area ↓, AT_1R ↓, macrophages ↓	[159]
	2018	Angiotensin II type 1 receptor peptide (GenBank accession no. NM_030985; amino acids 181–187)	Pneumococcal surface protein A (PspA)	Cyclic diguanylate monophosphate (di-GMP)	Spontaneously hypertensive Rats	anti-AT_1R IgG ↑, blood pressure ↓, AngII-AT_1R binding ↓	[160]
	2021	AJP001 (aa: ELKLIFLHRLKRLRKRLKRK) – angiotensin II	N/A	N/A	BALB/c	anti-Ang II antibody ↑, blood pressure ↓, IFNγ and IL-4 and IL-10 ↑, AngII ↑	[161]
Combination							
ApoB-100 and HSP60	2010	ApoB-100 (aa 661–680: IEIGLEGKGFEPTLEALFGK) and human HSP60 (aa 153–163: AELKKQSKPVT)	N/A	Keyhole limpet hemocyanin (KLH)	Apob^(tm2Sgy)LDLr^ (tm1Her/J) mice	41.3% atherosclerotic lesion area↓, peptide-specific IgG ↑, macrophage and $CD4^+$ and dendritic cell ↓, IFNγ and TNFα ↓, IL-10 ↑	[167]
	2012	Human ApoB peptide (aa 688–707: IEIGLEGKGFEPTLEALFGK), human HSP60 peptide (aa 153–163: AELKKQSKPVT), human HSP60 peptide (aa 303–312: PGFGDNRKNQ), and *Chlamydia pneumonia* peptides (aa 67–74: GDYVFDRI and aa 283–291: QAVANGGAI)	N/A	Alum	Apob^(tm2Sgy)LDLr^ (tm1Her) J mice	63.8% atherosclerotic lesion area ↓, peptide-specific IgG ↑, $CD4^+$ ↑	[162]
	2013	ApoB-100 (aa 661–680: IEIGLEGKGFEPTLEALFGK) and human HSP60 (aa 153–163: AELKKQSKPVT)	N/A	KLH	ApoB^(tm25gy)LDLr^ (tm1Her) mice	39.9% atherosclerotic lesion area↓, T-regulatory cells and macrophage ↑, $CD4^+$	[163]
	2014	Human ApoB-100 (aa 688–707: IEIGLEGKGFEPTLEALFGK), human HSP60 (aa 153–163: AELKKQSKPVT), and Chlamydia peptides (aa 67–74: GDYVFDRI and aa 284–292: QAVANGGAI)	N/A	N/A	ApoB^(tm2Sgy) / LDLr^ (tm1Her/J) mice	$CTLA4^+$ and $CD4^+CD25^+Foxp3^+$ T_{reg} ↑, 46.5% lesion area ↓, $CD4^+CD25^+Foxp3^+$ T_{reg}↑, $CD11c^+$ cells ↑	[164]
ApoB-100, CETP, and PCSK9	2021	Human ApoB (aa 3163–3182: KTTKQSFDLSVKAQYKKNKH), Human CETP (aa 461–476: FGFPEHLLVDFLQSLS), and Human PCSK9 (aa 207–223: NVPEEDGTRFHRQASKC)	Qβ VLP	Qβ VLP	C57BL/6 J mice	peptide-specific IgG ↑, CETP and ApoB and PCSK9 ↓, total cholesterol ↓	[90]

Lipid-Related Antigens							
Protein Target	**Year**	**Antigens**	**Carrier**	**Adjuvant**	**Model**	**Results**	**Reference**
CETP and HSP60	2012	Hsp65 DNA, Hsp65-CETP-PADRE-TT-CETP (HCPTC protein), or Hsp65 DNA + HCPTC protein	N/A	N/A	New Zealand white rabbits	aortic lesion area ↓, anti-CETP and anti-HSP65 antibodies ↑, LDL-C ↓, IFNγ ↓, IL-10 ↑	[165]